

MELADERM-trial: Melatonin cream against acute radiation dermatitis in patients with early breast cancer: a pivotal phase 2, double-blind, randomized, placebo-controlled trial

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Background

Radiation injury is a common serious adverse reaction of treatment of various cancers with ionizing radiation [1-8]. In treatment of breast cancer, the most common complication to radiation therapy is radiation dermatitis [9]. During the course of radiation therapy, most of the patients (74-100%) will experience radiation dermatitis [10]. Symptoms of acute radiation dermatitis include pruritus, discomfort, and local pain [11]. Radiation dermatitis can even limit the intended therapeutic dose of radiation delivered to the patient and lead to prolongation in treatment [9]. Furthermore, radiation dermatitis decreases patients' quality of life [12].

Ionizing radiation causes tissue damage by releasing free radicals leading to oxidative stress [13]. The ionizing irradiation of water molecules in the cells leads to aqueous radiolysis, creating free radicals [14]. These free radicals may react with macromolecules, such as DNA, RNA, proteins, and cell membranes, eventually leading to dysfunction of cells and cell death [13].

The sleep-hormone melatonin reduces oxidative stress [15, 16]. Melatonin is amphiphilic and distributes freely between all body compartments [17-19]. Studies have documented that melatonin is a strong free radical scavenger. The anti-oxidative effects of melatonin may therefore provide protective effects against the damage of ionizing radiation in clinical cancer treatment and radiological imaging procedures [20]. In animal studies, melatonin protects the healthy tissue against radiation injury [20]. However, melatonin has also been documented to possess oncostatic and pro-apoptotic effects in cancers in experimental studies [21, 22], and has been demonstrated to sensitize human breast cancer cells to ionizing radiation in cell culture studies [23, 24].

A report describing the expected incidence of cancers up until 2020, predicts a yearly incidence of approximately 4800 cases of breast cancer in Denmark alone [25]. These numbers suggest that if melatonin can protect against radiation injury, a large number of patients could be candidates for melatonin treatment, and thereby obtain a better quality of life. A previous study

demonstrated the radioprotective effect of melatonin in the treatment of breast cancer [11]. This study included patients receiving breast-conserving surgery, and randomized them to receive either melatonin or placebo in a cream, which was applied twice daily. This study demonstrated a protective effect of melatonin against radiation dermatitis in women receiving 50 Gy of radiation [11]. However, this study did not list the dose of melatonin applied, and only used the RTOG scale as an outcome parameter. No subjective outcomes were measured in this study [11]. The authors of the previous study have not been able to inform us of the dose of melatonin used in the study (personal communication).

Melatonin has the disadvantage of being unstable when exposed to air and light. A study demonstrated that melatonin in aqueous solutions at room temperature, and at neutral pH, degrades by 29% over 21 days [26]. The compound dimethyl sulfoxide (DMSO) has seen wide use both as a solvent and as an anti-inflammatory drug [27, 28]. Based on a report made available to the authors from the pharmaceutical company, Bioneer A/S, melatonin dissolved in DMSO undergoes no degradation over 45 days when stored at 25 °C temperature, away from light.

The aim of the present randomized double-blinded placebo-controlled clinical trial is to investigate if melatonin can protect against acute radiation dermatitis in patients with early breast cancer receiving radiation therapy, and whether this has an impact on the patients' quality of life.

Study design

The study is registered at clinicaltrials.gov (indsæt ID, når dette er sket). This is protocol version 1.0, 13/09/2018. The study will be a randomized, placebo-controlled, double-blinded clinical pivotal trial. Patients will be allocated in a ratio of 1:1 to the melatonin or placebo group. Patients will be stratified according to the type of surgery (lumpectomy or mastectomy). Randomization will be performed in blocks of randomized sizes. The study will be performed in the Department of

Oncology, Rigshospitalet, Denmark. Patients will be included into our study according to specific eligibility criteria (see Table 1).

