Study Protocol

Dose assessment of melatonin in sepsis trial

DAMSEL2

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Protocol approval

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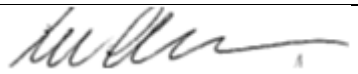
Signatures

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

NAME	POST HELD	ROLE	Signature	Date
Lee Allen	Consultant in Critical Care Medicine Aberdeen Royal Infirmary, Honorary Senior Lecturer	Chief Investigator		28/01/2020
Helen Galley	Professor of Anaesthesia & Intensive Care	Principal Investigator		28/01/2020
Nigel Webster	Professor of Anaesthesia & Intensive Care and Honorary Consultant in Intensive Care Medicine	Co-investigator		28/01/2020
Lorna Aucott	Senior Lecturer in Population Health	Trial Statistician		28/01/2020
Patricia Cooper	Pharmacist	Clinical Trials Pharmacist		28/01/2020

List of abbreviations

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Reporting Form
CTA	Clinical Trial Authorisation
DSUR	Development Safety Update Report
EudraCT	European Union Drug Regulating Authorities Clinical Trials
GCP	Good Clinical Practice
HDU	High Dependency Unit
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
TMF	Trial Master File
R&D	Research and Development
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
SmPC	Summary of Product Characteristics
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction

Summary

Antioxidant therapy targeted at mitochondria has the potential to reduce inflammation, mitochondrial damage and organ dysfunction in sepsis. Melatonin accumulates in mitochondria and both it and its metabolites have potent antioxidant and anti-inflammatory activity, preventing organ dysfunction in a rat model of sepsis. In a recent Phase I dose escalation study (DAMSEL 1) we showed that oral doses of melatonin in healthy subjects were well tolerated with no adverse events and resulted in levels of circulating melatonin and its major metabolite which had beneficial anti-inflammatory and antioxidant actions in *ex vivo* studies

DAMSEL 2 is a pilot Phase II study in patients with sepsis. Stage 1 will assess the pharmacokinetics of melatonin and its major metabolite after a single dose of 50 or 20mg exogenous melatonin in two small groups of patients with sepsis in order to make dosing and dosing interval decisions for Stage 2.

Stage 2 is a double blind randomised controlled trial of melatonin in patients with sepsis at the dose and dosing interval decided after Stage 1. Measurements of melatonin and its major metabolite, and an array of biomarkers of inflammation and oxidative stress will be made, plus transcriptome (mRNA) analysis. This study will inform a planned larger phase II trial.

1. INTRODUCTION

1.1 BACKGROUND

Around 40,000 people die from sepsis in the UK each year. Although the Surviving Sepsis Campaign -a performance improvement effort by hospitals across Europe, South America and the United States- has improved outcomes, the mortality rate remains at 31% overall, and >70% in patients who develop sepsis-induced multiple organ failure [1].

Oxidative stress in patients with sepsis has been consistently described over the last 20 years by us and others (reviewed in [2]). Oxidative stress initiates inflammatory responses via activation of the redox sensitive transcription factor nuclear factor κB (NFκB). Mitochondrial dysfunction initiated by oxidative stress is generally accepted as a playing a major role in sepsis induced organ failure [3].

Production of energy takes place in mitochondria resulting in production of reactive oxygen species (ROS) as by-products. Although ROS are damaging, they are essential in cell signalling and their activity is tightly regulated by a network of antioxidants. When antioxidant defences are overwhelmed, oxidative stress results, causing damage to lipids, proteins and nucleic acids within mitochondria and resulting in cell death [4].

It has been recognised that exogenous antioxidants may be useful in sepsis [2] and more recently the potential for antioxidants acting specifically in mitochondria has been highlighted [5,6]. Antioxidants targeted to mitochondria, including melatonin, reduced organ damage in a rat model of sepsis [7,8]. Although endogenous melatonin is primarily recognised for regulation of the sleep-wake cycle, higher concentrations have potent antioxidant activity [9] with highest levels in mitochondria, thus stabilising the mitochondrial membrane. Metabolites of melatonin also have antioxidant activity and products from the reactions with oxidant species are also antioxidants [9,10].

In clinical studies low doses (1-5mg) of exogenous melatonin have been given to patients with sepsis to try and normalise the sleep-wake cycle [11,12]. Our *in vitro* studies in a human endothelial cell model of sepsis show that higher dose melatonin and its metabolites are equally effective [10]. In a rat model of sepsis melatonin reduces oxidative damage [8,13]. The doses needed for antioxidant and anti-inflammatory actions is considerably higher than that used in sleep-wake cycle studies, but the actual dose required is unclear. Melatonin has been administered to patients with conditions other than sepsis at larger doses. Melatonin (10mg/day) decreased interleukin-6 (IL-6) levels in patients with cancer [14]; 300mg/day decreased oxidative stress in patients with amyotrophic lateral sclerosis [15]. In children with muscular dystrophy, 70mg/day melatonin reduced cytokines and lipid peroxidation [16].

Melatonin is also likely to be beneficial in sepsis [5,6,17]. We have recently undertaken a Phase I dose escalation study in healthy subjects [18] and found that the levels achieved of melatonin and its major metabolite, 6-hydroxymelatonin, are similar to those which reduce inflammation, oxidative stress and mitochondrial dysfunction in an *ex vivo* model of sepsis. Our studies and others show oral bioavailability of melatonin is low with rapid clearance and marked inter-individual variability [18-20]. This is due to variable first-pass extraction in the liver, as a result of genetic differences in cytochrome P450 enzymes, which convert melatonin to the 6-hydroxymelatonin metabolite before it enters the systemic circulation [21]. The majority of melatonin is converted to 6-hydroxymelatonin then sulphated and excreted in urine. In addition, 6-hydroxymelatonin can be generated by non-enzymatic means by reaction with peroxynitrite or hydroxyl radical and at extrahepatic sites such that during oxidative stress persistent production of 6-hydroxymelatonin might be expected [22]. In healthy subjects, although melatonin levels were variable and rapidly cleared, levels of 6-hydroxymelatonin were much less variable and remained stable for several hours after oral melatonin dosing [18]. *In vitro* and animal studies show that melatonin and its metabolites are equally effective [10,18,23] as melatonin. In healthy subjects we found that 6-hydroxymelatonin was still elevated 6h after oral melatonin dosing but had returned to baseline at 24h after the melatonin dose.

There is no evidence of adverse effects of melatonin administration, even at very high doses, from several studies in both healthy people and several disease conditions. In sepsis there have been several trials at lower doses with zero side effects. At the doses we propose there have been no reports of adverse effects in other patient groups but no studies in sepsis. The potential benefit is huge as melatonin has been shown to be very effective in both animal models of sepsis and in other oxidative stress mediated conditions. Further details are provided in the Investigator Brochure.

Thus there is compelling evidence from animal studies of the potential for a beneficial effect of melatonin in patients with sepsis, and we have shown that oral dosing of healthy subjects results in levels of melatonin and 6-hydroxymelatonin which concur with the levels required to reduce inflammation, oxidative stress and mitochondrial function in *ex vivo* studies. However there are still some unanswered questions requiring answer prior to undertaking a Phase II study of melatonin in patients with sepsis. Although we found that doses of 50mg or 20mg of melatonin produced levels of bioactive drug which were effective in *ex vivo*, we need to determine if these doses of melatonin produce similar circulating levels in the target patient group. We also need to determine how long the levels of 6-hydroxymelatonin remain elevated in order to determine a suitable dosing interval. Finally, relation of the levels of melatonin/6-hydroxymelatonin to a range of key biomarkers as surrogates for efficacy for a future Phase II trial is required.

