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PROTOCOL
CLINICAL INVESTIGATION OF MEDICAL DEVICE

Title of the study:	Perspective randomized study aimed at evaluating the effectiveness of the EmoLED medical device in the treatment of second and third stage pressure ulcers. (R.I.S.E._U.P.)
Reference number or identification code of the clinical investigation:	*EmoLED_005*
Protocol version number:	*3.0*
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APPROVAL OF THE PROTOCOL

The Investigator involved:

- approves this protocol;
- declares that the study will be conducted according to GCP, UNI EN ISO 14155:2020 and in accordance to this protocol.

Date: 16/07/2021

Lorenzo Targetti – Sponsor's legal representative	Firma
Dott. Stefano Gasperini – Scientific Coordinator	Firma
Prof. Claudio Macchi – Principal Investigator	Firma

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Information about the medical device

The device used in this study is a CE marked phototherapy medical device (emitting blue light) for the treatment of skin lesions. It is a IIa class portable device, internally powered. The trade name is EmoLED (product code 9800010001), the version is V.1 and it includes a software (version 3.0) that operates the applications. The accessories provided are: safety glasses for the operator, charging adapter for the internal batteries, a display for visual comfort and the device's case. The supplier is EMOLED S.r.l.

EmoLED is CE marked as a phototherapy device for the treatment of skin lesions. It is intended for use by medical personnel in a hospital or outpatient setting. The patients' demographic consists of individuals with skin lesions that are at least 16 old, regardless of their ethnicity. EmoLED is conceived and designed to treat chronic and acute lesions of the skin by healthcare professionals such as doctors and nurses operating in the tissue repair field. In particular the EmoLED treatment is part of the clinical practice and the Standards of Care (SoC) provided for the various types of lesions (e.g. chronic lesions or burns).

EmoLED treatment is additional to the conventional therapy consisting in the lesions cleansing and dressing with conventional or advanced medications. The recommended posology is of one treatment at every dressing change, to be performed after cleansing and before dressing.

The device is constituted by a "body" to hold, containing the battery pack, the motherboard and a touch screen, and by a rotating arm containing the optical part and the proximity sensor. The optical head has a cylindrical shape with a 50mm diameter and a 180 degrees rotation. The full rotation is prevented by mechanical means.

The front of the device contains a touch screen display through which it is possible to select the language and register as a user, manage the treatments by entering the size of the lesion and see information related to the treatment such as time and number of applications remained. The screen also displays information about the status of the device, such as the remaining charge and any error messages or warnings.

The casing is equipped with two openings on the left side containing:
- A power jack for connection to the power supply dedicated to charging the batteries;
- A microUSB port to download data related to the use of the device collected by the way during its use. Access to these data is subject to the entry of a security password provided only to authorized personnel.

On the back of the device there is an On/Off button.

The user interface is essential and intuitive, in order to minimize the risks related to usage error. The commands are simple and like "confirm"/"cancel"/"next". The information shown on the screen is easy to understand and the meaning of all the symbols used is given in the appropriate paragraph of the User Manual.

EmoLED is a portable device, powered by rechargeable lithium-ion batteries, non-invasive and non-contact, fast in the treatment and that can be disinfected.
The interface through touch screen allows, asking 2 simple questions to the user, to set the treatment in terms of number of applications; the duration of the individual application and the delivered dose are set at the factory and are not modifiable by the user.
The device is equipped with a movable and rotating part containing the Optical Head, from which the incoherent light beam generated by 6 LEDS that emit in the blue range (between 410 and 435 nm, Optical Power Density 120 mW/cm2) comes out, in order to facilitate the treatment of the lesions of interest. The emission of light radiation is subject to the correct positioning of the device towards the lesion: target treatment distance 40 ± 10 mm. The positioning is controlled by an electronic card containing a distance sensor.
The use of the device is excluded when charging the batteries.

Accessories supplied with the device:
- Power supply;
- Screen for visual comfort;
- UV filter glasses.

The visual comfort screen is located just behind the light beam area and provides physical protection against emitted components that can prove troublesome when reflected. The charger is a 24 V power supply with an integrated cable to connect the device and a separable cable to connect to the network.
On request, protective glasses with UV filtration and Optical Density (OD) of 4+ up to 400 nm and at least 2+ between 400 and 460 nm are provided. These PPE are recommended for both the operator and for all people within a radius of 1 meter from the source of light. The glasses available are the 450#53 model of the NOIR Laser Company.
The device is supplied by manufacturer with certified quality management system, traceability is guaranteed by the manufacturer's system which, through the serial number, is able to know both where the device in question is located, and the details of the internal components of the device.

The EmoLED device is very simple and intuitive in its use, the user manual illustrates the proper use. Moreover, the device operators will attend a manufacturer's training session in which the proper use of the device is explained and a demonstration test is performed. Emoled staff is always available for any question, request and for assistance.
The blue light emitted by the device interacts with the endogenous chromophores of the skin triggering reactions that lead to the activation of certain cellular pathways. In particular, the wavelength emitted by the device is absorbed by Protoporphyrin IX present in Cytochrome C, an essential protein for cellular respiration at the mitochondrial level. The energy absorbed is used by the cell to increase the production of ATP, a fundamental molecule for all the processes involved in tissue repair. In addition, blue light is able to stimulate the production of ROS (Reactive Oxygen Species) through the excitation of flavins and flavoproteines. Nowadays, multiple evidence has been produced about how ROS can be considered signal transducers of numerous cellular pathways. This

consideration validates the evidence that ROS (at physiological concentrations) are crucial for multiple cellular functions such as differentiation, proliferation, migration and contraction.
Therefore, it follows from those considerations that blue light can promote and restore the correct course of tissue repair in wound that are considered "difficult" or chronic.

Literature review and rationale of the clinical investigation

Wound healing is a complex and dynamic biological process that includes a series of organized phases, including coagulation, inflammation, matrix deposition, angiogenesis, proliferation, cell remodeling and wound contraction (1, 2). So that each step of the process is completed, complex interactions between various biological factors need to occur, such as growth factors and proteinases, matrix components and various cell types, such as platelets, macrophages, fibroblasts and endothelial cells (3). The interaction of these processes as a whole determines the restoration of tissue integrity and functional healing (5). Although much has been done towards the complete understanding of the processes that regulate this important biological function, there are many aspects that still need to be clarified. At the same time, if it is true, on the one hand, that technological advances have led to the creation of increasingly sophisticated medications, it is equally true, on the other hand, that there is a need to find new methods to further improve the healing process, regardless of the event causing the skin injury, reducing the pain and discomfort associated with the medication itself, possibly shortening the time needed to recovery, with relative reduction of related costs.
Pressure ulcers (PU), also known as decubitus sores, are areas of lesion localized to the skin and subcutaneous tissue. The development of decubitus ulcers occurs in institutional and community environments, and more frequently in assisted elderly, debilitated and motionless (e.g. orthopedic patients), in patients with severe acute disease (e.g. hospitalized in intensive care units) and in subjects with neurological deficits (e.g. spinal injuries).
Recognizing the considerable economic, health and social impact of pressure ulcers has led to considerable efforts to reduce their occurrence. Nevertheless, pressure ulcers continue to occur. Although not all pressure ulcers are iatrogenic, most of them can be prevented. Pressure ulcers are one of the most frequent iatrogenic lesions in developed countries.
Inadequate treatment methods, such as leaving vulnerable patients in potentially harmful positions for long periods, or massaging reddened skin areas, often continue to be perpetuated even if it has been proven that they are damaging or ineffective.
Pressure, that is often associated to a limitation in mobility of the patient, for a long time has been considered the most important extrinsic factor in the development of pressure ulcers.
However, recent research, and still ongoing, is revealing that shear forces, friction, and microclimate also play an important role in the etiology of pressure ulcers, and that, also, some significant and complex relationships between all these extrinsic factors exist.
In alert patients, the effects of prolonged pressure usually stimulate frequent light body movements to relieve the load and restore tissue perfusion. Unconscious, sedated, anesthetized or paralyzed patients cannot feel or respond to these stimuli and do not move spontaneously. As a result, skin and soft tissue can be exposed to prolonged and without relief pressure.
PUs are generally more common in anatomical locations that cover a bone prominence. In adults, the most common sites are the sacrum and the heel. Other frequently affected anatomical

