**Study Protocol**

**A randomised double blind, placebo controlled study of the efficacy of topical menthol for pain relief during topical photodynamic therapy**

| | |
|---|---|
| Study Acronym | **Menthol for PDT pain** |
| Sponsor | University of Dundee |
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**PROTOCOL APPROVAL**

**A randomised double blind, placebo controlled study of the efficacy of topical menthol for pain relief during topical photodynamic therapy**

EudraCT number 2015-002849-59

**Signatures**

**By signing this document I am confirming that I have read, understood and approve the protocol for the above study.**

| | | |
|---|---|---|
| **Professor Sally H. Ibbotson** | Sally H Ibbotson | 20.11.19 |
| Chief Investigator | Signature | Date |
| **Dr Robert S. Dawe** | Robert Dawe | 20.11.19 |
| Individual Responsible for Statistical Review | Signature | Date |

**LIST OF ABBREVIATIONS**
(including Study abbreviations)

| | |
|---|---|
| GCP | Good Clinical Practice |
| IMP | Investigational Medicinal Product |
| TMF | Trial Master File |
| SOP | Standard Operating Procedure |
| CRF | Case Report Form |
| AE | Adverse Event |
| SAE | Serious Adverse Event |
| AR | Adverse Reaction |
| UAR | Unexpected Adverse Reaction |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| CNORIS | Clinical Negligence and Other Risks Scheme |
| NRES | National Research Ethics Service |
| REC | Research Ethics Committee |
| MHRA | Medicines and Healthcare Products Regulatory Authority |
| PBU | Photobiology Unit |
| PDT | Photodynamic Therapy |
| NMSC | Non-melanoma skin cancer |
| VAS | Visual analogue scale |

**SUMMARY**

Topical photodynamic therapy (PDT) is widely used to treat superficial non-melanoma skin cancer (NMSC) and dysplasia, notably actinic keratosis and may also be effective in a range of other dermatological conditions. A major limitation of PDT is pain during irradiation, which occurs in most and can be severe in ~20% of patients. A lack of knowledge of the mechanism of PDT-induced pain has limited the development of effective approaches for prevention or relief of this adverse effect. We developed a mouse model of PDT-induced pain that enabled us to determine the molecular mechanisms involved. Use of the model in combination with an *in vitro* assay of PDT-induced excitation of nociceptive neurons led to our identification of menthol as a potential analgesic agent for minimising PDT-pain. This proposal describes the prospective randomised double blind, placebo controlled clinical trial we will undertake in which we investigate the use of topical menthol for PDT-induced pain relief in patients with actinic keratosis of the face and scalp who will be attending general dermatology and PDT outpatient clinics at Ninewells Hospital, Dundee.

This will be undertaken by comparison of 5% menthol in aqueous cream with aqueous cream as placebo and the primary outcome measures will be pain recorded on a visual analogue scale (VAS) during and up to 24 h after PDT. Secondary outcomes are phototoxicity, assessed by a semi-quantitative scoring system immediately after PDT, fluorescence assessed routinely after cream application and before irradiation and outcome based on clinical assessment three months after PDT and patient evaluation. Patients will be involved in the study from the first visit for PDT until the three-month assessment visit after PDT. Data analysis will be undertaken using within-subject paired analyses as patients act as their own control. Information from this study will inform us as to whether we should or should not incorporate topical menthol into PDT treatment regimens routinely in order to reduce pain and increase tolerance of treatment. The information will also provide us with additional information as to the mechanisms of PDT-induced pain and its possible prevention and/or relief.

## 1.0 INTRODUCTION

### 1.1 BACKGROUND

**Overview of background and purpose to the study**

Topical photodynamic therapy (PDT) is widely used to treat superficial non-melanoma skin cancer (NMSC) and dysplasia, notably actinic keratosis (AK) and may also be effective in a range of other dermatological conditions [1, 2].

Topically applied 5-aminolaevulinic acid (ALA), or its methyl ester (MAL), is absorbed (preferentially by diseased skin) and metabolised to the photosensitiser, protoporphyrin IX (PpIX) [3]. Irradiation during PDT causes photobleaching of PpIX and production of reactive oxygen species (ROS), particularly singlet oxygen, initiating cell destruction. A major limitation of PDT and the primary reason for lack of successful treatment delivery and refusal of therapy is pain during irradiation, which occurs in most and can be severe in ~20% of patients [3, 4]. A lack of knowledge of the mechanism of PDT-induced pain has limited the development of effective

approaches for prevention or relief of this adverse effect [3-5]. The use of low irradiance light delivery may limit pain but requires prolonged treatment times and is only applicable in specific situations and is not feasible for most patients treated with PDT [6, 7]. It is also unclear whether the choice of photosensitiser pro-drug influences pain severity. Clinical studies suggest that MAL use is either associated with less severe PDT-induced pain than the use of ALA [4, 8, 9] or that there is no difference between treatments [10, 11]. The European Medicines Agency has recently approved Ameluz® (BF-200 ALA) for PDT of AK of the face and scalp [12]. Ameluz® is a nanoemulsion containing 8% ALA-HCl, with improved stability. In a recent study 82% of patients experienced pain during Ameluz® PDT but this was not significantly different to MAL PDT [11]. We developed a mouse model of PDT-induced pain that enabled us to determine the molecular mechanisms involved. Use of the model in combination with an *in vitro* assay of PDT-induced excitation of nociceptive neurons led to our identification of menthol as a potential analgesic agent for minimising PDT-pain (see below). It is essential that we now determine whether the topical application of menthol is effective for PDT-induced pain relief in patients treated with PDT as this is a simple, safe approach that could easily be routinely incorporated into PDT regimes and if effective would markedly improve the management of such patients. Thus, this proposal describes the prospective randomised double blind, placebo controlled clinical trial we will undertake in which we investigate the use of topical menthol for PDT-induced pain relief in patients with AK of the face and scalp.

**Pain associated with PDT**

Most patients receiving PDT experience pain during irradiation. In Dundee, 16% of 4717 PDT treatments were associated with severe pain [4]. This limits successful and effective delivery of PDT and negatively impacts on the patient's experience of treatment. Unsurprisingly, given the nature of pain transmission, PDT is most painful in patients when performed on highly innervated skin such as the face and scalp [5, 13]. Interestingly, higher pain levels are experienced during PDT for AK and psoriasis than for basal cell carcinoma [13, 14], perhaps implicating neuromodulatory factors released from specific cell types. Characteristics of irradiation (emission spectrum and irradiance) appear to influence the perception of pain. Red light produces more pain than violet light and pain appears to correlate with the rate of photobleaching of PpIX [15, 16]. Reduction of irradiance may reduce pain but greatly increases treatment times [6, 7]. Other options for pain relief are limited as conventional non-invasive methods, such as topical analgesia with morphine or local anaesthetics are not significantly effective [5, 17, 18]. Local anaesthetic nerve block results in effective pain reduction [19] but is invasive, not feasible at all body sites and there may be risk of toxicity. Cooling is associated with slight reduction in PDT-induced pain [20], although there are concerns that this may reduce the efficacy of PDT [21].

**Mechanisms of PDT pain**

Little is known about the mechanism(s) of PDT-induced pain. At the time of writing a literature search using the terms “pain and photodynamic therapy” yielded 423 publications, although most relate to the prevalence of PDT-pain and the limited success of treatment. Inhibition of PDT-induced pain by nerve block [19] implicates classical modes of pain transmission in which voltage activated sodium channels (VASCs) are activated leading to action potentials in primary afferent fibres stimulating ascending pain pathways in the spinal cord, thalamus and cortex [22]. PDT pain is described as a burning sensation. Furthermore, demonstrations that cooling reduces PDT pain may implicate heat-sensitive nociceptors. The transient receptor potential (TRP) family of ion channels senses heat, among other noxious stimuli including the pungent vanilloid capsaicin [23].