Melatonin affects genes, epigenetic processes and transcriptional activators which regulate cytokine responses [10,24-26]. Around 10% of genes are controlled by circadian rhythm and although gene transcript changes have been reported in the blood of human volunteers after sleep deprivation [24] or when sleep is out of phase with melatonin secretion [26], the effects of exogenous melatonin on the transcriptome has not been studied. In addition, as mentioned above, metabolites of melatonin are also bioactive and their effects on the transcriptome are also not known. We also therefore propose to undertake studies of the transcriptome (mRNA expression) to determine effects of melatonin on gene transcriptome under conditions of sepsis.

This study will provide information on how the doses are tolerated in patients with sepsis, what levels of melatonin and its metabolites are achieved at each dose, and a suitable dosing interval. We will also determine the relationship between melatonin dose and biomarkers and determine the data distribution of these biomarkers and the degree of change after melatonin administration compared to placebo. This work will inform a subsequent funding application for a large Phase II trial and has the potential to translate to a novel therapeutic strategy in patients with sepsis.

1.2 RATIONALE FOR STUDY

The aim of this proposed study is to: Stage 1: administer a single dose of either 50mg or 20mg melatonin to patients with sepsis to determine pharmacokinetics of melatonin and its major metabolite, 6-hydroxymelatonin sulphate. The results from this part of the study will inform the dose and dosing interval for Stage 2 which is a double blind randomised controlled trial of melatonin in patients with sepsis.

Research questions

Stage 1

(i) What are the pharmacokinetics of 50mg and 20mg oral melatonin in patients with sepsis?
(ii) Which dose is most suitable based on the pharmacokinetics in (i)?
(iii) What is the most suitable dosing interval based on results from (i)?

Stage 2

(iv) What effect does 20mg oral melatonin have on the defined outcome measures compared to placebo?

Safety

There is no evidence of risk of melatonin administration even at very high doses from very many studies in both healthy people and disease conditions. In sepsis there have been several trials at lower doses with zero side effects. At the doses we propose there have been no reports of adverse effect in other patient groups but no studies in sepsis. The potential benefit is huge as melatonin has been shown to be very effective in both animal models of sepsis and in other oxidative stress mediated conditions. Melatonin is known to cause some drowsiness in some people regardless of dose [15,16,18] and is used to aid normalisation of sleeping patterns in blind people and as a treatment for 'jet lag'. Melatonin has also been given to ICU patients to aid normal sleep [11,12]. Drowsiness is not in itself harmful, but we will use existing assessment tools which are used routinely on the ICU (Richmond Agitation Sedation Scale or RASS) to monitor sedation requirements [27,28]. See also the Investigator Brochure.

2. STUDY OBJECTIVES

2.1 OBJECTIVES

To determine the pharmacokinetics of 2 doses of oral melatonin.

To determine plasma levels of melatonin/metabolite at the 2 doses.

To decide on which dose and dosing interval to use for Stage 2.

To undertake a double blinded randomised controlled pilot trial of the chosen dose (20mg) in patients with sepsis.

2.2 ENDPOINTS

The primary endpoint for Stage 1 is administration of 2 oral melatonin doses with no adverse reactions and approval by the DMC of dose and dosing interval decisions for Stage 2.

The endpoint for Stage 2 will be recruitment of 40 patients and final evaluation, including survival status at 28 days.

Secondary endpoints will include assessment of several biomarkers as endpoint surrogates and transcription changes. The ultimate goal of experimental therapies in sepsis is reduced mortality. However, using mortality as an endpoint in a pilot study such as this is clearly not feasible. We will use biomarkers which are surrogates of outcome from sepsis: arterial blood lactate, suPAR, isoprostane and CRP, plus markers of endothelial immune function. An additional panel of biomarkers will be measured using multiplex technology to include tissue specific injury markers, cytokines, adhesion molecules and chemokines:

MCP-1, Endocan, IL-1 beta, IL-2, IL-4, IL-6, IL-8, IL-18, Pentraxin 3, E-Selectin, SP-D, suPAR, VCAM-1, IL-10.

. Key clinical parameters including heart rate, mean arterial pressure, temperature, acute physiologial and chronic health evaluation (APACHE) II score, length of ICU stay, ICU- and 28 day- all cause mortality will be recorded. We will also measure mRNA changes in Stage 2.

3. STUDY DESIGN

Stage 1: Open label, 2 doses of melatonin (50mg and 20mg) cohort study in patients with sepsis.

Stage 2: Phase II pilot double blinded placebo controlled randomised trial of 20mg melatonin 8 hourly for 72 hours in patients with sepsis.

4. STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

The study will be in two stages:

Stage 1: The first sequential cohort of 5 patients will be assigned to a single oral dose of 50mg melatonin given in a liquid formulation via nasogastric tube or orally as a drink if patients can tolerate this. Usual medical care will be at the discretion of the clinical treating team. Blood samples (10ml) will be obtained prior to melatonin adminstration, after 10 and 30 mins, and 1, 2, 6, 12 and 24h. A second cohort of 5 patients will receive 20mg melatonin and blood will be taken as above.

Once Stage 1 is complete and a dose and dosing interval decision has been made, a substantial amendment will be submitted to update the study protocol accordingly, prior to moving to Stage 2.

Stage 2: In stage 2, 40 patients will be randomised to receive either melatonin or placebo. The dose (20mg) and dosing interval (8 hourly) of melatonin will be based on data from Stage 1. The dose will be based on the maxmimum tolerated dose of melatonin at which first pass metabolism to 6-hydroxmelatonin is complete (20mg). The study will be conducted in a double blind fashion. Melatonin or placebo will be adminstered for 72h or until discharge from ICU or HDU if discharged before 72h. Usual medical care will be at the discretion of the clinical treating team. Subjects will be followed up daily whilst on the ICU/HDU for 5 days then mortality status at 28d will be recorded from medical records.

We propose a 72h dosing period for these reasons: 1. The median length of stay of patients on the ICU in Aberdeen is 4 days. This means that patients will either die or get better in this time frame. 2. Melatonin has been given in other disease conditions for much longer periods even at higher doses with no ill effects. 3. Many previous anti-inflammatory trials in this group of patients have administered trial medication for 72h.

4.2 INCLUSION CRITERIA (for both Stage 1 and Stage 2)

Adult patients (16 years or over) on the ICU or HDU at Aberdeen Royal Infirmary with sepsis due to community acquired pneumonia who are within 24h of fulfilling the criteria for sepsis with clinical suspicion of pneumonia and the presence of chest X-ray changes consistent with pneumonia will be recruited. The criteria for sepsis are:

- clinical suspicion or evidence of acute infection
- systemic inflammatory response syndrome, defined by two or more of the following:
    1. Core temperature <36°C or >38°C;
    2. tachycardia: heart rate > 90 beats/min;
    3. tachypnoea: respiratory rate > 20 breaths/min or ventilated;
    4. leucocyte count >12 x 10^9/L or <4 x 10^9/L.