locations are ischium, ankle, elbow and hip.
Pressure ulcers are often difficult to diagnose, in particular it is easy to confuse them with moisture-caused injuries. A correct diagnosis is essential to determine both prevention and treatment plans.
For a proper management of pressure lesions it is essential to take into account their characteristics (location, color, size, amount of exudate, type of tissue, smell, edges) and to consider the condition of the peri-lesional skin (erythema, edema, hardening, maceration). To describe the state of a pressure ulcer, EPUAP (European Pressure Ulcer Advisory Panel) and NPUAP (National Pressure Ulcer Advisory Panel) recommend to divide pressure injuries stages from 1 to 4:

- Stage 1 - Persistent erythema
  Intact skin with redness not reversible to acupressure in a localized area, usually above a bone prominence. Stage 1 may indicate a patient at risk.

- Stage 2 - Partial thickness tissue loss
  Loss of the dermis at partial thickness, which looks like a shallow and open ulcer, with red - pinkish lesion bed, without slough. It may also present as a vesicle, intact or open/broken, serous and/or haematic.

- Stage 3 - Total Thickness Tissue Loss
  Loss of tissue at total thickness. Subcutaneous fat may be visible, but bones, tendons or muscles are not exposed. There may be slough, which does not hide the depth of tissue loss. It may include undermining wounds and tunneling.

- Stage 4 - Loss of subcutaneous tissue
  Loss of tissue at total thickness with exposure of bones, tendons or muscles. There may be slough or eschar on some parts of the lesion bed. Undermining wounds and tunneling are often present. Stage 4 injuries can extend to muscle and to support structures.

- Not classifiable lesion
  Loss of tissue at total thickness, where the base of the ulcer is covered by slough (yellow, brown, grey, green or brown) and/or eschar (brown, brown or black) in the bed of the lesion.

Principle of operation of the EmoLED medical device to help wound healing

The EmoLED device is a medical device for the healing of wounds, CE marked, whose operation is based on LED sources operating in the blue range. The choice of blue light was made on the basis of the absorption spectrum of the target chromophore - the Protoporphyrin IX present in Cytochrome C; actually, the emission range of EmoLED medical device is preferentially absorbed by this chromophore as it coincides with its maximum peak absorption in the visible light spectrum.
As for the use of light in medicine, the scientific literature is rich in data and evidence on its use for the treatment of acne (5) and psoriasis (6) and its effectiveness in accelerating the process of healing of an induced wound on animal models (7-9).

When considering the absorption coefficients of the skin chromophores in the visible light range, we note that Cytochrome C has the peak of maximum absorption in the range of blue, around 410 nm for both the reduced and oxidized form.

A way through which EmoLED can act in tissue repair is the chain of mitochondrial electronic transport. EmoLED in particular can act on the last two complexes that contain Cytochrome C, that is sensitive to visible light in the range of emission of the device.
The resulting effect is the strengthening of this process and the increase in ATP production, related to the development of a proton gradient dependent on the electron transport chain.
The increase of ATP production determines an increment of the available energy for the cell that can intensify its metabolic activity, a necessary process during the repair of an injury that involves activation of different cell types and an additional energy effort for the organism.
Other important effects of EmoLED device are mediated by the action of ROS, signal transducers of numerous cellular pathways that are involved in tissue repair. A moderate increase in ROS stimulates the production of pro-inflammatory agents (12-14).
Furthermore, through the action on the T lymphocytes present in the wound bed, ROS are able to promote the phenotypic transition of macrophages from M1 (pro-inflammatory) to M2 (pro-healing) (15, 16). This combination of effects suggests a positive action on the inflammatory stasis that characterizes some types of "non-healing" wounds: an increase in inflammation such as to induce the body to a consistent response and the consequent stimulation for the following phases.
Through the production of HIF-1α (Hypoxia-inducible factor 1-alpha) and the consequent release of proangiogenic factors and induction of eNOS (endothelial Nitric Oxide Synthase) ROS also play a role in promoting angiogenesis (17, 18) and therefore in the increase of the nutrient and oxygen supply in the wound bed, of great importance in the proliferation phase.

The device has been designed and set to deliver a power density of about 7 J/cm2 in an acceptable treatment time for both doctor and patient (60 seconds).

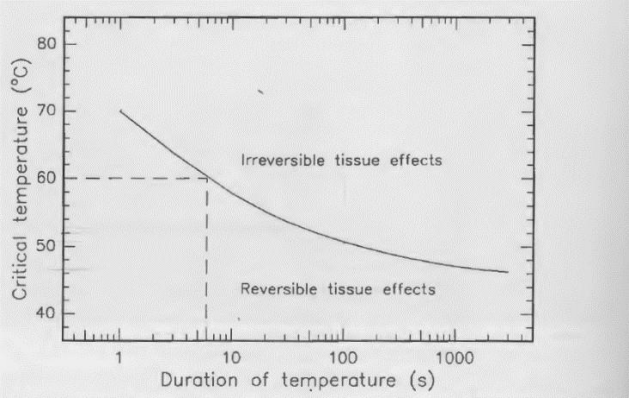
The choice of the duration of the treatment was also made on the basis of what is stated in literature on thermal damage: the treatment induces a temperature between 45 and 50 º C in the treated area, ideal condition to stimulate the reversible and physiological phenomena that we want to induce.

Table 3.6. Thermal effects of laser radiation

Temperature	Biological effect
37°C	Normal
45°C	Hyperthermia
50°C	Reduction in enzyme activity, Cell immobility
60°C	Denaturation of proteins and collagen, Coagulation
80°C	Permeabilization of membranes
100°C	Vaporization, Thermal decomposition (ablation)
> 150°C	Carbonization
> 300°C	Melting

The physiological phenomena induced by temperature (11)

The same volume shows a curve that links the duration of the thermal stimulus with the reversibility of the process induced by this stimulus.



Critical temperatures for the occurrence of cell necrosis in relation to the duration of exposure (11).