The heat/capsaicin activated TRP channel is TRPV1 [24] and this channel is a candidate for mediating PDT pain. However, PDT causes only modest temperature increases below the threshold for TRPV1 under normal conditions [24, 25] (Figure 1; Appendix 1) and thus there remains uncertainty about mechanisms involved.

**Pre-clinical Pilot Studies**

In pilot studies, we identified an electrophysiological correlate of PDT-evoked pain in cultured mouse primary afferent DRG neurons and developed a behavioural model for this phenomenon (Figures 2-9; Appendix 1).

**PpIX phototoxicity-induced pain behaviour in mice**

We examined the effect of laser irradiation (630 nm, 3.7 J/cm2) and ALA applied topically (4-6 h) to the tails of NIH SWISS mice. Neither topical ALA (n = 35), nor laser irradiation alone (n = 30), caused discernible pain behaviour in either mouse strain. By contrast, 20 ± 3 s (n = 30) of tail lifting, holding and licking occurred during 60 s following laser irradiation of ALA treated tails (Figure 2a; Appendix 1). Pain behaviour ceased within 5 minutes suggesting that the underlying pain was acute and transient. Tail lifting, holding and licking did not occur in the 60 s period following irradiation in mice treated with vehicle cream. We observed PpIX emission non-invasively using fluorescence spectroscopy with fibre-optic coupled laser excitation at 405 nm (Figure 2b; Appendix 1). Compared to vehicle treated mouse tails, the characteristic PpIX emission spectrum was enhanced by ALA treatment to levels similar to those achieved in the ALA treated human forearm (Figure 2b; Appendix 1). The *in vivo* data demonstrated that neither PpIX production nor laser irradiation alone was sufficient to generate pain in mice. By contrast, ALA PDT was associated with quantifiable pain behaviour.

**PpIX phototoxicity evokes action potentials in DRG neurons**

Primary afferent dorsal root ganglion (DRG) neurons transmit nociceptive pain from peripheral sites, such as the tail, to the spinal cord. We dissociated DRG from mice and performed flow cytometry on cells grown for 7 days in vitro. Flow cytometry revealed that cells treated with ALA (1 mM for 4 h) produced PpIX fluorescence that was not seen in untreated cells (Figure 3a; Appendix 1). We examined responses of

small DRG neurons (<25 μm) to ALA either alone or in combination with laser irradiation (630 nm) using the cell attached configuration to record action potentials. This approach preserves the intracellular milieu while action potentials are acquired as a combination of resistive and capacitive currents [26]. Spontaneous action potentials occurred very rarely in recordings from DRG neurons (grown for 1-15 days in vitro) under control conditions or in recordings from neurons exposed to ALA (1 mM, 4-7 h) prior to irradiation (Figure 3b; Appendix 1). By contrast, during laser irradiation (630 nm) neurons exposed to ALA exhibited bursts of frequent action potentials (Figure 3b; Appendix 1).

While 97% of neurons responded to PpIX irradiation (day 4-15 in vitro), there was considerable variability in the latency to the first burst of action potentials (486 ± 20 s, n = 135), which equates to a mean light dose of 47 ± 2 J/cm2 required to evoke a response. The instantaneous frequency of action potentials was 11.6 ± 1.3 Hz (day 7 in vitro). The mean frequency of firing during the entire period of irradiation was 0.23 ± 0.03 Hz. Interestingly, despite the known cellular toxicity of PpIX irradiation, resulting from the release of ROS, most DRG neurons survived the full 1200 s of laser exposure.

Using the whole-cell current-clamp recording configuration we examined the effects on membrane potential of ALA and irradiation either alone or in combination (Figure 3c; Appendix 1). DRG neurons exhibited infrequent spontaneous action potentials under control conditions. Neither ALA (-57 ± 3 mV, n = 7) nor laser irradiation alone (-60 ± 3 mV, n = 5) affected resting membrane potential or frequency of spontaneous action potentials (Figure 3c; Appendix 9). However, laser irradiation of ALA treated (4-7 h) DRG neurons led to sustained depolarisation (of 9 ± 1 mV, n = 3) and the appearance of frequent action potentials (Figure 3d; Appendix 9).

Taken together, the *in vitro* data demonstrate that neither PpIX production nor laser irradiation alone was sufficient to generate action potentials in nociceptive neurons. By contrast, PpIX irradiation depolarized neurons and initiated bursts of action potentials.

**Menthol suppresses PpIX phototoxicity-evoked action potentials and pain**

Menthol exhibits local anaesthetic effects through inhibition of VASCs on nociceptive neurons [27]. Menthol also causes a cooling sensation by activating TRPM8 receptors [28]. Both of these actions could provide analgesia to patients being treated with PDT. We examined whether menthol (300 μM and 600 μM) inhibited ALA PDT-induced action potentials recorded from DRG neurons.

Menthol inhibited action potential frequency in a concentration-dependent manner (Figure 4a; Appendix 1). Also, the percentage of cells that responded to PDT was reduced by menthol (600 μM) from 100% to 33%. Menthol (300 μM) had no effect on the threshold light dose; however, the reduction in the number of cells responding in

the presence of 600 μM menthol precluded analysis of the average threshold light dose in this case.

We next investigated whether menthol influenced ALA PDT-induced pain behaviour in mice. The application of menthol (16%) in aqueous cream had no effect on PpIX-mediated fluorescence determined using the photospectrometer with fibre-optic coupled laser excitation at 405nm (Figure 4b; Appendix 1). The application of menthol (2% and 16%) to the tails of mice 600s prior to laser (630 nm, 3.7 J/cm$^2$) irradiation caused a dose-dependent reduction in the duration of pain behaviour (Figure 4c; Appendix 1). These data suggest a role for TRP pathways [29] and indicate that menthol is an effective analgesic for treating ALA PDT-induced pain in mice and we will now investigate this in patients receiving PDT for AK.

### 1.2 RATIONALE FOR STUDY

As menthol is such a well-tolerated and safe treatment we would envisage that if the clinical study supported the *ex-vivo* findings and did indeed significantly reduce PDT-induced pain, then this would be routinely incorporated into PDT treatment protocols and significantly improve patient care and the tolerance of PDT. We will use 5% menthol as this is available for clinical use and in order not to miss a potential pain-relieving effect.

## 2 STUDY OBJECTIVES

### 2.1 OBJECTIVES

#### 2.1.1 Primary Objectives

The primary objective of this study is to examine whether the pain (as assessed by VAS pain scores) of topical PDT is significantly different when using menthol in aqueous cream applied before PDT compared with aqueous cream (placebo) application before PDT.

#### 2.1.2 Secondary Objectives

We will also examine possible differences in phototoxicity of the two regimens and assess patient evaluation of treatment. We will additionally assess fluorescence after cream application and before irradiation, using Wood's light as used in routine clinical practice. Efficacy of the two treatment regimens will also be assessed.

### 2.2 OUTCOMES

#### 2.2.1 Primary Outcomes

Pain will be assessed by the patient using a visual analogue scale of 0-10 cm. Maximal recall of pain experienced during PDT will be recorded immediately after PDT, and then pain scores at three, six and 24 hours after PDT. The patient-scored pain at the first time point will be undertaken with the aid of a blinded investigator and subsequently the patient alone will assess symptoms.