Interventions

Eligible patients with early breast cancer receive adjuvant radiation therapy over 15 to 30 daily fractions (5 fractions per week) of ionizing megavoltage photon radiation to a total of 40 - 60 Gy within 3-5 weeks according to the guidelines of the Danish Breast Cancer Cooperative Group [29]. In our study, the patients will administer approximately 1 g of cream containing melatonin (25 mg/g) and dimethyl sulfoxide (DMSO) (150 mg/g) or a placebo cream topically twice daily on the irradiated skin area. They are scheduled to do this every day from the first to the last fraction of radiation therapy, including the days where they do not receive radiation therapy. We have chosen placebo as our comparator due to it being safe, and in our view the most reasonable method of evaluating any effects of the intervention. On days where the patients receive radiation, the melatonin/DMSO or placebo cream will be applied no less than 2 hours prior to radiation.

Throughout the study, the patients will meet with the primary investigator once weekly and; the primary investigator will monitor compliance. In patients requiring steroid cream as a treatment for their radiation dermatitis, the steroid usage will be monitored. The patients will be recommended not to apply any other topical treatments to the irradiated area during the study. The use of other local therapeutics will be registered. Furthermore, patients will be informed that they should use safe contraception during the study. Safe contraception is defined as either an intrauterine device or hormonal contraception. A sterile permanent partner or abstinence from intercourse are also considered safe contraception. Should more than five years have passed since menopause the patient does not need to use contraception.

Recruitment

Patients with early breast cancer referred to radiation therapy will be screened through the electronic health records at Rigshospitalet (Sundhedsplatformen and Aria - Timeplanner (a program used locally for scheduling of radiation therapy)) by a secretary working in the Radiation Section, Department of Oncology. Before patients begin radiation therapy, they are referred to a dose-planning CT-scan in the Radiation Section, Department of Oncology, Rigshospitalet. When an eligible patient is identified through her referral for dose-planning, the secretary will inform them that we are conducting a study, and ask if an investigator can contact them. If the patient agrees, an investigator will be notified by the secretary. Patients will be screened by: diagnosis of early breast cancer, age, and previous radical tumor resection surgery. The investigator will then contact the patient (personally or by phone). The patients will be informed that we are conducting a study, and would like to invite them to participate. The patient will be offered a meeting, where she is allowed to bring a companion. The patient will receive both verbal and written information at this meeting. The patient will, before the information regarding the study is given, be informed that:

- It is an enquiry about willingness to participate in the study.
- The study has been approved by the Danish Data Protection Agency, the local ethics committee, and the Danish Medicines Agency.
- The subject has the right to a minimum of 24 hours for consideration.
- Choosing not to participate in the study will in no way influence the treatment they are offered at Rigshospitalet.

The information will be given in a room where both the investigator and the patient can sit comfortably without disturbances. The subject will be given enough time to read and listen to the information and ask questions. Both oral and written consent will be obtained either at this meeting or after at least 24 hours of consideration. A screening-list of patients will be created listing their CPR-numbers, name, inclusion/exclusion, and reason for exclusion if relevant.

Primary outcomes

The Radiation Therapy Oncology Group's (RTOG) acute radiation morbidity scoring criteria of the skin will be used as the primary outcome of the study [30]. The RTOG scale is widely used as a measure of radiation injury in the skin [30, 31]. This scale ranges from 0 to 4, being:

- 0: No change over baseline
- 1: Follicular, faint or dull erythema/epilation/dry desquamation/decreased sweating
- 2: Tender or bright erythema, patchy moist desquamation/moderate edema
- 3: Confluent, moist desquamation other than skin folds, pitting edema
- 4: Ulceration, hemorrhage, necrosis

The score will be performed at baseline, once weekly during radiation therapy, and once weekly for three weeks after the last radiation fraction.

Furthermore, an evaluation of the erythema will be performed with image analysis of clinical photographs after radiation exposure. Erythema has previously been evaluated by a validated method using software analysis (Image J, version 1.45S, National Institute of health, USA) of digital photos [32]. A "color space converter" function will be used to convert the clinical photos into grayscale in the software analysis. Erythema will be quantified by pixel color analyses where all white colored pixels represent erythema. An a*-value will represent degree of erythema (high a*-values represent a high degree of erythema). This method has previously been used in a study evaluating erythema developed as a result of ultraviolet radiation [33]. Photos for the analyses will be taken at baseline, once weekly during radiation therapy, and once weekly for three weeks after the last radiation fraction. Photographs will be taken with the same camera in the same lighting conditions for each picture.