This data on the physiological effects of temperature was the first constraint for the definition of treatment parameters.
In addition to studies related to the influence of temperature, the decision of treatment time is based on a finite element model (11) which correlates optical power, treatment time and final temperature induced by the treatment with the device in question. The initial model led to establish the conditions of optimal work, that were then tested in an animal model study.
The animal study, as reported in the article, allowed us to verify the effectiveness of the treatment sized this way relatively to the standard on an abrasion-induced wound with mechanical abrader on the back of the animal. The observations that were made showed the absence of thermal damage in the areas adjacent to the wound and confirmed that only one treatment is sufficient to trigger and accelerate the process of wound healing.
The mechanisms described above are in line with what is directly observed in a preclinical study on animal model, which is part of the Lightpatch project in the context of the BiophotonicPlus call 2012, where a fiber-optic version of the EmoLED device was used to treat injuries caused by abrasions on the back of CD1 albino male mice.
Previously, two clinical studies on acute lesions (skin sampling areas) were carried out, along with two studies on chronic injuries, one of them currently in progress.
The first study performed with EmoLED v.0 devices (completely analogous to the v.1 devices regarding the type of energy and power emitted) was performed on 20 patients afferent at the Great Burns Centre AOUP - Santa Chiara Hospital in Pisa. The collection areas were divided into two not bordering sub-areas of the same size (10x10 cm), one of which treated only with standard therapy – or application of dressing and bandage - while the second was treated with EmoLED for 30 seconds immediately after skin exportation.
The results showed, in the 15 days of observation, a tendency to heal faster in the treated area compared to the control area.
The second study was carried out on 20 patients belonging to the Santa Maria Annunziata Hospital in Ponte a Niccheri - regional reference center for melanoma - in Florence. Again, patients needed

an autologous skin transplant, but they were not hospitalized, so they went to the clinic for a visit every 3 days for the 15 days of observation.
The analysis show a trend towards faster healing in the areas treated with EmoLED compared to the untreated ones: considering the healing time and the post-operative course it seems significantly faster for the areas treated with EmoLED compared to those treated only with standard dressings.
The third multicenter study was performed on chronic leg ulcers with a wound presence of at least 8 weeks. The study involved the enrollment of 90 patients who have two wounds, one of which was used as a control wound, or had only one particularly large wound, which is only half treated with EmoLED. Enrolled patients were followed up on an outpatient basis for 10 weeks. The results significantly demonstrate that wounds or parts of wounds treated with EmoLED had a higher mean re-epithelialization at week 10 than wounds treated with standard dressings only. The 10 week healing trend is also significantly higher in wounds treated with EmoLED. The pain trend measured with the VASS scale undergoes a significant reduction already starting from the fourth week of treatment. The study shows in particular the efficacy of the device under study in venous type ulcers.
A further multicenter clinical study is currently underway with the EmoLED device which plans to observe patients with venous or mixed wounds over a period of 16 weeks. This trial is aimed not only at demonstrating therapeutic efficacy and safety, but also improving the quality of life of patients and the cost / benefit ratio for society and for health.
In this study are compared the control group -of patients who go to the clinic twice a week- and the treated group -of patients treated with EmoLED who visit once a week receiving blue light application in addition to standard treatment-. In this case, no data are yet available as enrollment is at 30% and only a small number of patients completed the study. However, no expected or unexpected side effects or device-related adverse events were found.
In conclusion, since no adverse event or side effect have been recorded and since the wounds treated with EmoLED healed faster and better than the control wounds, the risk-benefit ratio is assessed clearly shifted towards the benefit.

Study Objectives

The present clinical study aims to verify the effectiveness and safety of the blue light photobiomodulation therapy with EmoLED medical device in supporting the reparative process of the area of the second and third stage decubitus lesion, comparing this method with the effectiveness of the therapy commonly used in the clinical field.

Ethical considerations

This Study will be conducted with reference to the standards of Good Clinical Practice and in accordance with the principles of the Declaration of Helsinki, with the UNI_ISO_14155_2020 norm, and with all relevant local laws and regulations, applicable to clinical trials that use patients as research object. This clinical investigation cannot begin in the involved center before having received the favorable written approval of the "Area Vasta Toscana Centro" Ethics Committee. The medical assistance provided to the subject is responsibility of adequately qualified doctors, as the center involved in this study is specialized in the treatment of the pathology object of the clinical trial.
During this trial, the patients' right to physical and mental integrity, the right to privacy and the protection of data concerning them will be respected in accordance with Directive 95/46 / EC.
This clinical investigation was designed to cause as little pain, discomfort, fear and other foreseeable risks for the subject as possible, and the degree of discomfort related to the trial itself or to the treatment with the medical device in question is almost nil, considering the Clinical Evaluation Report of the device and the safety data obtained from post-marketing surveillance.
The degree of discomfort / pain of the enrolled patients is however subject to continuous verification during the clinical trial, through a special questionnaire on the detection of discomfort connected to participating in the study in question. The risk / benefit ratio will be constantly checked throughout the trial, taking into account any adverse events / device-related accidents or non-serious side effects reported by the main investigators, and the preliminary assessment of the benefits about patients who have completed the study process.
The Principal Investigator (PI) of the center is responsible for an adequate management of the study and for the coordination of the personnel involved, also ensuring that adequate medical assistance is provided to the subject for the whole duration of the trial and in case of any adverse event. The investigator is responsible to conduct the study in accordance with the protocol agreed with the sponsor and the authorities.

Informed consent procedures

The Principal Investigator (or their delegate), on the basis of the inclusion and exclusion criteria of the study, identifies the eligible patients. The Principal Investigator (or their delegate) then informs the patient (or their family member / caregiver / guardian) of the study in progress explaining in a clear and simple way the fundamental characteristics of the device, the expected effect, any risks associated with the trial, and briefly explains the characteristics of the device, the expected effect, the possible trial-related risks, the guarantees to protect the confidentiality of the collected information , and asks if the patient is interested in participating, leaving them at least 24 hours to decide. During this interview, the investigator verifies the understanding by the patient or their family member / caregiver / guardian of the information provided. If the patient manifests their

interest to partecipate in the study, they, or if the subject is unable to provide informed consent, their family member / caregiver / guardian, signs the Informed Consent and decides whether to inform their attending physician of their participation in the study. A copy of the Informed Consent, subscribed by the patient or their family member / caregiver / guardian and by the Principal Investigator and a copy of the study information will be given to the patients.

The informed consent shows the title of the investigation, the code and date of the protocol, the sponsor and the Principal Investigator; the objectives and characteristics of the study, the investigations to which the patient will be subjected and the consequences of their participation are described below; the benefits and risks deriving from the trial, the insurance coverage, the possible alternatives, the contact details of the Principal Investigator, the procedures envisaged at the end of the study, the possibility of informing the family doctor are then described. Finally, information on the processing of personal data is set out.

Any news about the trial and/or the medical device under investigation and any eventual amendment to the protocol of clinical investigation will be notified to the patients or their family member / caregiver / guardian as soon as the Principal Investigator receives any of them.

If the subject is unable to write, consent can be provided and recorded using suitable alternative means, if there is at least one impartial witness. In this case the witness affixes their signature and the date on the informed consent. The subject, or, if the subject is unable to provide informed consent, their family member / caregiver / guardian, receives a copy of the documentation or registration.