### 2.2.2. Secondary Outcomes

Phototoxicity as assessed by semi-quantitative scoring of erythema, oedema and urticaria will be assessed immediately after irradiation. Fluorescence will also be assessed after Ameluz and IMP applications using Wood's light. Efficacy of treatment (clearance, partial or no response) three months after PDT will be determined clinically. Patient evaluation of treatment will also be assessed.

# 3 STUDY DESIGN

## 3.1 STUDY DESCRIPTION

This protocol describes a prospective, single site, randomised, double blind placebo controlled study to assess the efficacy of menthol in aqueous cream for PDT-induced pain. Each subject will act as their own control. Potential participants with a diagnosis of actinic keratosis (AK) bilaterally affecting face or scalp who are referred for PDT will be identified by the Chief Investigator (CI), a consultant dermatologist, screening referrals and will be invited to participate by letter and a patient information sheet (PIS) will be sent out with the PDT clinic appointment details. Patients will also be identified in the dermatology outpatient or PDT clinics by either a dermatologist, specialist nurse or technologist and will be provided with details of the study and a PIS. At first visit at the PDT clinic the study will be described in full detail by a PDT study doctor, technologist or clinic nurse and written informed consent will be obtained prior to any study-related activities. Computer-generated block randomisation of the active/placebo to right or left sides and which side will be treated first will be undertaken by Tayside Pharmaceuticals. Patients will then receive their randomised treatment and this will also be undertaken by the Photobiology Technician or nurse as is the normal clinical practice in the PDT Unit.

A study clinician, technologist or nurse will obtain a medical history and the areas for treatment will be examined, the study fields will be marked out, lesions counted and the fields mapped and photographed. The maximal diameter of each comparable field will be 5x10cm. Topical PDT will be undertaken using the standard Ameluz® PDT regime (11). In brief, any hyperkeratotic areas will be surface prepared using a disposable ring curette (Stiefel™), without the need for local anaesthesia. Ameluz® gel will be thinly applied to both comparative fields, left to dry and occluded for three hours with Tegaderm and Mepore dressings. After removal of the dressings and surplus gel, the active treatment (menthol 5% in aqueous cream) or placebo (aqueous cream) will be applied to right/left side according to randomisation The IMPD/placebo will be provided as 1g in 2mL syringes, applied to the maximum field size field of 5x10cm or pro-rata to smaller field sizes. Ten minutes later any excess cream will be wiped off and irradiation will commence (LED 37.5 J/cm$^2$, irradiance approximately 80 mW/cm$^2$), with the randomisation to account for which side will be treated first. Menthol vapour will be in the room in order to mask the vapour of the IMP and to aid with ensuring blinding of both patient and assessor (research nurse or another technologist).

**Assessments**

Fluorescence will be examined using a semi-quantitative scoring system (0=none; 1=minimal; 2=moderate; 3=strong) after 3 h of Ameluz application and after menthol/placebo application.

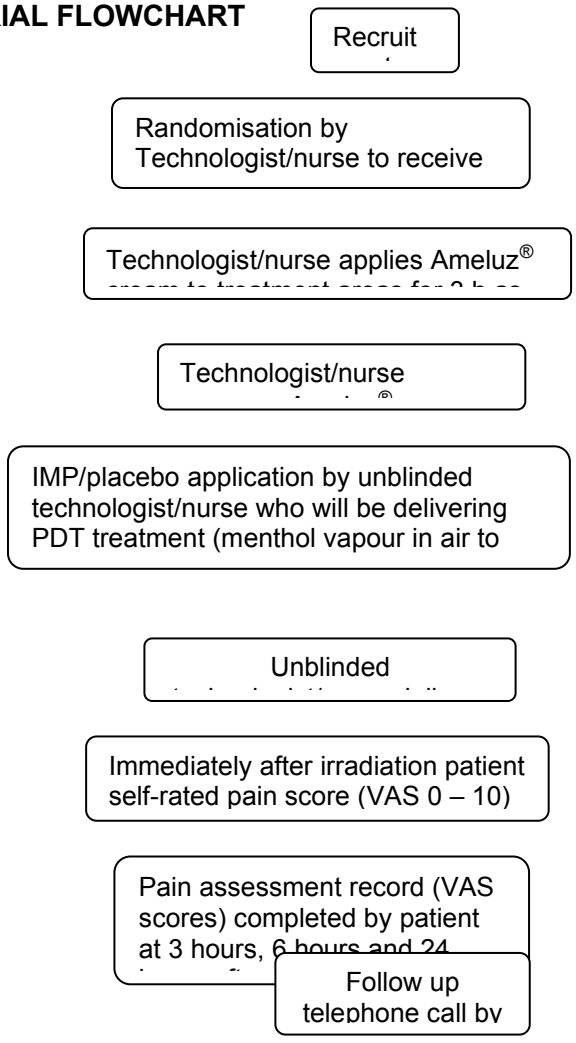
Pain scores will be recorded by the patient immediately after irradiation (as recall of maximal pain experienced during irradiation) and at 3, 6 and 24 h after irradiation

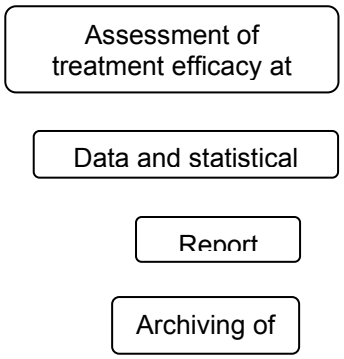
using a VAS score of 0-10cm at each time-point. The study technologist or research nurse will be present at the time of the first pain score but patients will fill in the VAS scores themselves at home for the 3, 6 and 24 h time-points and send these back to the PDT clinic by stamped addressed envelope. A study nurse will contact the patient by phone at one week, to ask about any adverse effects and to check that the VAS scores are returned.

Phototoxicity will be assessed by the study technologist or research nurse immediately after irradiation using a semi-quantitative scoring system for erythema (0=none; 1=mild; 2=moderate; 3=severe), oedema (1=yes; 0=no), exudation (1=yes; 0=no) and urticaria (1=yes; 0=no).

Efficacy will be assessed clinically approximately three months (± 2 weeks) after PDT, with clearance, partial response or no response recorded as outcomes. Patient evaluation of treatment and of blinding will also be assessed for each treatment site when the patient returns their pain scores after 24h, using the following two questions: [1] Which side do you think received menthol? Right/left/not sure; [2] Which side do you think was best? Right/left/no difference.

## 3.2 TRIAL FLOWCHART






### 3.3 STUDY MATRIX

| ACTIVITY | Day 1 | Day 2 | DAY 8 | Approx. 3 months |
|---|---|---|---|---|
| Informed consent | √ | | | |
| Eligibility screening | √ | | | |
| Standard skin preparation for PDT | √ | | | |
| Application of IMP/placebo (remove after 10 minutes) | √ | | | |
| PDT irradiation | √ | | | |
| Pain assessment (visual analogue scores) by subject immediately after PDT - clinic | √ | | | |
| Fluorescence assessment by technician | √ | | | |
| Phototoxicity assessment by technician | √ | | | |
| Pain assessment (visual analogue scores) by subject 3hrs after PDT - home | √ | | | |
| Pain assessment (visual analogue scores) by subject 6 hrs after PDT - home | √ | | | |
| Pain assessment (visual analogue scores) by subject 24 hrs after PDT - home | | √ | | |
| Follow-up phone call by research nurse | | | √ | |
| Assessment of treatment efficacy at 3 months after PDT | | | | √ |
| Patient evaluation of PDT & blinding | | √ | | |

## 4 STUDY POPULATION

### 4.1 NUMBER OF PARTICIPANTS

30 patients will be recruited to the study, with a view to 21 completing the study allowing for a 30% drop-out rate at the three month follow-up visit. The study will be performed in one centre and each participant will be in the study for three months.