The QLQ-BR23 is a questionnaire examining the quality of life in breast cancer patients, developed by the European Organization for Research and Treatment of Cancer (EORTC).

The QLQ-BR23 is a broadly used questionnaire that has been validated in Danish [34, 35]. The questionnaire includes several items of interest, but specifically for the present study, we focus on breast symptoms as a primary outcome. The remaining items from the questionnaire will be considered secondary outcomes, as described below. The QLQ-BR23 questionnaire will be filled out by the patients once weekly for the duration of the radiation therapy, and once weekly for three weeks after the last radiation fraction.

Secondary outcomes

The QLQ-C30 is a questionnaire concerning the quality of life in cancer patients in general, which is widely used and has been validated in Danish [36]. All items in the QLQ-C30 as well as the items from QLQ-BR23 (above) that are not mentioned under primary outcomes will be considered secondary outcomes. Patients will be asked to answer both questionnaires at baseline, once weekly during radiation therapy, and once weekly for three weeks after the last radiation fraction.

Patients will be asked about steroid cream usage for radiation dermatitis at baseline, once weekly during radiation therapy, and once weekly for three weeks after the last radiation fraction.

Demographic and background variables will be collected at baseline (see table 2 for a full list of demographic and background variables).

Participant timeline

The participant timeline is outlined in table 3. At the pre-screen consent, the patients will be informed that they have a right to 24 hours of consideration prior to deciding to participate in the trial or not. Oral and written consent will be obtained by the primary investigator at the time noted

in table 3, or at a maximum 24 hours hereafter. There will be no study-specific post-trial care for the participants, other than following the standard treatment regimens offered at Rigshospitalet.

Randomization and blinding

Patients will be randomly assigned to either a control or experimental group allocated in the ratio of 1:1 by use of a computer-generated randomization schedule. Patients will be stratified according to whether they have undergone lumpectomy or mastectomy. We will randomize the block size to between 4 and 8 patients per block. Each block will therefore consist of patients who have undergone either lumpectomy or mastectomy, allocated in a 1:1 ratio. Block size will be concealed to the investigators until the end of the trial (i.e. after data analysis and statistical evaluation). The allocation sequence will be generated by a staff member with no other involvement in the trial. Assignments will be placed in sequentially numbered, opaque, sealed envelopes with the randomization number printed on them. These envelopes will be packed by a staff member with no other involvement in the trial. Every assignment will contain a code that corresponds to tubes of the melatonin/DMSO or placebo preparation. The primary investigator will enroll and assign participants to intervention on the basis of the assignment code from the envelope. Envelopes as well as tubes with active and placebo cream will be stored in a locked cabinet at Rigshospitalet.

Outcome assessment will be performed by the primary investigator, who is blinded to treatment allocation. Participants are also blinded to treatment allocation. The primary investigator will be blinded when performing data analysis and statistical evaluations.

Should the investigator deem it necessary for the safety of the patient, the investigator can un-blind a patient. Treatment-allocation will be noted in a document on a secure folder on a drive on the server of the Region Hovedstaden. Should an investigator need to un-blind a patient, they can access this document from their computer. Treatment allocation will only be un-blinded if it is imperative to the treatment of the patient. The treatment allocation will in this case only be

revealed to one other investigator than the primary investigator. This investigator will oversee the treatment of the patient. Registration of adverse reactions is described in the “Harms” section.

Drop-outs and patient retention

We plan to promote participant retention and complete follow up by asking for contact information (phone number and e-mail) and preferred way of contact. We will schedule appointments with patients at same location, accommodating their calendar, as far as possible.

If a patient does not wish to complete all three follow-up visits, some of the outcomes can be filled in electronically from home, should the patients wish to do so. These outcomes are the QLQ-C30 and QLQ-BR23 questionnaires, and steroid usage outcomes. All data from drop-outs will be used in statistical analyses. Discontinuation of treatment will only occur in the following eventualities: the patient withdraws consent, or if the sponsor decides to terminate the study due to serious adverse events.