Pre-clinical tests and previous clinical experience

Preclinical animal model study observations

Preclinical observations aimed at characterize safety and efficacy of the EmoLED medical device have been preventively performed on murine models. On each anesthetized animal, two wounds (1 cm diameter) were inflicted using an abrasive method. The 30-seconds treatment with EmoLED device has been performed on one of the two wounds, then both were/have been medicated to avoid the development of infections. After the treatment, animals have been placed in individual, thermostat cages, until they fully came out from anesthesia.
The observations reported below were extrapolated from tests performed on biopsies taken at specific time points: after the treatment, each animal was observed over a given follow-up period before being sacrificed, and the areas under study were collected and embedded in a compound suitable for cryosectioning (frozen section). They were later used both for histological and immunohistochemical analyses. A large number of observations were collected and they are reported below by making reference to the observed phenomenon.
Inflammatory infiltrate: based on the data obtained 0, 1, 3, 6, 9, 12, 18 and 24 hours after inflicting the wound, it is noted that within the first few hours the amount of inflammatory infiltrate in the areas treated with the EmoLED device is higher compared to the untreated wound. The difference decreases between 9 and 18 hours until, after 24 hours, the situation is reversed and the amount of

inflammatory infiltrate in the untreated wound is higher than that in the treated wound.
Mast cells and Mast Cell Degranulation Index: although no particular differences in the number of mast cells were found between the treated and untreated samples, this was not the case when mast cell activation was studied: in fact, potent degranulation was observed in the treated sample at 3 hours, which was not seen in the wound not treated with the EmoLED device.
Macrophage populations: observations made 0, 3, 6, 9, 18 and 24 hours after inflicting the wound on the populations of M1 macrophages (proinflammatory) and M2 macrophages (pro-healing) show that the anti-inflammatory phase begins 6 hours after inflicting the wound in animals treated with the EmoLED device, whereas it is necessary to wait 18 hours for the inflammatory phase to end with the standard treatment. Moreover, after 18 and 24 hours, both the M1 and M2 populations are comparable in the treated and untreated samples, demonstrating that treatment with the EmoLED device does not induce responses other than normal physiological reactions.
Based on these studies, it can be concluded that the EmoLED device exerts its effect within the first few hours after wound infliction (and therefore within the first few hours after treatment). Our hypothesis is that treatment with the device exerts its effect early in the inflammatory phase of healing and promotes healing both in terms of time (observable based on the data obtained from the inflammatory infiltrate study) and quality, evident from the significant mast cell activation, resulting in greater histamine release, causing increased activation of various mediators and cell types that may be involved in the healing process.

In-human acute wound study observations and ulcer case study

The safety and efficacy clinical validation to obtain the CE marking for the EmoLED device for the treatment of acute surgical wounds is ongoing. In particular, the acute observations, whose detailed results are provided in the attached final Report on the clinical trial, have once again demonstrated the device's safety and efficacy in shortening the healing time of spontaneously healing wounds**.** Isolated observations in patients with chronic lesions, of various aetiologies, have shown that the device is capable of unblocking the healing process, thus allowing the wound to progress beyond the inflammatory phase by inducing re-epithelialisation and wound closure. Observations made using a thermal camera have also shown that, due to the compromised condition of the tissues surrounding the ulcers, the baseline temperature of the limb is lower than normal and that both during and after treatment with the EmoLED device, the temperature never exceeds 40°C.

Information related to the clinical investigation

Study description

The commercial purpose of this study is to value the clinical efficacy of a battery-powered device that uses blue LEDs.
The study aims to compare the response of an existing standard treatment for second and third stage pressure sores, with a protocol that plans the administration of the EmoLED treatment three times a week for 4 consecutive weeks in addition to the conventional therapy.

Primary and secondary objectives

The primary objective of the study will be to assess the clinical efficacy of a treatment made through a battery-powered portable medical device that uses blue LEDs, in addition to the conventional therapy, applied on second and third degree pressure sores, compared to the conventional treatment alone, at the end of the fourth week of observation. As secondary objectives, the study proposes the clinical evaluation of lesions during the 4 weeks of treatment, and the evaluation of the treatment's safety.

Endpoints

Primary endpoint of the study

The primary endpoint of the study is the assessment of the clinical state of second and third stage ulcers by reducing the index of the PUSH scale, in the two treatment groups at the end of the 4-week observation period.

Secondary endpoints

1. The assessment, during the weeks of observation, of the percentage of reduction of the lesion area compared to V0 in the second and third stage lesions in the two treatment groups;
2. Safety of treatment (number of related adverse events in the two groups).

Measurable parameters

After enrolment and signature of the informed consent, the salient data, in the appropriate case report form, and the photographic images of the 2° and 3° stage injuries of each patient will be collected.

Three times a week (in conjunction with the dressing change) data, on patient forms, will be recorded, while once a week and at the last visit, if healing has not occurred before, the photographic images of each lesion of 2° and 3° stage will be captured.

All these variables will be measured during the 4-week observation period. The date of recovery, if it occurs before the end of the observation period, will be recorded in the patient's case report form and the photographic image of the healed area will be acquired.

WEEK	W1			W2			W3			W4		
VISIT	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
CRF	P	P	P	P	P	P	P	P	P	P	P	P
PICTURE	P			P			P			P		P

Recording of clinical data

Data related to the evaluation of the endpoints are acquired during the visit for both study groups and will be inserted in pseudonymized form within a computerized database, where each subject will be associated with an alphanumeric identification code. The key of patient association code will be kept by the Principal Investigator for the duration of the study, it will not be communicated to the Sponsor and to the scientific coordinator and it will be eliminated at the end of the study, making the data completely anonymous.

The parameters of the Case Report Form and the photographic images of the lesions are both acquired and stored in digital format, during the length of the clinical trial and afterwards, for 10 years after the conclusion of the study.

Once the storage period indicated above has expired, the data will be anonymized so that it is no longer possible to trace, directly or indirectly, the identity of the interested subject. Anonymized data may be reused for further research and may therefore be stored indefinitely.

Each photograph of the lesions will be identified only with the patient code and the date of the visit.
The analysis of the photographic images will be performed through an appropriate analysis algorithm that measures in mm^2 the surface of the lesion.
If the patient has two or more decubitus lesions at the same stage, the doctor will choose to treat the lesion of which, in his opinion, the data acquisition (photography and size measurement) is better. If a patient has two or more lesions of different stages, the doctor will decide to include in the study only one of them, based on the number of lesions inserted at that time in the two groups, choosing the group with a smaller number: In this way, attempts are made to ensure a numerical trend as simultaneous as possible in the two groups of lesions (e.g. if the number of 2°stage lesions enlisted is less than the number of 3°stage lesions enlisted, then the 2°stage lesion will be included in the study). In the case of a numerical parity of the two groups of lesions, the doctor will decide to treat the lesion with a position that allows a better data acquisition (photographic image, measurement, etc.).

<u>Case Report Forms</u>

Case Report Forms are unique to every patient. It will be used the form of injury assessment already present at the Investigator Centre; the data collection by the Principal Investigator includes: patient's name and surname, date of birth, room number, medical record number, date of surgery, any allergies and the characteristics of the lesion under investigation -position and degree of the lesion-. Another section dedicated to the characteristics of the lesion, includes: dimensions (length x width), the amount of exudate and the type of tissue. All these features will be evaluated, according to the criteria of the international scale PUSH, with a score, specified in the legend in the form, of which the total will be reported at each visit.
The Pressure Ulcer Scale for Healing (PUSH) is a tool designed by the National Pressure Ulcer

Advisory Panel (NPUAP) and used to observe the trend of pressure ulcers over time, classifying them regarding the area of extension, the exudate and the tissue type, noting the partial score for each characteristic of the ulcer and summing those scores to obtain the total. Comparing the relevant total scores over time will provide an indication of the improvement or worsening of the healing of the pressure ulcer.

In addition, the data collected with the CRF, and other data from the medical records of patients enrolled, will be grouped and reported, in pseudonymized form, in a computerized database on three forms, generated with Forms of Microsoft, which will be filled by the health care personnel of the study team of the Experimental Center and which will be accessible to EMOLED staff of the Clinical Affairs Area. Specifically: in the Form "Patient Card", to be filled in during the enlistment phase, general clinical information is collected (alphanumeric ID associated with the enlisted patient, anamnesis, check of inclusion/exclusion criteria, Braden scale, etc.); in the Form "Patient Card Integration", also to be completed during the enlistment phase, are reported comorbidity, drug therapies and any allergies; while in the last Form "Patient Visit" the data relating to each of the three weekly visits provided for by the protocol are entered, namely those relating to the parameters of the PUSH scale (size of the lesion, exudate and type of tissue), the dressings and any notes.