4.3 EXCLUSION CRITERIA (for both Stage 1 and Stage 2)

Subjects will be excluded if they are

- <16 years old,
- have a life expectancy <24h,
- have metastatic cancer or immunosuppression,
- are receiving steroids (>20mg/d prednisolone or equivalent, used regularly for >2 weeks prior to ICU admission)
- women of child bearing potential without a negative pregnancy test or a history of surgical sterilization.
- patients receiving fluvoxamine or nifedipine,
- have overt hepatic failure
- unable to tolerate oral medication
- known to be hypersensitive to trial medication and/or excipients

5. PARTICIPANT SELECTION AND ENROLLMENT

5.1 IDENTIFYING PARTICIPANTS

Potential patients (i.e. those with sepsis) will be identified at daily ward rounds on ICU or HDU by usual clinical staff who will inform the Research Nurses (Specialist ICU Registered Nurses).

5.2 CONSENTING PARTICIPANTS

Consent will be obtained by research nurses or delegated study personnel. Following MHRA, research ethics committee and NHS R&D approval, written informed consent will be sought from the patient or more likely, their legal representative – a welfare guardian, a welfare attorney, a near relative or close friend, a clinical person not involved in the study, or another independent person nominated by the healthcare provider, according to the Medicines for Human Use (Clinical Trial) Regulations 2004. The ICU research nurses are highly experienced in recruiting critically ill patients to research studies and sensitive in their dealings with relatives. They are all trained to GCP.

Once recruited, each subject will be assigned a unique ID number and any identifiable information will be kept securely in a locked filing cabinet or password protected University computer in a locked office with limited access. Where possible we will seek consent from a Welfare Guardian or Welfare Attorney (if appointed) or a near relative/close friend/partner. If these are reasonably contactable, then a doctor responsible for the patient's medical treatment who is independent of the study, or a person nominated by the healthcare provider, will be approached for consent. This person will be told that they are being asked to give consent on behalf of the incapacitated adult, that they are free to decide whether they wish to make this decision or not, that they are being asked to consider what patient would want, and to set aside their own personal views when making this decision. The person asked for consent will be given sufficient information, in an understandable form, to ensure that they can make an informed decision. Participants will be withdrawn if the legal representative withdraws consent. There is no legal requirement to seek further consent from the patient if they regain capacity after being recruited into a CTIMP and the consent from the legal representative remains valid. If the patient regains capacity we will provide information to tell them about the study and if they subsequently wish to withdraw from the study then this will be respected. Participants or their legal representative will have up to 24h to decide. Consent to participate will be recorded in patients' notes by a medically qualified member of the research team. A record will be kept of those who refuse consent on the anonymous screening log.

5.3 SCREENING FOR ELIGIBILITY

Potential patients (i.e. those with sepsis) will be identified at daily ward rounds by usual clinical staff who will inform the Research Nurses (Specialist ICU Registered Nurses) who will then screen ICU/HDU notes and an anonymous record of the screening log will be kept. The research nurse will then confirm eligibility with a medically qualified member of the research team. Those patients who fulfil the inclusion criteria and do not fulfil the exclusion criteria will be invited to participate and a record of this will be made in the patient's medical records by a medically qualified member of the research team.

5.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

Patients thought to have sepsis will be screened and this pre-recruitment screening will be recorded anonymously with reasons for non-recruitment. Patients who do not meet the exclusion criteria may be screened daily since several inclusion criteria need to be fulfilled at the same time. Patients who are found to fulfil inclusion criteria but refuse consent or fulfil exclusion criteria will not be rescreened.

5.5 RANDOMISATION

5.5.1 Randomisation (Stage 2)

The IMP consists of randomised uniquely numbered kits each containing 2 active or placebo bottles.

Randomisation will.

be undertaken by the Centre for Healthcare Randomised Trials (CHaRT, UK CRN clinical trials unit) in the Health Services Research Unit, University of Aberdeen. CHaRT will provide the randomisation list to the IMP manufacturing site and a sealed list to the Clinical Trials Pharmacy. This list can be accessed for emergency unblinding.

5.5.2 Treatment Allocation

Dispensing will be handled by the Clinical Trials Pharmacist at NHSG. The drug will be administered as a liquid formulation. Upon receipt of a signed prescription, the pharmacist will allocate the treatment kit described above according to participant ID number.

5.5.3 Emergency Unblinding Procedures

The clinical trials pharmacist (or on-call pharmacist out of hours) will provide emergency unblinding by opening the sealed list. The Pharmacist will be contacted using usual hospital communication systems. Unblinding processes will be tested and documented prior to starting recruitment for Stage 2.

5.5.4 Withdrawal procedures

Should participants wish to withdraw during the actual study visit the process will depend on what stage of the study is reached. If the drug has not yet been administered, then the participant can withdraw immediately. If melatonin or placebo has been given, then no further drug will be given and no more samples will be obtained. Data and samples obtained with consent will be retained. Replacement subjects will be recruited. If the participant is withdrawn the reasons, if known, will be recorded and the subject will be monitored as planned.

6. INVESTIGATIONAL MEDICINAL PRODUCT AND PLACEBO

6.1 STUDY DRUG (see also IMP dossier and Investigator Brochure)

6.1.1 Study Drug Identification

Synthetic N-acetyl-5-methoxy tryptamine ('Melatonin') 1mg/ml liquid for oral use.

6.1.2 Drug Substance Manufacturer

Flamma S.p.A, Via Bedeschi 22, 24040 Chignolo d'Isola (Bergamo), Italy.

6.1.3 Manufacturing Authorisation Holder

The active melatonin bottles are manufactured and supplied by APL, Apotek Produktion & Laboratorier AB (Celsiusgatan 43 Malmö, 21214, Sweden) licensed by the Swedish competent authority for manufacture and release of the drug product for clinical trial use in the EU.

The placebo bottles are manufactured by Stockport Pharmaceuticals (Stockport NHS Foundation Trust, Stockport Pharmaceuticals Pharmacy Department, Stepping Hill Hospital, Poplar Grove, Stockport SK2 7JE, UK with MHRA manufacturing authorisation number: MIA (IMP) 13523). The oral melatonin will be manufactured by Apotek Produktion & Laboratorier AB (APL). Modepharma will source and arrange distribution of QP released oral melatonin to Stockport Pharma. Stockport Pharma will manufacture the matched placebo, will relabel the oral melatonin and will dispatch QP-released oral melatonin and placebo to the trial sites. The co-sponsors will enter commercial and technical agreements with Modepharma and Stockport Pharma in relation to these activities.

6.1.4 Labelling and Packaging

APL will supply the active IMP (1mg/mL) as an oral liquid in 100ml bottled closed with a child resistant cap. Bottles will be labelled as a stock special product in English for the Swedish market. QP batch release will be undertaken by APL and the IMP will be stored until dispatch at 15-25°C.
The IMP will be dispatched in a single consignment for Stage 1.