Conclusion of study

Recruitment of patients will be concluded when 48 patients have completed the study (see sample size calculations below, in the “Statistics” section). The study will be concluded, when all patients included have completed the study.

Handling of drugs

Storage

The active cream will consist of 25 mg/g melatonin as well as 150 mg/g DMSO, in a cream base. The placebo will consist of just the cream base. The cream base consists of polysorbate 80 (0,5%), cetostearyl alcohol (5%), paraffin oil (5%), glycerol monostearate 40-50 (6%), methyl

parahydroxybenzoate (0,1%), glycerol 85% (4%), sorbitol (7%), purified water (72,4%). The dose for each application will be 1 g, and the cream will be packaged in 30 g tubes. The tubes will be stored at room temperature, in a locked cabinet, in the office of one of the investigators. The cream will be produced and packaged by Glostrup Apotek in their manufacturing facility, according to EU GMP-guidelines. The tubes will be labelled with all required information listed in the EU GMP-guidelines concerning investigational medical products. All drugs will be delivered directly to the sponsor, who will document receiving the products according to GCP-guidelines (modtagekontrol).

Patients will be given one tube of cream at a time, and a second and third tube will be given to them, once the previous tube is empty or running low. Patients will be instructed to keep the tubes out of reach of children, away from sunlight and at room temperature. These instructions will also be listed on the label of the cream.

Administration

Prior to the first radiation treatment patients will receive a tube of cream with a number corresponding to their randomization number. They will be instructed in storage of the tube as described above. Patients will be seen once weekly throughout their radiation therapy. At this meeting, the patients will be asked how much cream they have left, and another tube of cream will be handed out when the patients are expected to run out of cream before the next meeting. Patients will receive a total of two to three tubes, depending on the number of radiation fractions they receive, each containing 30 g of cream.

Patients will be instructed to apply the cream twice daily, once in the morning and once in the evening. The patients will be instructed to apply approximately 1 g of cream in the treatment area twice daily. On days where the patients receive radiation fractions, the patients will be instructed to apply the morning dose no less than 2 hours prior to radiation. As part of the instruction, the investigator will weigh out 1 g of cream, so that the patient can see what amount of

cream they should apply each time. During the weekly visits, the patients will be asked if they are experiencing trouble applying the cream, and the investigator will help guide them. At every visit the patients will be asked to bring with them the tube of cream they are currently using. When first handed out, and during each visit, the tube will be weighed on a high-precision scale (range 0.01 mg to 200 g). Usage of cream will be calculated, usage will be evaluated. Furthermore, along with the first tube of cream, the patients will receive a schedule with the relevant dates for the patient; the patient will have to fill in every time they have applied the cream. When a patient is expected to run out of cream before the next visit, another tube will be handed to them. One tube of cream contains 30 g of cream, and the patients will apply 2 g each day they receive radiation. One tube should last for 15 days, assuming they administer the correct dose daily, and assuming they are able to completely empty the tube. Ideally patients will receive new tubs on Study visit week 2 and 4, if they have managed to use the instructed dose.

Statistics

Sample size calculations

In order to establish the needed number of patients for the study, we have performed 3 separate sample size calculations.

The sample size calculation for the RTOG acute radiation morbidity scoring criteria is based on the findings in a previous study examining melatonin as a radiation protector of the skin in patients receiving radiation therapy for breast cancer [11]. In this study, at two weeks follow-up, 41% of the patients receiving melatonin had toxicity grade 1-2 vs 90% of the patients receiving placebo.

Incidence in placebo group: 90%

Incidence in melatonin group: 41%

Alpha: 5%

Beta: 20%

Sample size needed: Two groups of 14 patients, totaling 28 patients.