The Braden scale, present in the Form "Patient Card", is used to assess the risk of the onset of pressure ulcers and, in particular for patients in prolonged stay or who are not able to move in total autonomy, is an assessment to be repeated at least once a week. The Braden scale takes into account 6 factors: sensory perception (ability to respond to discomfort dictated by compression), moisture of the skin (sweating, moisture linked to possible urinary and/ or fecal incontinence), motor activity (level of physical activity), mobility (ability to control/change the position of the body), nutrition (an adequate intake of calories, proteins, vitamins and minerals is essential to prevent the onset of new lesions) and, finally, friction and slipping (the friction force created with the solid surface and/or the linen can accelerate the onset of PU). Variables are ascribed to each factor and it is interpreted according to the principle that the lower the value resulting from the sum of all scores, the higher the risk of injury.

In the mentioned forms, the anonymity of patients will be guaranteed through the association of the patient's sensitive data with a progressive numerical ID. The register of this association will be accessible only to the medical staff of the structure that is part of the study team.

Photographic images acquisition

During the 4 weeks of observation, once a week, concomitantly with the visit, and during the last visit (V12), a photographic image of the wound will be collected in order to be able to assess both qualitatively and quantitatively the progress of healing. The images will be collected with a portable device suitable for taking photographs (eg. tablet).

The acquisition of the photographic image will take place after any cleansing / debridement of the wound, before treatment with EmoLED / bandage.

Each photograph will be named with the patient code and the date of the visit. The photo shall cover

the entire area of the lesion. Next to the lesion will be placed a special ruler for the measurement of the area of the lesion.

Guidelines for photos' standardization

In order to have accurate measurements it is necessary that the image registration procedure is standardized.

The wound must be well focused and framed frontally; in all the photos the ruler with the patient's identification code must appear and the date and must be in focus so as to have the 1 mm scale reference. The operator should try to take the picture by fully framing the wound (preferably considering a margin around the wound), and position themselves so that the ruler is always present in the frame.

It is also advisable to avoid artifacts from the movement of the subject or operator, as they cause blurring and therefore make it difficult to correctly view the ruler or the wound.

Bias

The staff participating in the study will be trained on the correct use of the device and accessories, as well as on the correct execution of the clinical protocol and on the compilation of the associated forms.

The EMOLED staff is always at the team's disposal for any clarification or doubt during the study.

In order to avoid bias during the procedure for the assignment of the patients enrolled to the experimentation group, it should be noted that the randomization grid is not distributed to the investigators: instead, opaque and sealed envelopes are distributed that inside have an adhesive label with the patient's ID and their attribution group that reflects the randomization grid. This adhesive label must be glued on the patient folder containing the informed consent and the Case Report Form.

Since this is an experiment with a medical device that emits blue light, in this case the masking of the assignment to one of the two groups cannot be carried out.

Being diabetes a condition that affects the timing of wound healing, and being widely distributed in the population under study, enlisted patients will also be randomized for this factor, so that the two treatment groups can be homogeneous.

The randomization grid is generated with the on-line software available on the website "randomization.com".

The 2° stage and 3° stage lesions are randomized separately, as well as diabetic and non-diabetic patients.

It is a 1:1 randomization, stratified, with 4 blocks of 10 patients distributed in the two groups (treated and control).

Patient selection

All patients under treatment who meet all the inclusion criteria listed below will be considered for

inclusion in this study. Patients will be evaluated using a standard procedure that includes the collection of the medical history and a physical examination of the patient.

Inclusion Criteria

- Patients with 2° or 3° stage lower limb/ sacrum located pressure ulcers;
- Patients with 2° or 3° stage pressure ulcers with a ≥ 2cm² lesion area;
- Patients with a hospitalization waiting time < 30 day;
- Men and women aged ≥ 50 years;
- Patients with a Braden scale score ≥ 11;

Exclusion criteria

- Patients who are participating in other clinical trials with drug or medical device;
- Patients with third stage pressure lesions, on the lower limbs or on the sacrum, with undermining wounds, tunneling or eschar;
- Patients with systemic or superficial infection at the time of recruitment, that need systemic antibiotic therapy;
- Patients with a history of self-harm who can voluntarily alter the course of healing;
- Patients under intravenous therapy with doses of corticosteroids above 40mg/day;
- Patients under immunosuppressant or cytostatic drugs therapy;
- Women who are pregnant or breastfeeding[1];
- Patients with neoplasia;
- Patients with pathologies that induce skin photosensitivity;
- Patients with a life expectancy of less than 30 days.

In order to be eligible for the planned treatment phase, all the inclusion and exclusion criteria must be met. Any concomitant pharmacological therapy must be maintained.

Number of patients expected to enroll

A total of 40 "evaluable" patients will be recruited in total, 20 patients with second stage PU and 20 patients with third stage PU, 10 per arm (Treated and Controlled) within each group, at the facility involved in this Clinical Study. By "evaluable" patient it is meant a patient enrolled who has respected the temporal sequence of treatments as indicated in this protocol. The expected time for enrollment is 10 months. However, the study will continue until the number of patients required by this protocol is reached.

Enrollment point

Once the patient has signed the Informed Consent, the Principal Investigator enters the data in the Enrollment Register, scrupulously checking all the inclusion and exclusion criteria; if all the criteria

are met, the patient is considered enrolled in the clinical trial and subsequently randomized.

Number of experimental medical devices expected to be used
Each experimental site will be equipped with a v.1 EmoLED medical device.

Time-frame of the study
The Study has an indicative duration of 12 months with an expected enrollment period of 10 months. The study, however, will continue until the number of patients required by this protocol is reached.

[1] The state of pregnancy or breastfeeding will be certified on the basis of the patient's declaration.

Medical and surgical procedures and study follow-up

The Control Group will follow the standard treatment indicated three times a week for the 4 weeks of observation. The experimental group will undergo, in addition to the standard treatment, treatment with Emoled three times a week for 4 consecutive weeks.
This treatment consists of irradiation with the blue light emitted by the device for one minute on the injured area. If the lesion has a greater extension than the irradiated area, multiple repeated applications will be performed, on adjacent areas, until the entire area is covered.
The treatment with EmoLED will be carried out in correspondence with the dressing change of the lesion.
The standard treatment for 2° and 3° stage PU consists of: cleansing with saline or ringer's lactate, hyaluronic acid gauze plus polyurethane foam every 48 hours or as needed. In addition, zinc cream or hyaluronic acid sodium salt + metallic silver is applied to prevent and/or treat the skin maceration of the surrounding area, and eventual debridement of the lesion, application of topical treatment indicated for that stage of the lesion (as provided by the "Protocol on pressure ulcers' dressing" intended as the "standard of care" of the structure) and subsequent bandage (that generally consists of a polyurethane film -thin hydrocolloid plate or a pressure discharge system made with hydrocolloids and polyurethane foam, known as "pressure relief system"), are planned.

During the 4weeks of treatment with the EmoLED device or until healing, treatments with other types of phototherapy (including photodynamic therapies), topical negative pressure therapy or additional skin grafts are not allowed.
If, during the 4 weeks of observation, clinical signs of infection occur, a Silver dressing will be preferably applied. In any case, each participating site is left free to choose the most suitable dressing among those available to the structure, as long as the same dressing is adopted both in the Control Group and in the Treated Group.
If at the conclusion of the patients' participation in the clinical investigation, any additional treatments to those normally provided for the clinical condition considered in this study, caused by the participation itself in this trial, are necessary, the Sponsor will bear the costs incurred for such treatments.