For Stage 1 the prescribed dose (50ml=50mg or 20ml=20mg) single dose will be administered either by drinking or via a nasogastric tube. For Stage 2 pharmacy will provide coded bottles for each patient containing either melatonin or placebo for the complete treatment period and the prescribed dose will be administered as for Stage 1 above at the required dose (20mg) and dosing interval (8 hours).
For Stage 2, to maintain blinding, placebo will be manufactured by Stockport Pharmaceuticals. The placebo is identical to the active but does not contain any melatonin active substance. A new batch of active bottles will be sourced from APL and both active and placebo bottles will be labelled and QP released by Stockport Pharmaceuticals. The IMP for stage 2 will be dispatched in several consignments.

Labelling will be undertaken according to Annex 13 of Volume 4 of The Rule Governing Medicinal Products in the EU: Good Manufacturing Practices". The Clinical Trials Pharmacy at Aberdeen Royal Infirmary will perform final assembly (clinical trials labelling) of the melatonin bottles as per Regulation 37 of Statutory Instrument 2004/1031.

Empty bottles and unused medication will be returned to Pharmacy who will maintain drug accountability records.

6.1.5 Storage

Storage will be at 15-25°C at all times. The drug product transit conditions will be 15-25°C using a temperature-monitored and GDP-compliant courier service. See IMP Dossier for stability summary.

6.1.6 Summary of Product Characteristics

Melatonin is an IMP but is not marketed and so there is no SmPC. Please see Appendix 1 and the IMP dossier which provides drug substance and manufacturer information.

6.1.7 Accountability procedures

Drug accountability will be the responsibility of the Clinical Trials Pharmacist.

6.2 PLACEBO

The placebo will be exactly the same in every detail except for the active ingredient (melatonin). For Stage 2, the active drug product will be supplied by APL as for Stage 1 and the placebo will be manufactured by Stockport Pharmaceuticals in the UK and decanted into identical bottles as the active IMP; the bottles will be supplied by APL.

6.3 DOSING REGIME

All patients will receive the trial medication via nasogastric tube or as a drink. In Stage 1 the first cohort of 5 subjects will receive a single dose of 50mg (50ml) and the second cohort of 5 subjects will receive 20mg (20ml) melatonin.

In Stage 2 subjects will be randomised to receive doses of 20mg (20ml) melatonin or placebo at 8 hour intervals. Subjects will receive repeated doses for up to 72h whilst on the ICU or HDU. Patients who are discharged from ICU/HDU before 72h will only receive the trial drug for the duration of the ICU stay. Trial medication of 20mg will be given three times daily for up to 3 days.

6.4 TREATMENT DISCONTINUATION

Treatment will be stopped if a patient develops an unexpected reaction thought to be due to melatonin administration. This might include, but is not limited to, vomiting, diarrhoea or suspected anaphylaxis. There are several expected severe adverse events in this patient population which have been identified in Appendix 3 and it would be any events which are unexpected which we may consider to be criteria for stopping the treatment in an individual patient. If a patient deteriorated due to the underlying disease process it may also be appropriate to discontinue treatment at the discretion of the clinical team. In addition, treatment would be stopped if consent was withdrawn.

6.5 PARTICIPANT COMPLIANCE

Melatonin or placebo will be administered to patients on the ICU/HDU site and will be observed by study personnel. The volume prescribed and the volume given will be recorded in the Case Record File (CRF).

6.6 OVERDOSE

Doses will be prescribed and administered to patients by clinical staff. In Stage 1 subjects will be given only a single dose. If a higher dose than intended is given, or a dose is

given before the intended intervals between doses in Stage 2 then an overdose could perhaps occur. However, there are no previous reports of toxicity from any dose of melatonin and no reports of overdoses. Any patient in whom an overdose occurred would therefore simply be monitored carefully for 24h after overdosing and an adverse event recorded.

6.7 OTHER MEDICATIONS

6.7.1 Permitted Medications

All medication required at the discretion of the clinical team is permitted, except for fluvoxamine or nifedipine. If these drugs were required then no further doses of melatonin or placebo would be given. These drugs interfere with the metabolism of melatonin but are not considered to be harmful to the patient.

6.7.2 Prohibited Medications

Participants will not be recruited if they are taking fluvoxamine or nifedipine.

7. STUDY ASSESSMENTS

7.1 SAFETY ASSESSMENTS

Patients will be monitored continuously with usual nursing care.

7.2 STUDY ASSESSMENTS

Recruited subjects will be Intensive Care Unit or High Dependency Unit patients.

Stage 1: The first sequential cohort of 5 patients will be assigned to a single oral dose of 50mg melatonin given in a liquid formulation via nasogastric tube or as a drink. All other clinical treatment will be at the discretion of the clinical team. Blood samples (10ml) will be obtained prior to melatonin adminstration, after 10 and 30 mins, and 1, 2, 6, 12 and 24h. A second cohort of 5 patients will receive 20mg melatonin and blood will be taken as above. Concentrations of melatonin and 6-hydroxymelatonin will be analysed as detailed in section 2.3 above.

Stage 2: In stage 2, 40 patients will be randomised to receive either 20mg (20ml) melatonin or placebo. The study will be conducted in a double blind fashion. Melatonin or placebo will be adminstered for a maximum of 72h or until discharge from ICU/HDU if <72h, and subjects' 28d mortality status will be recorded. Blood samples (10ml) will be taken prior to study drug adminstration, and after 1, and 24h, then daily during the ICU stay up to a maximum of 5 days for the measures as detailed in Section 2.2. Blood samples taken solely for research purposes will be taken from existing cannulae into appropriate blood sampling tubes and processed on the ICU by a trained research nurse and placed into a dedicated -20°C freezer on the the ICU. They will then be transported to the research laboratory in the Institute of Medical Sciences for storage at -80°C according to Standard Operating Procedures in a dedicated freezer in the laboratory. Routine blood tests taken as part of usual medical care will be handled and processed as usual and results of these will be recorded. Processes for sending samples off site for analysis will comply with a pre-defined Material Transfer/Technical Agreement and the agreed Analytical Protocol according to UoA-NHSG-SOP-022. These samples will be stored in Aberdeen until all samples are collected then sent by courier on dry ice for analysis. Remaining samples (if any) will be returned to the Chief Investigator after analysis is complete for sample reconciliation and disposal or storage records to be maintained.

Heart rate, mean arterial pressure, temperature, acute physiologial and chronic health evaluation (APACHE) II score, will be recorded at least daily during the ICU/HDU stay

for up to 5 days. Length of ICU/HDU stay, ICU/HDU- and 28 day- all cause mortality will be recorded.

8. DATA COLLECTION

Clinical data will be collected from ICU/HDU charts and entered into a CRF. Biochemical and pharmacokinetic data will be reported to the Chief Investigator and the research team at the end of Stage 1. For Stage 2 biochemical data will be reported to the Chief Investigator and the research team at the end of Stage 2. stage and will be recorded on a dedicated trial database..

9. STATISTICS AND DATA ANALYSIS

9.1 SAMPLE SIZE CALCULATION

For Stage 1 data analysis will be simple descriptive statistics for pharmacokinetic data in each dose cohort, to include Cmax, Tmax, AUC and time to return to baseline for both melatonin and 6-hydroxymelatonin. For Stage 2, as a pilot, this study does not require a formal sample size calculation. Although melatonin levels after a single oral dose can be variable, as explained the levels of 6-hydroxymelatonin are less variable and are also bioactive. We therefore anticipate that 20 patients per group will allow an initial exploration of potential effects to enable a formal sample size to be calculated for a definitive subsequent trial.