### 4.2 INCLUSION CRITERIA

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. Adults >18 years. Target population is men or women ≥50 years (only post-menopausal women)
2. Presence of actinic keratoses (AK) on the face and scalp involving both right and left comparable sites.
3. Free of significant physical abnormalities (e.g. tattoos, dermatoses) in the potential treatment area that may cause difficulty with examination or final evaluation.
4. Able to understand and adhere to protocol requirements.

### 4.3 EXCLUSION CRITERIA

1. Unable to give written informed consent.
2. Allergy to menthol, aqueous cream or excipients
3. Participation in a drug trial or other interventional study within 30 days of recruitment to this study
4. Pre-menopausal women, pregnancy, breast feeding, planning to conceive
5. Chronic pain

## 5 PARTICIPANT SELECTION AND ENROLMENT

### 5.1 IDENTIFYING PARTICIPANTS

Potential participants with a diagnosis of actinic keratosis (AK) who are referred for PDT will be identified by the chief investigator (CI), a consultant dermatologist via screening referrals and will be invited to participate by letter and a patient information sheet (PIS) will be sent out with the PDT clinic appointment details. Potential participants will also be identified during consultation by a Dermatologist, Specialist Nurse or technologist in the Dermatology or PDT clinics. Patients will be invited to participate in the study and will be given a Participant Information Sheet (PIS), which will include contact details for members of the research team. In the event of the patient not receiving the study PIS in the clinic, one will be sent with their appointment letter for the PDT clinic. All subjects will be given at least 24 hours to read the PIS and have the opportunity to discuss the study and have any questions answered before deciding if they wish to participate in the study. If willing to proceed, written informed consent will be obtained by a Dermatologist or a delegated suitably qualified member of the research team.

### 5.2 CONSENTING PARTICIPANTS

Where a participant requests to speak with a Dermatologist from the study team the consent process will not be completed until the participant has spoken to the doctor and had all their questions answered to their satisfaction.

### 5.3 SCREENING FOR ELIGIBILITY

Potential participants with a diagnosis of actinic keratosis (AK) bilaterally affecting face or scalp who are referred for PDT will be identified by the Chief Investigator (CI), a consultant dermatologist, screening referrals and will be invited to participate by letter and a patient information sheet (PIS) will be sent out with the PDT clinic appointment details. Patients will also be identified in the dermatology outpatient or

PDT clinics by either a dermatologist, specialist nurse or technologist and will be provided with details of the study and a PIS. At first visit at the PDT clinic the study will be described in full detail by a PDT study doctor, technologist or clinic nurse and written informed consent will be obtained prior to any study-related activities.

## 5.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

A screening log will be populated and maintained to list reasons for ineligibility and non-recruitment. These patients will then proceed to standard clinical care

## 5.5 RANDOMISATION

### 5.5.1 Randomisation

Randomisation will be to left or right side within each individual as we cannot consider pain experiences on right vs. left within an individual as being independent. We want to compare pain immediately after PDT illumination (time-point of most interest) and, as another way of comparing between the interventions "pain experience over-time" (while trying to reduce the multiple comparisons "problem"), to compare within-subjects Areas Under the Curve of pain over time.

Patients will be randomly allocated to either active or placebo treatment to right or left side using a computer-generated block randomisation sequence, which will also include randomisation as to which side will be treated first. The randomisation code will be generated by Tayside Pharmaceuticals and will be concealed from study investigators.

### 5.5.2 Treatment Allocation

Patients will be allocated treatment to both right side and left side e.g. right side to receive treatment A; left side to receive treatment B. Patients should be unable to distinguish treatments by smell as menthol has a strong smell, which will likely permeate the air around the patient. In addition, menthol vapour will be present in the room so that there is an overall menthol smell, which will be difficult to localise.

### 5.5.3 Blinding and Emergency Unblinding Procedures

Those assessing patients (Consultant Dermatologist, study technologist or research nurse) will not be aware of which side has received which intervention. It is possible that all patients (from sensation [cooling] of menthol) might not remain fully blinded. Patients will be instructed not to tell those assessing them if they suspect they know which intervention is applied to each side. After treatment, patients will be asked to provide an opinion as to which treatment was applied to each side to allow assessment as to how complete patient blinding was.

Given the nature of the study, it is very unlikely that unblinding will be required. If required, it will be conducted by a PBU member of staff unconnected to the trial.

### 5.5.4 Withdrawal procedures

Although a participant is not obliged to give reason(s) for withdrawing prematurely, if the participant appears lost to follow up, the CI will make a **reasonable** effort to ascertain the reason(s), while fully respecting the individual's rights, and will demonstrate that everything possible was done in an attempt to find any participant lost to follow-up. Those lost to follow-up or withdrawn will be identified and a

descriptive analysis of them provided, including the reasons for their loss and its relationship to treatment and outcome.

# 6 INVESTIGATIONAL MEDICINAL PRODUCT

## 6.1 STUDY DRUGS/IMPS

### 6.1.1 Study drug identification

Active IMP is formulated into a cream and supplied as 1g in a 2ml syringe for topical administration.

**Composition of Active IMP - Levomenthol 5% Cream**

| Component | Reference | Amount |
|---|---|---|
| Menthol | Ph Eur | 50mg (5% w/w) |
| Aqueous Cream | BP<br>PL 04917/0057 | to 1g<br>(50mg menthol + 950mg aqueous cream) |

Placebo is provided as a cream (1g in a 2ml syringe) for topical administration.

**Composition of placebo cream -** Aqueous Cream BP.

| Component | Reference | Amount |
|---|---|---|
| Aqueous Cream | BP<br>PL 04917/0057 | 1g (100%) |

### 6.1.2 Study IMP Manufacturer

The investigational drug substance is Menthol 5% in Aqueous Cream. This product is manufactured by Tayside Pharmaceuticals, Ninewells Hospital & Medical School, Dundee, DD1 9SY, MIA (IMP) 14076 (see IMPD).

The placebo is Aqueous Cream BP, which is a licensed product, PL 04917/0057

### 6.1.3 Marketing authorisation holder

Tayside Pharmaceuticals MIA (IMP) 14076

### 6.1.4 Labelling and Packaging

Study IMPs will be packaged and labelled by Tayside Pharmceuticals.

### 6.1.5 Storage

Study IMPs will be released by Tayside Pharmceuticals to NHS Tayside Pharmacy who will supply them to the PBU. IMPs will be stored in a local PBU storage area which will be audited prior to use by the NHS Tayside Clinical Trials Pharmacist. Temperature control logs will be maintained.

### 6.1.6 IMP Safety Information

The simplified IMPD will be held in the Pharmacy Site File (PSF) and in the Trial Master File (TMF). No adverse effects are expected. A minor cooling or tingling

sensation may be expected with IMP contact with skin. Contact with eyes or mucosal surfaces will be avoided as these are not within the study treatment sites.

### 6.1.7 Accountability procedures

An IMP Accountability Log will be maintained in the TMF.

## 6.3 DOSING REGIME

IMP will be applied topically by a study technologist or nurse at study Visit 1. After removal of the dressings and surplus Ameluz® gel, the active treatment (menthol 5% in aqueous cream) or placebo (aqueous cream) will be applied to right/left side according to randomisation. Ten minutes later any excess cream will be wiped off and irradiation will commence using a standard red light LED (37.5 J/cm$^2$, irradiance 80 mW/cm$^2$), with the randomisation to account for which side will be treated first. Menthol vapour will be in the room in order to mask the vapour of the IMP and to aid with ensuring blinding of both patient and assessor (research nurse or another technologist).