The sample size calculation for the image analysis is based on the study evaluating erythema after exposure to ultraviolet radiation [33]. Data for the sample size calculation were obtained directly from the authors. We make the assumption that the erythema based on ultraviolet radiation can be a reasonable substitute for acute radiation dermatitis based on erythema being a part of the RTOG acute radiation morbidity scoring criteria grade 1 and 2 [30]. Based on the study used for the RTOG sample size calculation, we assume that the erythema will be most pronounced 2 weeks after conclusion of radiation therapy. We also make the assumption that melatonin applied twice daily in a 2.5% cream in which it is completely dissolved, will provide an effective dose comparable to the 12.5% used in the study evaluating ultraviolet radiation [17], due to a deposition or depot-effect in the skin [37]. Furthermore, we have been informed by the authors of the previous study utilizing the 12.5% cream that the cream was not homogenous, and contained small granules of undissolved melatonin in both the 2.5%, 5% and 12.5% creams. This suggests that the required dose, to obtain an effect, is in fact smaller than 12.5%. The sample size is calculated based on the a^* -values, where a high a^* -value corresponds to a higher degree of erythema.

Mean (SD) a^* -value for placebo at all time points: 18.4 (3.47)

Mean (SD) a^* -value for 12.5% melatonin cream at all time points: 13.44 (2.36)

Alpha: 5%

Beta: 20%

Sample size needed: Two groups of 8 patients, totaling 16 patients.

The sample size calculation for the QLQ-BR23 breast symptom score is based on a study investigating quality of life following radiation therapy, comparing mastectomy to breast conservation therapy in patients with breast cancer [38]. The data used for the sample size-

calculation are based on the patients receiving breast-conserving therapy, on their last day of radiation. The 142 patients scored a breast symptom score mean (SD) of 82.2 (15.1).

Mean (SD) α -value for breast symptoms: 82.2 (15.1)

We aim to reduce the breast symptom score to 70.

Alpha: 5%

Beta: 20%

Sample size needed: Two groups of 24, totaling 48 patients.

Because we have three sample size calculations, resulting in required sample sizes of 28, 16, and 48 patients, respectively, and when considering the risk of drop-outs, we estimate to include a total of approximately 80 patients. This is based on the inclusion schedule, where the required 48 evaluable patients finalizing all data for the outcome parameters have to be completed, will require an inclusion larger than 48 patients. This is because there will be 8 weeks from inclusion to obtaining the data point at two weeks after ended radiation therapy. Thus, we will include continuously in randomized blocks (see randomization above) until patient 48 has completed the 8 weeks data point. If we get less than 24 patients in one group completing the 8-weeks data point, additional blocks will be included, until we have at least 48 patients. The staff-member responsible for the randomization sequence will inform the investigators how many patients they will need to include to reach this point because of the randomization of the block size. During 2016, Rigshospitalet treated 194 patients who matched our inclusion criteria. Therefore we find it feasible to finish inclusion in approximately 6 months.

Statistical analyses

Normality of data will be assessed through visual inspection of histograms and Q-Q plots. Normally distributed data will be analyzed with parametric tests (e.g. unpaired t-tests), and not normally

distributed data will be analyzed with non-parametric tests (e.g. Mann-Whitney U test). Our primary outcomes are the RTOG scores and the image analysis two weeks after the last radiation fraction, as well as the QLQ–BR3 breast symptoms on the last day of radiation therapy. Our secondary outcomes will be analyzed as follows: We will analyze steroid usage through two different outcomes. Days of steroid usage will be analyzed through either parametric or non-parametric tests. Any steroid usage (yes/no) will be analyzed through χ^2 or Fisher's Exact test. The RTOG scores, the quality of life questionnaires, and the image analysis values for the entire period will be analyzed in a mixed linear model, which takes correlation between patients into account. Subgroup analyses will be performed for mastectomized and lumpectomized patients respectively, if feasible. Other subgroup analyses may be performed based on background variables (fractionation schedule, histopathology and demographic data). Missing data will be taken into consideration when using the mixed linear model. A detailed statistical analysis plan will be formulated and be supervised by a bio-statistician prior to study start.

Data management

All outcomes will be collected by the primary investigator at time points listed in table 3. All data entered into the electronic case report form (eCRF) will be validated through “required fields” and format (e.g. date-format) where applicable. In the clinical photographs, patients will be marked with patient-ID written on tape, fixed to the patients' chest. The time-stamp of the photograph will be noted in the eCRF. a*-values will be imported into the eCRF.