9.2 PROPOSED ANALYSES

Melatonin and 6-hydroxymelatonin will be measured in house using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and enzyme immunoassay respectively. These methods have been established and validated in-house. For stage 1, concentration-time data will be used to determine peak concentrations in serum, time to peak concentration in blood, minimum concentration in serum, area under the serum concentration-time curve from immediately prior to dosing to the last sample and extrapolated to infinity, the apparent terminal elimination half-life. Summary statistics will be reported for all pharmacokinetic parameters, weight, age and clinical data. Intention to treat analysis will be undertaken.

Arterial blood lactate will be analysed on the ICU using blood gas analysers in routine use. CRP will be measured by the Pathology Department as is routine. Other biomarkers will be measured in house using luminex multiplex technology, to include tissue specific injury markers, cytokines, adhesion molecules and chemokines: MCP-1, Endocan, IL-1 beta, IL-2, IL-4, IL-6, IL-8, IL-18, Pentraxin 3, E-Selectin, SP-D, uPAR, VCAM-1, IL-10.

As part of the multiplex biomarker analysis, linear discriminant analysis, hierarchical regression and hierarchical cluster analysis will be used to explore relationships between biomarkers and other indices and will provide useful information regarding proxies of patient outcome for both this and future studies.

Blood will be treated to stabilize gene transcription and then stored at -70^{o}C. Total RNA will be extracted at a later date and purified for analysis of mRNA by the University of Aberdeen Genome Centre. Bioinformatic analysis will include quality control, normalisation, fitting of statistical models to identify differentially expressed features and downstream analysis such as gene set enrichment.

Any data analyses will be pre-specified and governed by a comprehensive analysis plan finalised before analysis begins, which will be authored by Dr Aucott. The primary analyses will be on an intention to treat basis. Broadly the plan will investigate initial exploratory data and descriptive analyses followed by appropriate univariate analyses to examine the two groups (melatonin and placebo) for the various measurements and patient characteristics. These will be put together along with baseline co-variates, confounders and effect modifiers, defined *a priori*, into appropriate multiple regression

models. Multilevel Linear Modelling will be conducted including variables with measurements taken over time thus allowing for related measures at potentially irregular time intervals.

10. ADVERSE EVENTS

Investigators who are is clinically qualified at consultant level will be responsible for the detection and documentation of events meeting the criteria and definitions detailed below.

There are likely to be adverse and severe adverse events including death, in patients with sepsis, as described in Appendix 3. The Chief Investigator and the clinical team will make decisions as to whether any such events are unexpected in terms of timing or severity and considered related to the study drug, with advice from the DMC as necessary.

The only expected adverse event as a result of the IMP is drowsiness manifesting as a decrease in sedation requirements as determined using the RASS score [24,25].

10.1 DEFINITIONS

ICU or HDU admission is a pre-requisite for study participation. AEs and SAEs as will be recorded and reported to the Sponsor within the specified time period as appropriate.

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 the 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 (i.e. the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- requires hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect.

10.2 DETECTING AEs AND SAEs

All AEs and SAEs will be recorded from the time of study entry until the end of the study visit, defined as 24h for Stage 1 and up to 5 days for Stage 2 whilst patients remain on the ICU/HDU.

10.3 RECORDING AEs AND SAEs

Depending on severity, when an AE/SAE occurs, the Investigator will review any documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event and record all relevant information in the CRF and on the AE or SAE form.

Information to be collected will include dose of any medication, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

10.4 EVALUATION OF AEs AND SAEs

Seriousness, causality, severity and expectedness will be evaluated. Please also refer to Appendix 3.

10.4.1 Assessment of Seriousness

The Investigators will make an assessment of seriousness as defined in Section 10.1 and whether any events are usual and expected and/or considered related to the study drug, with advice from the DMC as necessary. Please also refer to Appendix 3.

10.4.2 Assessment of Causality

The Investigator will make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions:

Unrelated: where an event is not considered to be related to the study drug.

Possibly: although a relationship to the study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably: the temporal relationship and absence of a more likely explanation suggest the event could be related to the study drug.

Definitely: The known effects of the study drug or its therapeutic class, or based on challenge testing, suggest that study drug is the most likely cause.

All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably, definitely) to the study drug will be considered as ARs/SARs. All AEs/SAEs judged as being related (e.g. possibly, probably, definitely) to an interaction between the study drug and another drug will also be considered to be AR/SAR.

Alternative causes such as natural history of the underlying disease and other risk factors and the temporal relationship of the event to the treatment in these critically ill patients will be considered. Please also refer to Appendix 3.

10.4.3 Assessment of Severity

The Investigators will make an assessment of severity for each AE/SAE and record this on the CRF according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

10.4.4 Assessment of Expectedness

Events considered to be unexpected or unusual in this patient population, and/or possibly related to the study drug, will be considered AEs, SAEs, ARs, SARs and SUSARs. Please also refer to Appendix 3.

10.5 REPORTING OF SAEs / SARs / SUSARs

Once the Investigator becomes aware that an SAE has occurred in a study participant, they will report the information to the Sponsor within 24h of becoming aware of the event as per the current SOP. The SAE form will be completed as thoroughly as possible

with all available details of the event, signed by the Investigator or their delegate. If all the required information is not available at the time of reporting, the Investigator will ensure that any missing information is provided as soon as this becomes available. It will be indicated on the report that this information is follow-up information of a previously reported event.

10.6 REGULATORY REPORTING REQUIREMENTS

An annual Development Safety Update Report (DSUR) will be submitted to the MHRA and the main REC listing all SARs and SUSARs. The Chief Investigator is responsible for submitting annual DSURs to the MHRA and the main REC on the anniversary of the Clinical Trial Authorisation approval.

The Chief Investigator is responsible for informing the MHRA and the main REC of these safety issues. Fatal or life threatening SUSARs will be reported to MHRA no later than **7 calendar days** and all other SUSARs will be reported no later than **15 calendar days** after they are first aware of the reaction.

10.7 FOLLOW UP PROCEDURES

After initially recording an AE or recording and reporting an SAE, the Investigator will follow each participant as per the current SOP. As detailed in the protocol, AEs and SAEs will be followed up whilst the patient remains on ICU, for a maximum of 24h for Stage 1 and 5d for Stage 2. SUSARs will be followed up until resolution as per the SOP.

11. PREGNANCY

Pregnant subjects will be excluded, and it is very unlikely that a patient will become pregnant whilst admitted to the ICU.

12. TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

12.1 TRIAL STEERING GROUP

A Trial Management Group consisting of the study team members and 3 independent members including one lay member will have oversight of the trial conduct. It will be Chaired by an independent Intensive Care Clinician (Dr Alison Pittard, Leeds) as per the current SOP. The group will meet at least annually.

12.2 PROJECT MANAGEMENT GROUP

The trial will be managed on a day to day basis by the project management group (Appendix 4). The Chief Investigator or delegated personnel will be responsible for checking the CRFs for completeness, plausibility and consistency. Any queries will be resolved by the Investigator or delegated member of the trial team.

A Delegation Log will detail the responsibilities of each member of staff working on the trial.