## 6.4 DOSE CHANGES

No planned dose changes in IMPs

## 6.5 PARTICIPANT COMPLIANCE

As both IMPs will be applied topically by a study technologist at study Visit 1, there will be no requirement to monitor participant compliance as this will not be relevant.

## 6.6 OVERDOSE

No risk of overdose

## 6.7 OTHER MEDICATIONS

### 6.7.1 Permitted medications

Usual medications are permitted except for menthol preparations used on the treatment day.

### 6.7.2 Prohibited medications

Menthol preparations used on the treatment day

### 6.7.3 Concomitant Medications

All usual medications will be permitted except for menthol preparations. Concomitant medications will be logged in the Case Report Form (CRF).

# 7 STUDY ASSESSMENTS

## 7.1 STUDY ASSESSMENTS

As detailed above – pain, fluorescence, phototoxicity, efficacy and patient evaluation will be assessed.

## 7.2 SAFETY ASSESSMENTS

Pain and phototoxicity will be assessed by per protocol and intention to treat analyses

# 8 DATA COLLECTION & MANAGEMENT

## 8.1 DATA COLLECTION

Data will be collected in the CRF and appropriate clinical information will also be recorded in patient notes. Both patient notes and CRF will act as source data.

## 8.2 DATA MANAGEMENT SYSTEM

The data management system will be Excel. The study system will be based on the protocol and CRF. The CRF will not collect more information than is required to meet the aims of the study and to ensure the eligibility and safety of the participant. Data collection and validation will be carried out as described in TASC SOP on Excel in CTIMPs.

# 9 STATISTICS AND DATA ANALYSIS

## 9.1 SAMPLE SIZE CALCULATION

We were not able to obtain variance for within-subjects pain differences from similar studies. An assessment of pain experience with PDT in our unit found a standard deviation of between subjects VAS pain score of 2.7cm. Rounding this up to 3cm and conservatively estimating that within-subject's pain score SD will be two-thirds of this (2cm) then we estimated that 21 subjects should give us 90% power to detect a mean difference in VAS scores of 1.5 cm as significant at the 5% level. To allow for dropouts, assuming dropout rate by 3 months of up to 30%, we will recruit 30 subjects.

## 9.2 PROPOSED ANALYSES

The main analysis will the difference between within-subject pain using appropriate paired methods (paired Student's t-test if assumptions met). The difference will be expressed with 95% confidence interval. VAS scores immediately after PDT and the areas under the curve for pain scores plotted over each of the time points will be assessed. Within subject comparisons of fluorescence, phototoxicity, efficacy and patient evaluation will be secondary outcome measures, using paired statistical analyses.

**Amendment**

Since the original concept and set up of the study, there have been changes in the practice of PDT for AKs on the face and scalp, with low illuminance daylight PDT now favoured when practicable because this form of PDT is less painful than conventional PDT. Therefore, we will perform (statistical analysis by someone not involved in patient treatments and assessments, to ensure blinding) an interim analysis. With the same assumptions as before with a power of 90% we expect to detect a difference (at alpha = 0.1 level, two tailed testing) of 3 cm or more (on VAS) in pain between groups with 9 subjects should such a difference exist. We will therefore recruit a minimum of 9 participants and if on this interim analysis such a difference is detected then the study will be continued as originally planned in order to determine whether the difference can be detected at alpha = 0.05 level of significance, unless the dermatologist analysing the data advises stopping early as a greater difference already detected. If it seems unlikely that there will be a difference of ≥ 3 cm in pain

on VAS based on this interim analysis, then (with the changes in use of PDT for AKs on face and scalp) the data analyst will advise stopping the study.

# 10 ADVERSE EVENTS

## 10.1 DEFINITIONS

An **adverse event** (AE) is any untoward medical event affecting a clinical trial participant. Each initial AE will be considered for severity, causality or expectedness and may be reclassified as a serious event or reaction based on prevailing circumstances.

An **adverse reaction** (AR) is where it is suspected that an AE has been caused by a reaction to a trial drug

A **serious adverse event** (SAE), **serious adverse reaction** (SAR) or **suspected unexpected serious adverse reaction** (SUSAR) is any AE, AR or UAR that at any dose:

- results in death
- is life threatening
- requires hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- Or is otherwise considered serious

Note: Hospitalisations for treatment planned prior to randomisation and hospitalisation for elective treatment of a pre-existing condition will not be considered as an SAE. However any adverse events occurring during such hospitalisation will be recorded.

## 10.2 RECORDING AND REPORTING AES AND SAES

Topical menthol may cause a sensation of cooling and hyperalgesia and, with repeated applications may cause some skin irritation. However, with a single 10 minute application to a small area, as proposed in this study, we do not anticipate any adverse effects and, indeed, any cooling, hyperalgesic effect may be desirable with respect to potential pain relief. The clinical data and Reference Safety Information (RSI) are summarised in **Appendix 2**.

All AEs and SAEs will be recorded from the time a participant consents to join the study until the last study visit. Participants with unresolved AEs at the last study visit will be followed up until resolution or 30 days after last patient, last visit (LPLV), whichever is sooner. SUSARS will be followed until resolution.

The CI or delegate will ask about the occurrence of AEs and hospitalisations at every visit during the study. AEs will be recorded on the AE Log in the CRF. SAEs will be submitted on an SAE form to the TASC Safety Section (pharmacovigilance.tayside@nhs.net) within 24 hours of becoming aware of the SAE. SAEs will be assessed for expectedness and causality by the Investigator. The evaluation of expectedness will be made based on the knowledge of the reaction and

the relevant product information **(simplified IB; Appendix 2).** Refer to TASC SOP 11 “Identifying, Recording and Reporting Adverse Events for CTIMPs”.

### 10.3 REGULATORY REPORTING REQUIREMENTS

The Sponsor, together with the CI, is responsible for reporting SUSARs to the competent authority, the MHRA, the Research Ethics Committee (REC) and any other competent authorities. Fatal or life threatening SUSARs will be reported within 7 days and non-fatal and non-life threatening SUSARs within 15 days.

### 10.4 ANNUAL REPORTING REQUIREMENTS

The following reports will be submitted each year as a condition of the authorisation to undertake a clinical trial or as a condition of a favourable opinion from a REC.

The Development Safety Update Report (DSUR) will be prepared jointly by the TASC Safety Section and CI and submitted to the MHRA on the anniversary of date of Clinical Trial Authorisation (CTA).

An NRES CTIMP Safety Report Form will be sent to REC along with the DSUR. Reports of SUSARs in the UK, urgent safety measures and any other safety reports submitted, for example, reports of a data monitoring committee, will also be accompanied by a Safety Report Form.

A NRES Annual Progress Report for CTIMPs will be prepared and submitted by the CI to REC, and copied to Sponsor, on the anniversary date of the REC favourable opinion.

### 10.5 URGENT SAFETY MEASURES

The CI or other clinician may take appropriate immediate urgent safety measures in order to protect the participants of a CTIMP against any immediate hazard to their health or safety. The MHRA, REC and Sponsor will be notified in writing within three days.