Our data will be stored electronically in Research Electronic Data Capture (REDCap), an electronic database used throughout the Capital Region of Denmark. All confidential data will be stored in a secure server for 15 years after patient enrollment in the study. Only the investigators and the Good Clinical Practice (GCP) Unit will have access to these data. After conclusion of the study, a final anonymized dataset will be available to RepoCeuticals ApS who has sponsored the

study through a grant through a formalized research contract between the Capital Region of Denmark and the company. Ownership of data is shared between the investigators and RepoCeuticals ApS.

Monitoring

This study will be conducted in compliance with the protocol, EU ICH-GCP guidelines and the applicable regulatory requirements. The standard procedures for quality control and quality assurance are followed in compliance with the ICH-GCP guideline, and investigator/sponsor allows direct access to data/documents for monitoring, auditing, and inspection from both Danish Medicines Agency and the Good Clinical Practice (GCP)-unit. The study will be audited by the GCP Unit of the University of Copenhagen. The GCP Unit will be independent from the investigators and sponsors, and have direct access to the electronic case report forms as well as the trial master file. The GCP Unit will also perform data monitoring. The GCP Unit is an independent unit that manages clinical trials in Denmark. Further information can be found at <http://www.gcp-enhed.dk>. No interim analyses have been planned. Currently, a monitoring schedule has not been formulated.

Harms

Adverse reactions to melatonin

Melatonin is non-toxic in both physiological and pharmacological doses [39]. In mice, rabbits, cats, and dogs, melatonin doses of 800 mg/kg, administered intravenously, have demonstrated no toxicity [40].

A study administered 1 g of melatonin orally to patients for 25-30 days, with drowsiness as the only adverse reaction [41]. These patients had substantial clinical tests (hematological and blood chemistry tests, urine analysis, heart rate and blood pressure monitoring and ECG) performed, and no reactions to melatonin were demonstrated [41]. Another study investigated the effect of intravenously administered melatonin in healthy subjects, as well as patients with Parkinson's disease and epilepsy. They applied 1.25 mg/kg melatonin as a bolus injection. One of the patients, who had 1.25 mg/kg melatonin intravenously, received further two doses of 0.5 mg/kg. Correspondingly, melatonin was non-toxic and no adverse reactions were observed [42].

Furthermore, melatonin has been used as an intravenous infusion in neonates with sepsis, in neonates undergoing abdominal surgery, and in preterm infants [43-45]. A total of 224 patients were included (of who 105 received melatonin) and no adverse reactions were reported, at a dose of 10 mg/kg intravenously [43-45].

Melatonin has also been given in very large doses up to 100 mg intravenously in adults, with no adverse reactions [46]. Other studies investigated melatonin when used as a pre-medication for gynecological laparoscopic surgery, and demonstrated no adverse reactions at a dose of 5-20 mg of sublingual melatonin [47, 48]. No adverse reactions were reported in a study investigating pre-medication with 5 mg melatonin prior to laparoscopic cholecystectomy [49].

Drowsiness, confusion and depression has been described as side-effects of melatonin in a study examining if melatonin could augment antidepressant treatment in patients with depression [50]. Furthermore, a few studies report headache [51] and fatigue as side-effects of melatonin [52].

Adverse reactions to DMSO

Dimethyl sulfoxide (DMSO) is regularly used in the treatment of interstitial cystitis either as monotherapy, or serving as a vehicle for anti-inflammatory agents [27, 53-55]. DMSO has previously been applied as a solvent for intravesical drug administration in the treatment of bladder cancer [56]. An adverse reaction of DMSO is a garlic-like breath odor and taste, due to pulmonary elimination of a small percentage of the DMSO as dimethyl sulfide. One study demonstrated that up to 48% of patients with interstitial cystitis reported adverse reactions, including urethral irritation and nausea [53]. However, the dose in this study was a total of 25 g of DMSO (50 ml fluid of 500 mg DMSO/ml), which is a much higher dose than we aim to apply [53]. These adverse reactions are self-limiting, lasting up to 24-48 hours [27, 53, 54]. DMSO has demonstrated a self-limiting mild burning sensation as an adverse reaction when administered topically [57], but DMSO dose locally was approximately 50 times larger than in current study [57], and DMSO was applied every 8 hours, opposed to the current application two times a day [57].