12.3 DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established to oversee the safety of subjects in the trial and will comprise of 3 consultants in intensive care who are external to Aberdeen . The terms of reference of the DMC and the names and contact details of the Chair are detailed in Appendix 5. The DMC will be provided with data after Stage 1 and after each 10 patients are randomised to Stage 2. The data provided will be blinded. No data will be unblinded (unless requested for safety concerns), until the end of the study, unless emergency unblinding is required. . The DMC will agree terms of reference and other procedures prior to the study starting. The DMC will monitor the trial data in 'virtual' meetings after Stage 1 and after every 10 patients randomised to

Stage 2 and be regularly provided with all relevant data. The DMC will make recommendations as to any modifications that are required to be made to the protocol and advise on study termination if they are of the opinion the severity of unexpected adverse events in the study population is unacceptable. If the DMC has safety concerns and request data to be unblinded, a third party will oversee unblinding.

12.4 INSPECTION OF RECORDS

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

12.5 STUDY MONITORING

A monitor, designated by the Sponsor, or an appointed local monitor will visit the Aberdeen study site prior to the start of the study, during the course of the study and will undertake a close down visit at study end.

The trial will be monitored by NHS Grampian. We will comply fully with the University of Aberdeen/NHS Grampian joint standard operating procedures (SOPs) for reporting of serious adverse events to sponsors, the MRHA, the NHS R&D director and the research ethics committee.

12.6 RISK ASSESSMENT

An independent risk assessment has been carried out by the sponsor.

12.6.1 Potential Risks

There have been no reports of serious side effects in patients receiving melatonin [15,17,18]. Non-serious side effects are limited to transient drowsiness [18].

12.6.2 Minimising Risk

Risk minimisation strategies:

1. DMC approval after each dose cohort in Stage 1.
2. Stage 1 is open label.
3. DMC will approve continuation after each cohort of 10 patients in Stage 2.

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).

A favourable ethical opinion will be obtained from the appropriate REC and local NHS R&D approval will be obtained prior to commencement of the study.

13.2 REGULATORY COMPLIANCE OF THE STUDY

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the appropriate Regulatory Authority. The protocol and study conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004, and any relevant amendments.

13.3 INVESTIGATOR RESPONSIBILITIES

The Chief Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff. Delegated tasks will be documented on the Delegation Log and signed by all those named on the list.

13.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants or legal representatives will receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant or their legal representative will be performed by the Investigator or designated person and will cover all the elements specified in the Participant Information Sheet/Informed Consent.

The participant or their legal representative will be given every opportunity for clarification of any points they do not understand and, if necessary, ask for more information. The participant/legal representative will be given sufficient time to consider the information provided. It will be emphasised that consent may be withdrawn at any time without loss of benefits to which they otherwise would be entitled.

The participant/legal representative will be informed and agree to study records being inspected by regulatory authorities but understand that inspection is undertaken by authorised personnel and their data will remain confidential.

The Investigator or delegated member of the trial team and the participant/legal representative will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant/legal representative will receive a copy of this document and a copy will be filed in the patient's notes and the Trial Master File (TMF).

13.3.2 Study Site Staff

The Investigator will be familiar with the IMP, protocol and the study requirements. The Investigator will ensure that all staff assisting with the study are adequately informed about the IMP, protocol and their trial related duties.

13.3.3 Data Recording

The Investigator is responsible for the quality of the data recorded in the CRF.

13.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the Sponsor, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents);
- A signed copy of the Chief Investigator's responsibilities letter issued by the Sponsor.

The Chief Investigator, with the agreement of the Sponsor, will ensure all other documents required for compliance with the principles of GCP are retained in a TMF and that appropriate documentation is available in local ISFs.

13.3.5 GCP Training

Where appropriate all relevant research staff will have GCP training or will undergo GCP training. A training log will be kept in the Trial Master File.

13.3.6 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 locked filing cabinet in a locked office only accessed by the Principal Investigator. 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, Regulatory Authorities, or the REC. The Investigator and study site 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.

13.3.7 Data Protection

All Investigators and study site 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. Access to collated participant data will be restricted to those clinicians treating the participants.

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.

14. STUDY CONDUCT RESPONSIBILITIES

14.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant, must be reviewed and approved by the Sponsor. Amendments to the protocol will be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to participants being enrolled into an amended protocol.

14.2 PROTOCOL VIOLATIONS AND DEVIATIONS

The Investigator will not implement any deviation from the protocol without agreement from the Chief Investigator and appropriate REC, Regulatory Authority and R&D approval except where necessary to eliminate an immediate hazard to trial participants.

In the event that an Investigator needs to deviate from the protocol, the nature of and reasons for the deviation should be recorded in the CRF. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate.

14.3 STUDY RECORD RETENTION

All study documentation will be kept for at least 15 years after publication of the study data.

14.4 END OF STUDY

The end of the study is defined as completion of analysis of the last participant's samples including data analysis.

The Investigators 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 REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants and ensure that the appropriate follow up is arranged for all involved.

A summary report of the study will be provided to the REC and Regulatory Authority within 1 year of the end of the study. An end of study report should also be issued to the funders at the end of funding.

14.5 CONTINUATION OF DRUG FOLLOWING THE 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. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

15.2 PUBLICATION

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

The results will be publicly available in a lay friendly poster style format called 'Focus on Research' on the funding body's website.

15.3 PEER REVIEW

The data from the study will be peer reviewed as part of the publication process.

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APPENDIX 1: Drug substance and manufacturer

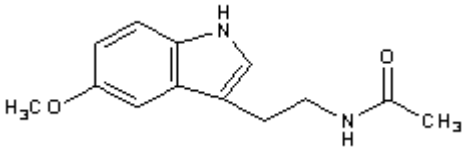
This presents information relating to Melatonin Liquid containing 1mg/mL of N-acetyl-5-methoxytryptamine.

Nomenclature

Chemical Name (IUPAC)	N-[2-(5-methoxy-1H-indol-3-yl)ethyl acetamide
Common Name	Melatonin
Other names	N-acetyl-5-methoxytryptamine, melatonin

Structure

Structural Formula



Molecular Formula	$C_{13}H_{16}N_2O_2$
Molecular Weight	232.2
Description	Off-white powder.
pH and pKa	pKa 4.7 pH~2.5
Melting Point	116-118°C.
Solubility	Solubility at 20°C is 0.1 mg/ml in water, 8mg/ml in ethanol.
Hygroscopicity	Not considered to be hygroscopic.

Manufacture

Melatonin liquid is manufactured in accordance with Good Manufacturing Practice at the following facility:

Company Name	Apotek Produktion & Laboratorier AB,
Street address	Prismavägen 2,
Town	141 75 Kungens Kurva,
Country	Sweden

For further information see the IMP Dossier and Investigator Brochure.

APPENDIX 2:

Melatonin and Placebo oral liquid (see IMP Dossier for full details)

Table 1 Composition of Melatonin Oral Solution

Amount (mg/mL)	Reference to Standard	Function	Amount (mg/mL)
Melatonin	1	Internal monographs for pharmaceutical raw ingredients	Active ingredient
Methyl parahydroxybenzoate	1	Ph.Eur	Preservative
Potassium sorbate	1	Ph.Eur	Preservative
Glycerol (85 per cent)	100	Ph.Eur	Solubilizing agent
Hydrochloric acid (5M)	q.s. (~ 0.7 mg/mL)	Ph.Eur	pH-adjustment
Water purified	Ad 1 mL	Ph.Eur	Solvent

The Placebo will be identical but without melatonin.