## 11 PREGNANCY

Pregnancy is not considered an AE or SAE, unless there is a congenital abnormality or birth defect. Any unexpected pregnancy occurring during the clinical study and the outcome of the pregnancy, will be recorded on a TASC Pregnancy Notification Form and submitted to the TASC Safety Section (pharmacovigilance.tayside@nhs.net) within 24 hours of becoming aware of the pregnancy. The pregnancy will be followed up until the end of the pregnancy. If the study participant is a male, informed consent for follow up must be sought and obtained from his female partner. This study will only be undertaken in women who are >50 years old and post-menopausal.

# 12 TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

## 12.1 TRIAL MANAGEMENT GROUP

The trial will be co-ordinated by a Trial Management Group, consisting of the CI, Trial Technologist and research nurse who will meet on a weekly basis. The TMG will also be responsible for oversight of data monitoring.

## 12.2 TRIAL MANAGEMENT

A Research Nurse will oversee the study and will be accountable to the CI. The Research Nurse will be responsible for checking the CRFs for completeness, plausibility and consistency. However, this will remain the overall responsibility of the CI. Any queries will be resolved by the CI or delegated member of the trial team.

A study-specific Delegation of Responsibilities & Signature Log will be prepared, detailing the responsibilities of each member of staff working on the trial.

## 12.3 INSPECTION OF RECORDS

The CI and the institutions involved in the study will permit trial related monitoring, audits, REC review, and regulatory inspection(s). In the event of an audit, the CI will allow the Sponsor, representatives of the Sponsor or regulatory authorities direct access to all study records and source documentation.

## 12.4 RISK ASSESSMENT

A study risk assessment was carried out by the TASC Research Governance Manager prior to Sponsorship approval being granted.

## 12.5 STUDY MONITORING

The Sponsor has determined the appropriate extent and nature of monitoring for the trial and will appoint appropriately qualified and trained monitors. Thus, any potential risks will be minimized,

# 13 GOOD CLINICAL PRACTICE

## 13.1 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the principles of good clinical practice (GCP). In addition to Sponsorship approval, a favorable ethical opinion will be obtained from an appropriate REC. Authorisation from an appropriate competent authority(s) and appropriate NHS R&D permissions(s) will be obtained prior to commencement of the study.

### 13.1.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality. All records will be kept in a secure storage area with limited access to study staff only. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee or Regulatory Authorities. The CI and study staff involved with this study will not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals

for the purpose of the study. Prior written agreement from the Sponsor or its designee will be obtained for the disclosure of any said confidential information to other parties.

### 13.1.2 Data Protection

The CI and study staff involved with this study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. The CI and study staff will also adhere, if appropriate, to the current version of the NHS Scotland Code of Practice on Protecting Patient Confidentiality. Access to collated participant data will be restricted to the CI and appropriate study staff.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

### 13.1.3 Insurance and Indemnity

The University of Dundee and Tayside Health Board are Co-Sponsoring the study.
**Insurance.** – The University of Dundee will obtain and hold Professional Negligence Clinical Trials Insurance cover for legal liabilities arising from the study.
Tayside Health Board will maintain its membership of the Clinical Negligence and Other Risks Insurance Scheme ("CNORIS") which covers the legal liability of Tayside in relation to the study].
Where the study involves University of Dundee staff undertaking clinical research on NHS patients, such staff will hold honorary contracts with Tayside Health Board meaning they will have cover under Tayside's membership of the CNORIS scheme.
**Indemnity**.The Co-Sponsors do not provide study participants with indemnity in relation to participation in the Study but have insurance for legal liability as described above.

## 14 STUDY CONDUCT RESPONSIBILITIES

### 14.1 PROTOCOL AMENDMENTS

The CI will seek Sponsor approval for any amendments to the Protocol or other study documents. Amendments to the protocol or other study docs will not be implemented without approval from the Sponsor and subsequent approval from the appropriate REC and/or Regulatory Authority, as appropriate, and NHS R&D Office. Refer to TASC SOP 26 "Amendments to CTIMPs"

### 14.2 PROTOCOL DEVIATIONS, BREACHES AND WAIVERS

The CI will not implement any deviation from the protocol without agreement from the Sponsor, except where necessary to eliminate immediate hazard to trial participants.

In the event that the CI needs to deviate from the protocol, the nature of and reasons for the deviation will be recorded in the CRF, documented in a TASC Deviation & Breach Log and notified to the Sponsor. If this necessitates a subsequent protocol amendment, this will be submitted to the Sponsor for approval and then to the appropriate REC, Regulatory Authority and local NHS R&D for review and approvals as appropriate. It is Sponsor policy that waivers to the Protocol will not be approved.

In the event that a serious breach of the protocol or GCP is suspected, this will be reported to the Sponsor immediately using the form “Notification to Sponsor of Potential Serious Breach or Serious Deviation”. Refer to TASC SOP 25 “Escalation and Notification of Serious Breaches of GCP or the Trial Protocol for CTIMPs”.

### 14.3 STUDY RECORD RETENTION

Archiving of study documents will be carried out as specified in TASC SOP 13: Archiving Clinical Research Data for Clinical Trials of Investigational Medicinal Products. For studies where the data does not form part of an application for a Marketing Authorisation (MA) all study documentation will be kept for at least 5 years. For studies where the data does form part of an application for a Marketing Authorisation (MA) all study documentation will be kept for at least 15 years.

### 14.4 END OF STUDY

The end of study is defined as last patient last visit (LPLV). The Sponsor and CI have the right at any time to terminate the study for clinical or administrative reasons. The end of the study will be reported to the Sponsor, REC, Regulatory Authority and NHS R&D Office within 90 days, or 15 days if the study is terminated prematurely. The CI will ensure that any appropriate follow up is arranged for all participants. A final report of the study will be provided to the Sponsor, REC and Regulatory Authority within 1 year of the end of the study.

## 14.5 CONTINUATION OF DRUG AT END OF STUDY

Not applicable

## 15 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

### 15.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

### 15.2 PUBLICATION AND 15.3 PEER REVIEW

The evaluated data will be used for peer-reviewed publication and presentation at scientific meetings. Trial investigators may present orally and publish in writing study results. A study summary will also be made available to Investigators for dissemination within their clinical areas (where appropriate and at their discretion).

## 16 REFERENCES

1. Morton CA et al., European Guidelines for topical PDT: Part 1. J Eur Acad Dermatol Venereol 2013; 27: 536-44
2. Morton CA et al., European guidelines for topical PDT: Part 2. J Eur Acad Dermatol Venereol 2013; 27: 672-9
3. Morton CA et al., Guidelines for topical PDT. Br J Dermatol 2008; 159:1245-66