Adverse events

The subjects will be questioned about adverse events once weekly during treatment. An adverse event is defined as any medical event that leads to an unwanted effect in the subject after the administration of the intervention medication, without any necessary connection between the adverse event and the intervention. An adverse reaction is defined as harm or unwanted reaction to a drug, no matter the dosage. We have defined six pre-specified self-reported symptoms (nervousness, confusion, depressed mood, dizziness, headache, or garlic like odor), based on the most commonly reported adverse reactions of melatonin and DMSO available in the literature [41, 50-53]. Patients will also be asked to report additional symptoms of adverse effects, if any. Both adverse reactions and adverse events will be reported in the eCRF.

Serious adverse events (SAE) are defined as any medical effect that result in death, are life-threatening, lead to hospitalization, result in disability or permanent damage, or any other important medical event. All serious adverse events will be registered in EudraCT..

Serious adverse drug reactions (SARs) will be reported annually. Most likely this will not be applicable to this study, as it will last less than a year. In this case, SARs will be reported on conclusion of the study.

Sudden unexpected serious adverse reactions (SUSARs) will be reported immediately to the Danish Medicines Authority and the local ethics committee. Adverse event registration will commence on the first study day and be finalized after the last study day. In case of serious adverse reactions, the sponsor can decide if the study will be terminated. It is the sponsor's responsibility to evaluate if an adverse event is a SUSAR. SUSARs that are lethal or life-threatening will be registered and reported to the Danish Medicines Agency as fast as possible and at the latest 7 days after the sponsor has obtained knowledge of such an adverse event. All other SUSARs will be reported to the Danish Medicines Agency within 15 days. Due to not expecting any serious adverse reactions, all SARs will be considered unexpected and therefore considered SUSARs.

Ethical considerations

The study will be performed in accordance with the Helsinki II declaration. Patients will be included after oral and written consent. If patients do not wish to participate in the study, or choose to withdraw before administration of melatonin in any of the series of radiation therapy, they will receive standard treatment for their cancer. Patients can withdraw from the study at any time without stating a reason. Withdrawal from the study will not have any consequence for further treatment. Should an investigator deem it necessary for the safety of the patient, they can withdraw the patient from the trial. It is important to clarify if melatonin has a protective effect against

radiation injury, due to it having a large impact on the quality of life in patients. Positive results from this study will guide further studies and potentially change current practice in radiation oncology. The protective effect of melatonin has the potential to be used in several other types of cancer treatment, where ionizing radiation is used, to minimize the radiation injury and therefore decreased quality of life in patients. All information regarding participants in this study will be kept protected and no personal data will be published. The anonymity of all participants will be secured and data kept confidential.

Insurance

For this study no additional insurance policies have been arranged that exceed the *Danish Act on the Right to Complain and Receive Compensation within the Danish Health Service*

(Patientforsikringsloven). Patients participating in this study will be covered by the mentioned Act in accordance with “Bekendtgørelse af lov om klage- og erstatningsadgang inden for sundhedsvæsenet” in the same way as patients that are treated otherwise in the hospital. This is normal practice for investigator-initiated trials in Denmark.

Approvals

The study will be performed in accordance with the Helsinki II declaration. Approval from Danish Data Protection Agency in the Capital Region of Denmark (Datatilsynets fællesanmeldelse i Region H) will be sought in accordance with Data Protection Act (Databeskyttelsesloven), and the study will not start subject accrual before this approval has been given. The same applies for the local ethics committee as well as Danish Medicines Authority (Lægemiddelstyrelsen). Permission will be

sought to register and store data manually and electronically from participants in the study through their electronic health records and the Danish National Patient Register.

Confidentiality

All information about the patient will be noted in the eCRF, which is stored in a secure server (see Data management).

The sponsor, investigators, monitors and Danish Medicines Agency will have access to required information (outlined in Table 2) from the patients' electronic health records.

Protocol amendments

Currently there are no protocol amendments. Should any protocol amendments arise, these will be communicated to the investigators, the journal in which the protocol will be published, clinicaltrials.gov, the Good Clinical Practice Unit, the Danish Data Protection Agency (if relevant), the local ethics committee, and the Danish Medicines Authority.