Appendix 3

Expected events in patients with sepsis

System	Expected events
Cardiovascular	Arrhythmia, hypotension, cardiac arrest, requirement for vasoactive drugs
Respiratory	Respiratory failure, need for re-intubation
Nervous	Confusion/delirium, neuro/myopathy
Gastro-intestinal	GI/hepatic failure, bleeding, diarrhoea
Renal	Oliguria, renal failure, requirement for RRT
General	Readmission to ICU/admission from HDU to ICU, digital ischaemia/necrosis

RRT= renal replacement therapy

APPENDIX 4: Project management group

Dr Lee Allen MBChB FFICM
Consultant Critical Care Medicine and Honorary Senior Lecturer,
NHS Grampian.

Professor Helen Galley, PhD FIBMS FRCA FSB FFICM
Professor of Anaesthesia and Intensive Care
University of Aberdeen.

Dr Lorna Aucott BSc PhD
Medical Statistician
University of Aberdeen.

APPENDIX 5: Data Monitoring Committee

Charter for the Data Monitoring Committee (Draft)

1. Introduction	
Name (and sponsor's ID) of trial plus ISRCTN and/or EUDRACT number	DAMSEL 2 Sponsor Ref: 3/035/14, EudraCT No. 2014-002840-42, ISRCTN70688534
Objectives of trial	DAMSEL 2 is a pilot Phase II study in patients with sepsis. Stage 1 will assess the pharmacokinetics of melatonin and its major metabolite after a single dose of 50 or 20mg exogenous melatonin in two small groups of patients with sepsis in order to make dosing and dosing interval decisions for Stage 2. Stage 2 is a double blind randomised controlled trial of melatonin in patients with sepsis at the dose and dosing interval decided after Stage 1. Measurements of melatonin and its major metabolite, and an array of biomarkers of inflammation and oxidative stress will be made. This study will inform a planned larger phase II trial.
Outline of scope of charter	The purpose of this document is to describe the roles and responsibilities of the independent DMC for the DAMSEL2 trial, including the timing of meetings, methods of providing information to and from the DMC, frequency and format of meetings, statistical issues and relationships with other committees.
2. Roles and responsibilities	
A broad statement of the aims of the committee	To protect and serve DAMSEL2 trial patients (especially re: safety) and to assist and advise Principal Investigators so as to protect the validity and credibility of the trial. To safeguard the interests of trial participants, assess the safety and efficacy of the interventions during the trial, and monitor the overall conduct of the clinical trial.
Terms of reference	The DMC should receive and review the progress and accruing data of this trial and provide advice on the conduct of the trial. The DMC will inform the Sponsor if, in their view: (i) the results are likely to convince a broad range of clinicians, including those supporting the trial and the general clinical community, that one trial arm is clearly indicated or contraindicated, and there was a reasonable expectation that this new evidence would materially influence patient management or (ii) it becomes evident that no clear outcome would be obtained.
Specific roles of the DMC	Interim review of the trial's progress including updated figures on recruitment, data quality, and main outcomes and safety data. A selection of specific aspects could be compiled from the following list:- • comment on dose and dosing interval decision following Stage 1 • assess data quality, including completeness (and by so doing encourage collection of high quality data) • monitor recruitment figures and losses to follow-up • monitor compliance with the protocol by participants and investigators

	• comment on trial conduct – organisation and implementation of trial protocol • monitor evidence for treatment harm (eg toxicity data, SAEs, deaths) • decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated either for everyone or for some participant subgroups • suggest additional data analyses • advise on protocol modifications suggested by investigators or sponsors (eg to inclusion criteria, trial endpoints, or sample size) • monitor continuing appropriateness of patient information • monitor compliance with previous DMC recommendations • consider the ethical implications of any recommendations made by the DMC • assess the impact and relevance of external evidence
3. Before or early in the trial Input to the Protocol	All potential DMC members will have sight of the protocol before agreeing to join the committee. Before recruitment begins the trial will have undergone review by the funder and sponsor, scrutiny by other trial committees and a research ethics committee. DMC members will be independent and constructively critical of the ongoing trial, but also supportive of aims and methods of the trial.
	The DMC will meet early in the course of the trial, to discuss the protocol, the trial, any analysis plan, future meetings, and to have the opportunity to clarify any aspects with the principal investigators. The DMC will meet after Stage 1.
Issues specific to sepsis	Sepsis is inherently associated with expected adverse events. The Chief Investigator has defined expected events and the DMC will be asked to comment on these and the strategy for reported Adverse Events, prior to recruitment starting
Specific regulatory issues	This study is a CTIMP and requires a Clinical Trial Authorisation from the MHRA, in addition to REC and NHS R&D approval.
Issues specific to the treatment under study	Melatonin has been used at high oral doses (over 100mg) in several disease conditions and has been given to patients with sepsis at repeated low doses (<10mg). In this study we will be giving 20mg in repeated doses, depending on the results of Stage 1. Phase 1 studies have shown no toxicity of melatonin, but high repeated doses have not been given to patients with sepsis before.
Confirmation of agreement with this Charter	DMC members could formally register their assent by confirming (1) that they agree to be on the DMC and (2) that they agree with the contents of this Charter.
4. Composition Membership and size of the DMC	Membership of the DMC will comprise at least one clinician experienced in the clinical area and at least one statistician. The members will be independent of the trial (e.g. should not be involved with the trial in any other way or have some competing interest that could impact on the trial). Any competing interests, both real and potential, should be declared. A short competing interest form should be completed and returned by the DMC members to the trial coordinating centre (Annex 1). The members of the DMC for this trial are: (1) Dr Dan Martin (Chair), London. (2) Dr Malachy Columb (Statistical advisor), Manchester. (3) Professor Peter Andrews, Edinburgh