4. Attili SK et al., A review of pain experienced during topical PDT- our experience in Dundee. Photodiagnosis Photodyn Ther 2011; 8: 53-7
5. Warren CB, et al. Pain associated with aminolevulinic acid-PDT of skin disease. J Am Acad Dermatol 2009; 61: 1033-43
6. Wiegell SR et al., Continuous activation of PpIX by daylight is as effective and less painful than conventional PDT for actinic keratoses: a randomised controlled single-blinded study. Br J Dermatol 2008, 158: 740-6
7. Moseley H et al., An open pilot study of ambulatory PDT using a wearable low-Irradiance organic LED light source in the treatment of non-melanoma skin cancer. Br J Dermatol 2009; 161: 170-3
8. Gholam P et al., Treatment with 5-aminolaevulinic acid methylester is less painful than treatment with 5-aminolaevulinic acid nanoemulsion in topical PDT for actinic keratosis. Dermatology 2011; 222: 358-62
9. Moloney FJ & Collins P. Randomized double-blind prospective study to compare 5-aminolaevulinic acid methylester with 5-ALA PDT for extensive scalp actinic keratosis. Br J Dermatol 2007; 157: 87-91
10. Valentine RM et al. A quantitative comparison of 5-aminolaevulinic acid- and methyl aminolevulinate-induced fluorescence photobleaching and pain during PDT. Photochem Photobiol 2011; 87: 242-9
11. Dirschka T et al. PDT with BF-200 ALA for the treatment of actinic keratosis: results of a multicentre randomized observer-blind phase III study in comparison with a registered methyl-5-aminolaevulinate cream and placebo. Br J Dermatol 2012; 166:137-46
12. Tzogani K et al., The European Medicines Agency approval of 5-ALA (Ameluz) for the treatment of actinic keratosis of mild to moderate intensity on the face and scalp. J Dermatolog Treat. 2014; 25: 371-4
13. Grapengiesser S et al., Pain caused by PDT of skin cancer. Clin Exp Dermatol 2002; 27: 493-7
14. Radakovic-Fijan S et al., Topical aminolaevulinic acid-based PDT as a treatment option for psoriasis? Results of a randomized observer-blinded study. Br J Dermatol 2005; 152: 279-83
15. Cottrell WJ et al., Irradiance-dependent photobleaching and pain in delta-Aminolevulinic acid-PDT of superficial basal cell carcinomas. Clin Cancer Res 2008; 14: 4475-83
16. Mikolajewska P et al., Topical aminolaevulinic acid- and aminolaevulinic acid methyl ester-based PDT with red and violet light: influence of wavelength on pain and erythema. Br J Dermatol 2009, 161: 1173-9
17. Holmes MV et al., A randomized double-blind placebo-controlled study of the efficacy of tetracaine gel (Ametop®) for pain relief during topical PDT. Br J Dermatol 2004; 150: 337-40
18. Langan SM & Collins P. Randomized double-blind placebo-controlled prospective study of the efficacy of topical anaesthesia with a eutectic mixture of lignocaine 2.5% and prilocaine 2.5% for topical 5-aminolaevulinic acid-PDT for extensive scalp actinic keratoses. Br J Dermatol 2006; 154: 146-9

19. Paoli J et al., Nerve blocks provide effective pain relief during topical PDT for extensive facial actinic keratoses. Clin Exp Dermatol 2008; 33: 559-64
20. Pagliaro J et al., Cold air analgesia in PDT of basal cell carcinomas and Bowen's disease: An effective addition to treatment. Dermatol Surg 2004;30: 63-6
21. von Felbert V et al., PDT of multiple actinic keratosis: reduced pain through use of visible light plus water-filtered infrared A compared with light from light-emitting diodes. Br J Dermatol 2010; 163: 607-15
22. Mogil J.S. Animal models of pain: progress and challenges. Nat Rev Neurosci 2009; 10: 283-94
23. Patapoutian A et al., Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov 2009; 8: 55-68
24. Caterina MJ et al., The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997; 389: 816-24
25. Palsson S et al., Kinetics of the superficial perfusion and temperature in connection with PDT of basal cell carcinomas using esterified and non-esterified 5-aminolaevulinic acid. British Journal of Dermatology 2003; 148: 1179-1188
26. Hales TG et al., GABA has excitatory actions on GnRH-secreting immortalized hypothalamic (GT1-7) neurons. Neuroendocrinology 1994; 59: 297-308
27. Pan R et al., Central mechanisms of menthol-induced analgesia. J Pharmacol Exp Ther 2012; 343:661-672
28. Liu B et al., TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. Pain 2013; 154:2169-217
29. Babes A et al., Photosensitization in porphyrias and photodynamic therapy involves TRPA1 and TRPV1. J Neuroscience 2016; 36: 5264-78

## Appendix 1
## Figures

**Figure 1**. Temperature increase caused by laser irradiation of A, the *in vitro* recording chamber and B, a mouse tail (see text).

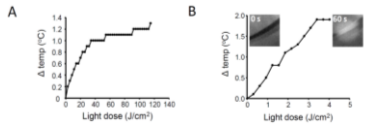



**Figure 2**. A, Pain and B, PpIX fluorescence in mouse tails treated with vehicle or ALA cream

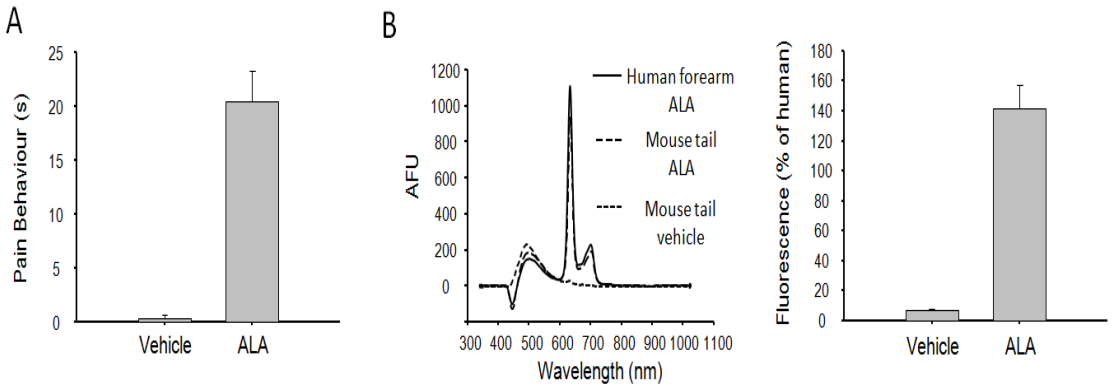



**Figure 3**. A, Flow cytometry showing fluorescence in DRG cultured cells. Action potentials recorded from DRG neurons treated with ALA B, recorded extracellularly and C, using the whole-cell patch-clamp approach.

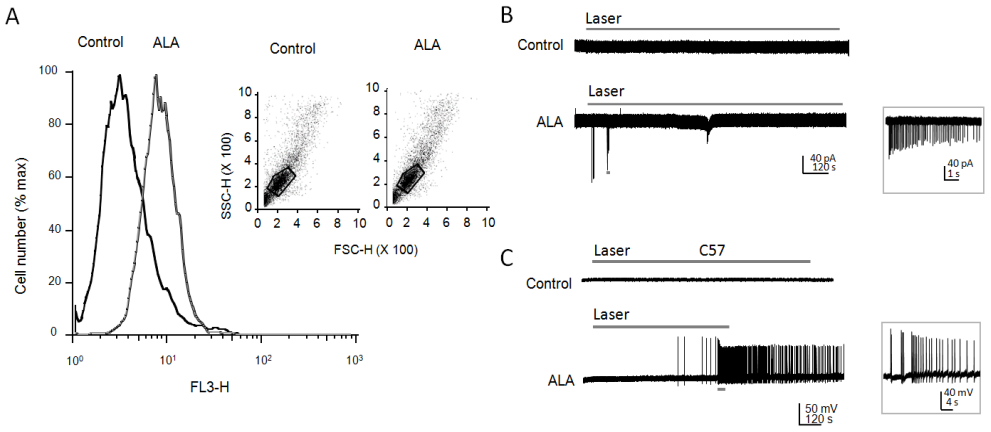
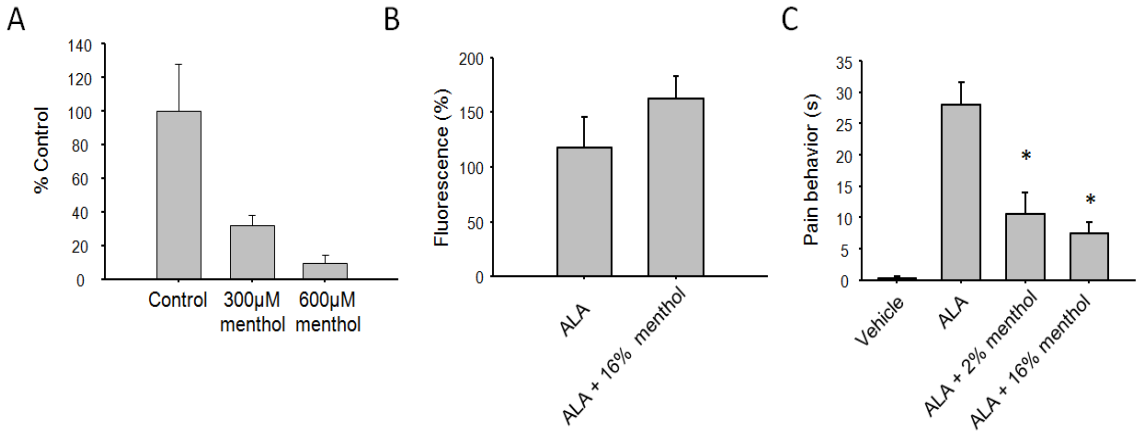