Known or predictable factors that may compromise the results and their interpretation

An incorrect recording of photographic images does not make it possible to determine the area of the lesion via software. This problem is tackled by providing precise instructions on the correct use of the tools available for the retrieval of images.
Poor management of bedridden patient placement may affect the reliability of the result. This problem is contained by well explaining to the involved staff the importance of the adherence to the protocols of the structure regarding the positioning of patients, and including in the trial only suitably trained and experienced personnel in the management of the bedridden patient.

Subjects discontinuation and withdrawal from the clinical investigation

Patients who will not receive treatment with EmoLED and/or the provided standard dressings for more than twice within the 4-weeks observation period will be excluded from the study. Patients who have not followed any eventual therapy or medical indications prescribed by the personnel involved in the trial will also be excluded.

However, the patient has the right to withdraw from the study at any time without giving any explanation to the investigator. After the patient's discharge or abandonment of the study, the case report form is identified, scanned and archived. If a patient withdraws from the study, the Principal Investigator or their delegate records the event in the identification register and on the case report form and archives everything.

Clinical investigation monitoring plan

In order to verify that the rights, safety and well-being of enlisted patients are protected, that the reported pseudonymized data are reliable and strong and that the clinical investigation is conducted in compliance with the requirements of the current legislation, the sponsor ensures adequate monitoring of the conduct of this clinical investigation. The monitoring of the clinical investigation is entrusted to the adequately trained sponsor staff.

To guarantee the conformity with ICH/GCP guidelines, staff will be responsible for the study to be carried out in full observance of the Standard Operating Procedures, of the Protocol and other written instructions.

The main responsibilities of the staff are to ensure adherence to the Protocol, to make sure that the data are accurately and fully registered and reported and to verify the presence of the Informed Consent and that is was obtained and registered for each subject before their participation in the study.

Researchers will be contacted on a regular basis throughout the whole duration of the study to check and verify the various documents (case report forms and other relevant documents containing the original data) related to the study in order to verify the adherence to the protocol and to ensure the completeness, consistency and accuracy of the recorded data. The documents subject to verification will be identified with the progressive alphanumeric ID code.

Monitoring staff will conduct a site initiation visit (SIV), a minimum of 2 visits during the Study (MOV) to be scheduled approximately one after 1 or 2 months after SIV based on the number of patients enrolled, one at 2/3 enrollment and another at the conclusion of the observations on the last patient.

Finally, once all remaining issues have been resolved (open queries, clarifications, etc.) the Centre Closing Visit (COV) will be carried out. If further monitoring visits are required, they shall be programmed by the personnel with the Principal Investigator.

Financial arrangements

An appropriate financial agreement is concluded between the sponsor and the centre involved in the clinical investigation following the approval of the trial by the relevant Ethics Committee.

This contract must indicate which are the contact persons of the trial, what are their tasks and their qualifications, it establishes an indicative date of the beginning of the experimentation and the number of patients to enlist, it establishes the obligations of the promoter (device delivery, free supply of additional materials, any additional fees, etc.) and of the facility (guarantee of proper management of the study, communication with the sponsor, etc.); it also establishes the payment of 300 euros per patient, divided into the visits forseen by the protocol, which the sponsor will pay to the structure, and the manner in which this will happen.

The contract also describes the method of processing personal data, defines the ownership of scientific data and establishes the start of the convention and the method of withdrawal.

The sponsor undertakes to provide free use of the EmoLED device to the facilities, which receive it for the exclusive purpose of the study, and for as long as necessary to carry out the study.

The delivery of the device will be recorded on special forms. Facilities shall assume the responsibility for ensuring the safekeeping of equipment and related user material. Access to the device shall be granted only to personnel involved in the study. At the end of the activities requiring the use of the device or, in any case at the end of the trial, the sponsor will take charge of the pick-up of the device with the drafting of a report.

Quality assurance, control procedures, data management and record keeping

All the information gathered during the Study will be considered strictly confidential. The patients' consent to the registration of personal data will be requested at the time of recruitment; nevertheless, the case report forms will contain the Identification Code of the patient and their date of birth only.
In order to guarantee the correct traceability of all parties involved, the number of the Study and the naming of the Center will be included as well.
At the end of the Study, all data will be archived using the appropriate safety measures at the Coordinating Site for a minimum of ten years.
If a patient withdraws their consent to data processing, they will be immediately destroyed to ensure total confidentiality. In addition each investigator will keep a copy of the study's documentation for at least ten years after its conclusion.

The Principal Investigator creates and is responsible for updating and maintaining the Patient Identification Register, or Enrollment Register, and is responsible for the implementation of appropriate organizational measures to protect such information in order to prevent unauthorized access and dissemination or accidental destruction or loss (e.g. keeping under lock and key study material containing sensitive data).

The register shall contain the personal data of the patients giving their consent to participate in the study, the identification code assigned to the patient, the indication of any exit from the study, with the relative motivation and the date of recruitment.
The patient code consists of a two-digit number, starting from 01, and progressively in order of enlistment. The patient code is reported in the case report forms and in all documents that record data related to the trial, such as questionnaires, list of visits, photographic recordings and so on.
Only the Principal Investigator and, at the discretion of the Principal Investigator, the members of their team are aware of the identity of the patients enrolled in the study.
Both the center involved in the study and the sponsor qualify as independent data controllers and undertake to process personal data, of which they are aware for any reason during the clinical investigation, in accordance with the provisions of the regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, as well as the related national legislative and administrative provisions in force, and any subsequent amendments and/or additions.

For the purposes of this clinical trial, the data referred to in Article 4 No. 1 of the GDPR and data falling into the "particular" categories of personal data, in particular health data referred to in Article 9 of the GDPR, will be processed. The Principal Investigator is identified by the involved investigator site as an authorized person to be treated in accordance with Article 29 of the GDPR, as a person designated in accordance with Article 2 quaterdecies of the Code, and must acquire from the patient duly informed the consent document as well as to participate in the trial, also to the processing of data, after having informed clearly and completely, before the trial begins, each patient about the nature, purpose, results, consequences, risks and methods of processing personal data.
In the event of a personal data breach, the Prinicipal Investigator informs the involved investigator site without undue delay after becoming aware of the breach. The involved investigator site shall notify the competent supervisory authority of the infringement without undue delay and, where possible, within 72 hours of becoming aware of it. Where the notification to the supervisory authority is not made within 72 hours, it shall be accompanied by the reasons for the delay. The notification should describe the nature of the personal data breach including the approximate number of subjects concerned, the contact details of the data protection officer, the likely consequences of the personal data breach and the measures taken or to be taken to fix the personal data breach and also, where appropriate, to mitigate its possible adverse effects. If the breach of personal data presents a high risk to the rights and freedoms of natural persons, the involved investigator site shall communicate in plain and clear language the nature of the breach to the data subject without undue delay, unless the data controller has made personal data incomprehensible to anyone who is not authorized to access it, or has subsequently adopted measures to avoid the occurrence of a high risk for the rights and freedoms of the subjects.