The Chair's role.	The Chair has previous experience of serving on DMCs and experience of chairing meetings and will facilitate and summarise discussions. The Chair has been chosen by the investigators running the trial since he was also Chair of DAMSEL 1. The Chair will facilitate and summarise discussions.
The responsibilities of the DMC statistician	The DMC membership will include a statistician to provide independent statistical expertise (Dr Malachy Columb).
The responsibilities of the trial statistician	The trial statistician, Dr Lorna Aucott will produce (or oversee the production of) the report to the DMC and will participate in DMC meetings, guiding the DMC through the report, participating in DMC discussions.
The responsibilities of the Chief and Principal Investigators	The Investigators will be available to attend open sessions of the DMC meeting if asked.
5. Relationships	
Relationships with Principal Investigators, sponsor and regulatory bodies	A diagram can help to clarify relationships when there are several inter-related committees. A short statement of the responsibilities of the other committees should be given if these are not provided in the protocol.
DMC is advisory	The DMC does not make decisions about the trial, but rather makes recommendations to the Sponsor and Chief Investigator.
Payments to DMC members	Members will be reimbursed for travel and accommodation.
Disclosure of competing interests	Competing interests will be disclosed. These are not restricted to financial matters – involvement in other trials or intellectual investment could be relevant. Although members may well be able to act objectively despite such connections, complete disclosure enhances credibility. (See Annex 1) DMC members should not use interim results to inform trading in pharmaceutical shares, and careful consideration should be given to trading in stock of companies with competing products.
6. Organisation of DMC meetings	
Expected frequency of DMC meetings	The exact frequency of meetings will depend upon pre-specified plans, and on trial events. The wishes of the DMC and needs of the Sponsor will be considered when planning each meeting. It is recommended that the DMC meet at least yearly.
Meetings format	Meetings will be a combination of face to face, video- or tele-conference depending on the DMC Chair's preference and circumstances. Some funding is available for travel where appropriate.
Organisation of DMC meetings	Meetings will comprise a mixture of open and closed sessions. Closed meetings will comprise the DMC and Trial Statistician as required and Open will involve these and the Investigators, Sponsor representative or other personnel if requested by the DMC.
7. Trial documentation and procedures to ensure confidentiality and proper communication	
Intended content of material to be available in open sessions	Open sessions: Accumulating information relating to recruitment and data quality (e.g. data return rates, treatment compliance) will be presented. Toxicity details based on pooled data will be presented and total numbers of events for the primary outcome measure and other outcome measures may be presented, at the discretion of the DMC.

Intended content of material to be available in closed sessions	Closed sessions: In addition to all the material available in the open session, the closed session material will include efficacy and safety data by treatment group.
Blinding	Stage 1 is open label. Stage 2: blinding will be maintained unless there is an issue when data will be unblinded if requested by the DMC.
Who will see the accumulating data and interim analysis	The people who will see the accumulating data and interim analysis will be specified by the DMC Chair. DMC members do **not** have the right to share confidential information with anyone outside the DMC, including the PI.
Identification and circulation of external evidence (eg from other trials/ systematic reviews)	Identification and circulation of external evidence will be the responsibility of the Trial team.
Communication of decisions/ recommendations that are reached	The DMC will report its recommendations in writing to the Sponsor's representative. This will be copied to the trial statistician. The report from the DMC may include a summary paragraph suitable for trial promotion purposes. (See Annex 2.)
After the meeting	The DMC members should store the papers safely after each meeting so they may check the next report against them. After the trial is reported, the DMC members should destroy all interim reports.
8. Decision making Recommendations available to the DMC	Possible recommendations could include:- • No action needed, trial continues as planned • Early stopping due, for example, to clear benefit or harm of a treatment, futility, or external evidence • Stopping recruitment within a subgroup • Extending recruitment (based on actual control arm response rates being different to predicted rather than on emerging differences) or extending follow-up • Stopping a single arm of a multi-arm trial • Sanctioning and/or proposing protocol changes
Rules or guidelines	The DMC will review and agree any interim analysis plan. Reasons will be recorded for disregarding a stopping guideline.
How decisions or recommendations will be reached within the DMC	• The decision making methods and criteria that will be adopted for guiding deliberations will be pre-defined • The process of decision making, including whether there will be voting or other formal methods of achieving consensus will be defined. The method of deliberation should not be revealed to the overseeing committee as this may reveal information about the status of the trial's data. • The role of the Chair - to summarise discussions and encourage consensus; it may be best for the Chair to give their own opinion last. If the DMC cannot achieve a unanimous decision this, a vote may be taken. It is important that the implications (eg ethical, statistical, practical, financial) for the trial be considered before any recommendation is made.

When the DMC is quorate for decision-making	All members should attend. Members who cannot attend in person should be encouraged to attend by teleconference. If, at short notice, any DMC members cannot attend at all then the DMC may still meet if at least one statistician and one clinician, including the Chair will be present. If the DMC is considering recommending major action after such a meeting the DMC Chair should talk with the absent members as soon after the meeting as possible to check they agree. If they do not, a further teleconference should be arranged with the full DMC.
DMC members who cannot attend the meeting may still input	If the report is circulated before the meeting, DMC members who will not be able to attend the meeting may pass comments to the DMC Chair for consideration during the discussions.
Members who do not attend meetings	If a member does not attend a meeting, it should be ensured that the member is available for the next meeting. If a member does not attend a second meeting, they should be asked if they wish to remain part of the DMC. If a member does not attend a third meeting, they should be replaced.
9. Reporting	
To whom will the DMC report their recommendations/decisions, and in what form	This will be by email letter to the Sponsor's representative. A timescale will be specified.
Minutes	Separate records may be required for open and closed sessions. The DMC Chair should sign off any minutes or notes.
Disagreement between the DMC and the body to which it reports	If the DMC has serious problems or concerns with the Sponsor's decision a meeting of these groups should be held. The information to be shown would depend upon the action proposed and the DMC's concerns. Depending on the reason for the disagreement confidential data will often have to be revealed to all those attending such a meeting. The meeting will be chaired by someone not directly involved with the trial.
10. After the trial Publication of results	At the end of the trial there will be a meeting to allow the DMC to discuss the final data with investigators/sponsors and give advice about data interpretation
Information about the DMC in published trial reports	DMC members should be named and their affiliations listed in the main report, unless they explicitly request otherwise. A brief summary of the timings and conclusions of DMC meetings should be included in the body of this paper.
Approval of publications by the DMC	The DMC may wish to be given the opportunity to read and comment on any publications before submission.
After the trial has been published	The DMC may discuss issues from their involvement in the trial when permission is agreed with the Sponsor.

Annex 1: Suggested competing interests form

Potential competing interests of Data Monitoring Committee members for *[Insert trial name (and sponsor's ID)]*

The avoidance of any perception that members of a DMC may be biased in some fashion is important for the credibility of the decisions made by the DMC and for the integrity of the trial.

Possible competing interest should be disclosed via the trials office. In many cases simple disclosure up front should be sufficient. Otherwise, the (potential) DMC member should remove the conflict or stop participating in the DMC. Table 1 lists potential competing interests.

Table 1: Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the sponsor
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial
- Emotional involvement in the trial
- Intellectual conflict e.g. strong prior belief in the trial's experimental arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) in competing products
- Involvement in the publication

Please complete the following section and return to the trials office.

☐ **No,** I have no competing interests to declare
☐ **Yes,** I have competing interests to declare (please detail below)

Please provide details of any competing interests: _____

_____

_____

Name: _____

Signed: _____ Date: _____

Annex 2: Suggested report from DMC to TSC where no recommendations are being made

[Insert date]

To: Sponsor's Representative

Dear *[X]*

The Data Monitoring Committee (DMC) for the DAMSEL2 trial met on [meeting date] to review its progress and interim accumulating data. [List members] attended the meeting and reviewed the report.

We congratulate the trial organisers and collaborators on the progress and conduct of the trial and the presentation of the data. The trial question remains important and, on the basis of the data reviewed at this stage, we recommend continuation of the trial according to the current version of the protocol *[specify protocol version number and date]* with no changes.

We shall next review the progress and data *[provide approximate timing]*

Yours sincerely,

*[*Name of meeting Chair*]*
Chair of Data Monitoring Committee

On behalf of the DMC (all members listed below)

DMC members:
(1) [*Insert name and role*]
(2) [*Insert name and role*]

(3) [*Insert name and role*]