**Figure 4**. A, Concentration-dependent inhibition of action potential frequency by menthol. B, Menthol does not affect PpIX-mediated fluorescence in ALA-treated mouse tails. C, Menthol caused a dose-dependent inhibition in PpIX fluorescence-evoked pain behaviour in mice.



## Appendix 2

**A randomised, double-blind placebo controlled study of the efficacy of topical menthol for pain relief during topical photodynamic therapy**
**Eudract No: 2015-002849-59**
**Product: menthol in aqueous cream 5%**

**Simplified Investigator Brochure: Overview of clinical data to support proposed study**

Thorough Medline and Old Medline searches encompassing all years (through PubMed, NLM) were undertaken combining the search terms (menthol or levomenthol) AND (skin or cutaneous) AND (adverse effect) yielding 65 relevant publications. Eleven abstracts of reports concerning topical application to the skin without occlusion were considered of potential relevance and these publications have been assessed and a summary of relevant adverse effects is reported.

Topical menthol is a vasodilator (Craighead et al., 2017). In the studies in which topical menthol has been used, no serious or notable advents have been reported. Skin irritancy may occur (Liu et al., 2016, Yosipovitch et al., 1996), however irritancy is more likely with repeated applications of topical menthol, and with the single 10 minute application proposed in this study we would not expect this to be a factor. A hyperalgesic tingling, prickling, cooling sensation can be experienced as an expected and often desirable effect of topical menthol (Wasner et al., 2004, Andersen et al., 2016). However, again, in the proposed study, as it is only applied for 10 minutes and then patients are immediately treated with photodynamic therapy, we would not expect there to be any notable adverse effects. The safety profile of topical peppermint and menthol and lack of any notable adverse effects have been highlighted (Nair, 2001, Higashi et al., 2010, Sabzghabaee et al., 2011). The cooling sensation is likely to be one of the proposed mechanisms for pain relief and a desirable effect of topical menthol (Gillis et al., 2010, Andersen et al., 2015).

In a study by Fallon and colleagues, cancer treatment-related neuropathic pain was studied in 51 patients and 40 completed a 6 week treatment course of daily topical menthol in aqueous cream with beneficial effects on pain (Fallon et al., 2015). Other objective parameters were

also studied in that trial and there was a lack of any notable adverse effects with topical menthol using this repeated daily treatment regime over 6 weeks. Of the eleven patients who discontinued treatment, only two did this because, as stated in the manuscript, they "disliked the cream", but no other notable effects of topical menthol were described.

In the study of Anderson and colleagues (Anderson et al., 2016), 14 healthy volunteers were investigated in a randomised double-blinded study of trans-cinnamaldehyde provoked neurogenic inflammation and hyperalgesia compared with trans-cinnamaldehyde combined with 40% levomenthol applied simultaneously. The topical menthol reduced pain, hyperalgesia and inflammation, and other than the reporting of a cooling sensation in association with the combined trans-cinnamaldehyde and menthol treatment, there were no other side effects documented.

Thus, in conclusion, whilst there may be some expected effects of topical menthol in terms of minor skin irritation and a cooling and hyperalgesic sensation, no other significant adverse effects are anticipated in the proposed study, with a single application of 10 minutes only of topical menthol.

**Reference safety information (RSI)**

Topical menthol may cause the effects of skin irritation, cooling and hyperalgesia and these would be expected, albeit minor and possibly even desirable effects. We would not expect any serious adverse reactions in association with topical menthol as used in this study. Thus, any serious adverse events would be considered as unexpected.

**References**

ANDERSEN, H. H., GAZERANI, P. & ARENDT-NIELSEN, L. (2016) High-Concentration L-Menthol Exhibits Counter-Irritancy to Neurogenic Inflammation, Thermal and Mechanical Hyperalgesia Caused by Trans-cinnamaldehyde. *Journal of Pain,* 17**,** 919-929

ANDERSEN, H. H., POULSEN, J. N., UCHIDA, Y., NIKBAKHT, A., ARENDT-NIELSEN, L. & GAZERANI, P. (2015) Cold and L-menthol-induced sensitization in healthy volunteers-a cold hypersensitivity analogue to the heat/capsaicin model. *Pain,* 156**,** 880-889

CRAIGHEAD, D. H., MCCARTNEY, N. B., TUMLINSON, J. H. & ALEXANDER, L. M. (2017) Mechanisms and tie course of menthol-induced cutaneous vasodilation. *Microvascular Research,* 110**,** 43-47

FALLON, M. T., STOREY, D. J., KRISHAN, A., WEIR, C. J., MITCHELL, R., FLEETWOOD-WALKER, S. M., SCOTT, A. C. & COLVIN, L. A. (2015) Cancer treatment-related neuropathic pain: proof of concept study with menthol-a TRPM8 agonist. *Supportive Care in Cancer,* 23**,** 2769-2777

GILLIS, D. J., HOUSE, J. R. & TIPTON, M. J. (2010) The influence of menthol on thermoregulation and perception during exercise in warm, humid conditions. *European Journal of Applied Physiology,* 110**,** 609-618

HIGASHI, Y., KIUCHI, T. & FURUTA, K. (2010) Efficacy and Safety Profile of a Topical Methyl Salicylate and Menthol Patch in Adult Patients With Mild to Moderate Muscle Strain: A Randomized, Double-Blind, Parallel-Group, Placebo-Controlled, Multicenter Study. *Clinical Therapeutics,* 32**,** 34-43

LIU, D. J., COLLAKU, A. & DOSIK, J. S. (2016) Skin irritation and sensitization potential of fixed-dose combination of Diclofenac 1% and Menthol 3% topical gel: results of two Phase 1 Patch studies. *Drug Research (Stuttgard Germany),*

NAIR, B. (2001) Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. *International Journal of Toxicology,* 20**,** 61-73

SABZGHABAEE, A. M., NILI, F., GHANNADI, A., EIZADI-MOOD, N. & ANVARI, M. (2011) Role of menthol in treatment of candidial napkin dermatitis. *World Journal of Pediatrics,* 7**,** 167-170

WASNER, G., SCHATTSHNEIDER, J., BINDER, A. & BARON, R. (2004) Topical menthol--a human model for cold pain by activation and sensitization of C nociceptors. *Brain,* 127**,** 1159-71

YOSIPOVITCH, G., SZOLAR, C., HUI, X. Y. & MAIBACH, H. (1996) Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. *Archives of Dermatological Research,* 288**,** 245-248