Clinical evaluation plan deviations
In the case of minor and isolated deviations from the protocol, these are recorded in the case report form, which is provided with a space to record any changes or comments the doctor deems appropriate (e.g. treatment not carried out, change of dressing applied, lack of a detection).
In any case, no major deviations from the protocol must be initiated (e.g. change in the timing or

method of treatment with the device, modification of the observation times or of the surveys carried out, modification of the inclusion criteria), nor modifications to the same, without the Ethics Committee of reference has expressed in writing a favorable opinion on a specific amendment, except when this is necessary to eliminate the immediate risks for the subjects or when the change concerns exclusively logistical or administrative aspects of the study.
If, during the study, a change to the protocol is deemed necessary, the investigator communicates this need to the sponsor who, having summoned the investigator of the coordinating center, the scientific manager of the study and any other experts in the field, discusses the proposed changes and, if necessary, submits a request for amendment to the Ethics Committee to approve the changes.

Accidents and their reporting

Since this is a Post Market Clinical Follow-up (PMCF) clinical study, which is then carried out using a medical device already marked CE and conducted within the use intended by the manufacturer, the provisions laid down for products placed on the market concerning surveillance shall apply regardless of whether the device is used in the context of a clinical study.

Although the information in our possession and the physical characteristics and performance of the device do not suggest the possible occurrence of an accident or a serious accident and that therefore may cause death, a serious deterioration in the health of the patient/ user/ other person or a serious threat to public health, as defined and reported below, by the European Regulation on Medical Devices 2017/745 (article 2 definitions 64 /65 /66), this section of the protocol shall consider the possibility of such an event occurring.
Even in clinical practice, since 2018, in the structures where the device is in use, there have been no adverse events or side effects, and its efficacy has been confirmed in different types of ulcers (19, 20).

Definitions
In accordance with European Regulation 2017/745 of medical devices, the following definitions are applied:

- «**accident**»: any malfunction or alteration of the characteristics or performance of a device made available on the market, including the error of use caused by the ergonomic characteristics, as well as any inadequacy in the information provided by the manufacturer and any undesirable side effect;
- «**serious accident**»: any accident which, directly or indirectly, has caused, may have caused or may cause one of the following consequences: a) the death of a patient, user or other person; b) the serious deterioration, temporary or permanent, of the health conditions of the patient, the user or another person; c) a serious threat to public health;
- «**serious threat to public health**»: an event which could lead to an imminent risk of death, a serious deterioration in the health of a person or a serious illness which may require prompt corrective action and which may cause a significant rate of human morbidity or mortality or which is unusual or unexpected for that particular time and place;

- «**corrective action**»: an action aimed at eliminating the cause of a potential or current non-compliance or other undesirable situation;
- «**field safety corrective action**» means a corrective action taken by a manufacturer for technical or medical reasons in order to prevent or reduce the risk of serious accidents related to a device made available on the market;
- «**intended use**»: the use for which a device is intended, according to the indications provided by the manufacturer on the label, in the instructions for use or in the material or in the promotion or sales declarations and as specified by the manufacturer in the assessment clinic;
- «**label**» means written, printed or graphic information appearing on the device itself or on the packaging of each unit or on the packaging of several devices;
- «**instructions for use**»: information provided by the manufacturer to inform the user of the intended use and correct use of a device and any precautions to be taken.

Reporting by health professionals to the Ministry of Health and the manufacturer

Public or private health workers who in the exercise of their business detect a serious accident involving a medical device are required to notify the Ministry of Health and the Manufacturer at the same time, immediately and without any undue delay, with the terms and procedures established by the law.

Specifically, in accordance with Art. 9 of Legislative Decree 46/92, public and private health professionals must communicate data relating to serious accidents involving a device belonging to one of the classes I, IIa, IIb or III, to the Ministry of Health. The Ministry of Health classifies and evaluates the data concerning the following accidents:

a) any dysfunction or deterioration of the characteristics or performance, as well as any deficiency in the labeling or instructions for use of a device which may cause or have caused the death or serious deterioration of the state of health of the patient or of a user;

b) any technical or medical cause connected to the characteristics or performance of a device which has determined the consequences referred to in letter a) and which has led to the withdrawal from the market by the manufacturer of devices belonging to the same type, and communicates the acquired data to the manufacturer or their authorized representative established in the Community.

The communication is made directly or through the health facility where the reported incident occurs, in compliance with any regional provisions that require the presence of the contact persons for the supervision of medical devices. The communication must also be sent to the manufacturer or their authorized representative, also through the supplier of the medical device by submitting the appropriate form *"Accident report by health professionals to the Ministry of Health (Article 9 of Legislative Decree no. 46 of 1997; Article 11, Legislative Decree no. 507 of 1992; Article 11, Legislative Decree no. 332 of 2000)"*.

The pdf file generated by the procedure must be sent to Office 5 of the Directorate General for Medical Devices and Pharmaceutical Service, at the address dgfdm@postacert.sanita.it. Among the tasks assigned to the health worker there is also the one of communicating to the manufacturer or the authorized representative any other inconvenience which, although not presenting itself with the characteristics of the accident, may allow the adoption of measures to guarantee protection and health of patients and users.
In order to encourage and facilitate any notification to the manufacturer, for the devices that the manufacturer markets, in the User Manual of the medical device supplied to the user together with the device, there is a User Reporting Form, which must be completed and forwarded to the company through the channels indicated.

Reporting by the manufacturer to the Ministry of Health
The legislation also establishes the obligations regarding the supervision of accidents with medical devices for the manufacturer or their authorized representative, in particular the immediate communication to the competent authority of all the accidents of which they have become aware and of all the corrective actions on the field that have been undertaken to avert or reduce the risk of death or serious deterioration in health associated with the use of a medical device.

EMOLED Srl, as a manufacturer of Medical Devices and within the company Quality Management System, defines and implements specific procedures and practices relating to vigilance and market surveillance that deems appropriate and proportionate to the risk class of its devices on the market according to the applicable, cogent and collateral legislation and as defined by the guidelines on medical devices made available by the European Commission, available at the following link: https://ec.europa.eu/growth/sectors/medical-devices/current-directives/guidance_en.
The models to be used by the manufacturer, as also reported on the website of the Ministry of Health in the section concerning the supervision of medical devices, are those attached to the guidelines MEDDEV 2.12-1, Rev. 8, available on the website of the European Commission at page Medical devices > Guidance at the following link:
https://ec.europa.eu/growth/sectors/medical-devices/current-directives/guidance_en.
Reports must be sent to Office 5 of the Directorate General for Medical Devices and Pharmaceutical Service, at the address dgfdm@postacert.sanita.it , attaching both the relative xml file to the pdf file, as per the recommendations of the Ministry of Health.

EMOLED reports to the relevant competent authorities:

a) any serious incident relating to devices made available on the Union market, except for the expected side effects which are clearly documented in the product information and quantified in the technical documentation and which are the subject of trend reports;

b) any field safety corrective action (FSCA) relating to devices made available on the Union market, including field safety corrective actions taken in a third country in relation to a device that is legitimately made available also on the Union market if the corrective action in question is not only caused by the device made available in the third country.

Timing for reporting

The deadline for the above reports is commensurate with the seriousness of the serious accident and in accordance with the prescriptions ascribable to Article 87 of the European Regulation. This timing is to be understood starting from the moment in which a connection, even potential, is established between the accident and an EMOLED brand device.

The timing is as follows:

a) Manufacturers report any serious accident immediately after establishing the causal link, even if only reasonably possible, between the accident and their device, and no later than 15 days after becoming aware of the accident;
b) In case of a serious threat to public health, the manufacturer sends the report immediately and no later than 2 days after becoming aware of the threat;
c) In the event of death or an unexpected serious deterioration in the health of a person, the report is transmitted immediately after the manufacturer has ascertained or as soon as it assumes that there is a causal link between the device and the serious accident and in any case within 10 days after the date on which the manufacturer becomes aware of the serious accident.