Funding

A grant covering all study expenses has been donated by the pharmaceutical company RepoCeuticals ApS for this study. Both the active and placebo treatments used in the trial have been

donated by the company, which has no influence on the design, interpretation or publication of data.

None of the investigators have any interests in RepoCeuticals, financial or otherwise.

Conflicts of interests

None of the investigators have any conflicts of interests to declare.

Dissemination policy

Three publications will be written on the basis of this study:

- A protocol article, describing our methods
- An article concerning the acute radiation dermatitis measured with the RTOG scale, image analysis and the need for steroid treatment;
- An article concerning the impact of acute radiation dermatitis on the patients' quality of life.

The articles will be published in international peer-reviewed scientific journals. Both negative, positive, and inconclusive results will be published. The determination and documentation of authorship will be based on the authorship criteria formulated by the International Committee of Medical Journal Editors (ICMJE). Participants will be offered a copy of the articles when published.

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Table 1: Inclusion and exclusion criteria

Inclusion criteria
Diagnosed with early breast cancer
Over 49 years old
Have had radical tumor resection surgery
Follows treatment regimens and follow-up at Rigshospitalet
Written informed consent after written and verbal information
Exclusion criteria
Inability to understand Danish, written or spoken
Mental illness*
Previous therapy with ionizing radiation in the thoracic or neck area
Use of bolus for radiation therapy**
Pregnancy***

** Defined as having a diagnosis and being in medical treatment, or if anticipated poor compliance. ** A bolus is a material which has dose absorption properties equivalent to tissue. It is placed on the irradiated area to alter dosing or target of the radiation therapy. *** Patients will be asked to take a pregnancy test prior to inclusion*

Table 2: Demographics and background variables

Demographics	Source
Age	CPR-number ¹
Weight	Patient
Height	Patient
Ethnicity	Patient
Bra size	Patient
Breast volume	Radiation treatment plan
Skin-type ²	Investigator evaluation
Comorbidities	Patient/electronic health record
Connective tissue diseases	Patient
Disease and treatment	Source
Tumor type	Pathology report
Tumor size	Surgical report
Location of tumor	Surgical report
Lymph node status	Pathology report
Metastases	Electronic health record
Axillary dissection	Surgical report
Chemotherapy prior to radiation	Electronic health record
Type of chemotherapy	Electronic health record
Radiation dosage	Radiation treatment plan
Hypo-/hyperfractioning of radiation therapy	Radiation treatment plan

¹Central Person Register in Denmark. ²Fitzpatrick scale.

Table 3: Participant timeline

Activity/assessment	Pre-study screening/ consent	Pre-study baseline/ randomization	First radiation fraction	Study visit week 1	Study visit week 2	Study visit week 3	Study visit week 4	Study visit week 5	Last radiation fraction	Follow-up week 1	Follow-up week 2	Follow-up week 3
Prescreening consent	•											
Screening log	•											
Consent form	•											
Inclusion/exclusion form	•											
Randomization		•										
Demographics questionnaire		•										
Instruction in application of cream		•										
Handover of drug/placebo ^a		•			•		•					
Evaluation of compliance			•	•	•	•	•	•	•			
RTOG score evaluation ^b			•	•	•	•	•	•	•	•	•	•
Clinical photography			•	•	•	•	•	•	•	•	•	•
QLQ-C30 ^c			•	•	•	•	•	•	•	•	•	•
QLQ-BR23 ^c			•	•	•	•	•	•	•	•	•	•
Steroid usage evaluation			•	•	•	•	•	•	•	•	•	•
Monitoring of adverse events			•	•	•	•	•	•	•	•	•	•
Serious adverse event form ^d												
Conclusion of participation												•

^a Patients will receive extra trial drug/placebo when required throughout study, therefore, the marked visits might vary from patient to patient depending on compliance. ^b RTOG score = Radiation Therapy Oncology Group cooperative group common toxicity criteria. ^c QLQ-C30 and QLQ-BR23 =European Organization for Research and Treatment of Cancer quality of life questionnaires. ^d Serious Adverse event form will be filled in as needed throughout the trial.