To ensure timely reports, in accordance with the provisions of the applicable legislation, EMOLED may, if necessary, adopt the policy of submitting incomplete initial reports, but within the prescribed time frame, making use of the possibility of completing and possibly correcting the information initially forwarded with the following forwarding of complete final report. If, after becoming aware of an accident potentially to be reported, there still is uncertainty about the need to report the accident, the manufacturer in any case sends a report within the prescribed time frame as defined above.

If the manufacturer receives from the Ministry of Health a report made by a user, the manufacturer must evaluate the appropriateness of the report and then send an Initial Accident Report (or a Follow-up / Final Report) to the Ministry of Health, in the case of the event meets the criteria for a report; otherwise provide the Ministry of Health with the reasons why the event is not to be reported and the details relating to the use of the information (eg. insertion in the "file" of complaints), if manufacturer does not consider that the event meets the criteria for an alert.

The manufacturer will submit a follow-up report to the Ministry of Health if the investigation time reaches the limit communicated to the Ministry of Health as part of the initial report, after which a final report must be submitted which is a written statement of the result of the investigation and any action.

Reporting incidents criteria

Any event that meets all three basic reporting criteria listed below is considered an INCIDENT and must be reported to the Ministry of Health, and possibly to the Competent Authorities of the Member States where the investigation is carried out (basic reporting criteria A - C as well as defined by the MEDDEV 2.12-1 rev.8 guidelines - Ref. Par. 5.1.1). Furthermore, where the manufacturer identifies such an event that caused or could have caused indirect damage / death / serious deterioration of the state of health, he must report the accident.

The basic reporting criteria are:

A: an event happened
This also includes situations in which, following tests performed on the device or following the analysis of the information provided with the device or any other scientific information, factors emerge that could lead or have led to an event. Typical events include, but are not limited to:

a. A malfunction or deterioration in characteristics or performance.

A malfunction or deterioration must be interpreted as an inability of the device to operate in accordance with its intended use, even if the device is used according to the manufacturer's instructions.

a. Unexpected adverse reaction or unexpected side effect
b. Interactions with other substances or products
c. Device degradation / destruction (e.g. fire)
d. Inappropriate therapy
e. An inaccuracy / imprecision in labeling, instructions for use and / or promotional materials.

Inaccuracies include omissions and deficiencies. Omissions do not include the absence of information that should generally be known to intended users.

B: it is suspected that the medical device is a contributing cause of the accident
In assessing the link between the device and the accident, the manufacturer must take into account:

- the opinion, based on available evidence, of health professionals;
- the results of the preliminary accident assessment, carried out by the manufacturer himself;
- evidence of similar previously occurring incidents;
- other evidence held by the manufacturer.

This judgment can be difficult when multiple devices and/or drugs are involved. In complex situations, it must be assumed that the device may have caused or contributed to the accident.

C: the event caused, or could have caused, one of the following outcomes:

- death of the patient, of the user or of another person;
- serious deterioration in the state of health of the patient, user or other person;

Serious deterioration in health can mean:
a) a serious illness;
b) permanent impairment of a bodily function or permanent damage to a bodily structure;
c) a condition requiring medical or surgical intervention to prevent serious illness or permanent damage (examples are a clinically relevant increase in the duration of a surgical procedure and / or a condition requiring hospitalization or a significant prolongation of ongoing hospitalization);
d) fetal distress, fetal death, or any congenital anomaly or birth defects.

Not all accidents lead to death or a serious deterioration in health. The non-occurrence of such an event may have been determined by other favorable circumstances or by the intervention of medical personnel. Therefore, it is sufficient that an accident associated with a device has occurred and that the accident was such that, if it happened again, it could lead to death or a serious deterioration in the state of health.

Conditions under which a report is not required

Please note that the non-reporting of any event, for one of the reasons listed below to the relevant Competent Authority, does not exclude the obligation of communicating them by the user to the manufacturer, immediately and without any delay that cannot be justified.
The communication to EMOLED S.r.l. can be done by any means (telephone / fax / e-mail) to the channels listed in the User Manual supplied with the Device. Furthermore, in order to encourage and facilitate any report to the manufacturer, for the devices that they markets, in the User Manual supplied to the user together with the medical device, there is a Reporting Form to be filled in to the Manufacturer for any report from the users.

Here are some of the conditions that may not require reporting:

- Inadequacy of a device found by the user before use: all deficiencies of the device detected (and which could not be identified) by the user before use of the device itself, must not be reported.
- Event caused by the patient's condition: when the manufacturer becomes aware that the main cause of the event is related to the patient's condition, the event does not require reporting. These conditions may pre-exist or occur during the use of the device. To justify the non-reporting, the manufacturer must have information demonstrating that the device was functioning in accordance with the established performance and therefore could not have caused or contributed to death or serious deterioration in health. This conclusion must be shared by a person qualified to make a medical judgment. The manufacturer is recommended to involve a clinician in this decision.
- Exceeding the deadline or expiration date of the device: when the only cause of the event was the exceeding of the expiry date of the device, as indicated by the manufacturer, and the failure modes are not unusual, the accident must not be reported.
- Correct operation of the protection system against a fault: events that have not led to serious deterioration of the state of health or death must not be reported, as a design feature has prevented a fault from constituting a danger (in compliance with the appropriate standards or to the documented design inputs). EMOLED S.r.l. establishes, implements and documents a Risk Management System, at the same time as the company Quality Management System, for the devices it markets in accordance with the current, cogent and collateral legislation applicable to it. It defines and maintains updated a risk analysis of the EMOLED Device v.1 (Code 9800010001), including its SanaLight Software in version 2.1 as defined in the reference

risk analysis document for that product / product family in its latest review (9800010001_RAN - Risk Analysis) provided by the manufacturer together with the rest of the documentation given to the investigator.

As concerns the risks associated with the use, according to what has emerged and reported in the analysis performed, the only risk that is identified for the device is inherent to its operation and does not vary either with the context or with the variation of the user. This consists in the direct exposure of the eyes to the light emitted by the device.

The documentation accompanying the device takes this danger into account, and clearly defines it as misuse. All the implemented means of protection are not able to eliminate it completely, but the introduction of a positioning control system for the device, as explained in the User Manual, greatly limits the risk of involuntary eye exposure. Overall and on the basis of the analysis, evaluations and controls of the risks set out in the risk analysis document of the device, and of the analysis of the individual residual risks, the sum of the overall residual risk is considered ACCEPTABLE, considering the benefits brought by the testing and training method.

- Expected and foreseeable side effects: scientific literature reports that the use of blue light can lead to temporary and transient hyper-pigmentation in the peri-woundal skin. In the cases described, this hyper-pigmentation disappears in a few tens of seconds. No direct observations from EMOLED have been found to date.
- Usage errors and abnormal uses: It is defined as "usage error", an action or non-action, which has a different result from that expected by the manufacturer or by the user of the medical device. It is defined as "abnormal use", an action or non-action by the user of a medical device, as a result of a behavior that goes beyond any possibility of risk control by the manufacturer.

Usage errors relating to medical devices that led to death or serious deterioration of the state of health or to a serious danger to public health must be reported by the manufacturer to the Ministry of Health when the manufacturer notices a significant change in the trend (typically an increase in frequency) or a significant change in the way a problem presents itself that could potentially lead to death or serious deterioration in health or represent a danger to public health, or the manufacturer takes corrective action to prevent a death, a serious deterioration in health or a serious danger to public health. Abnormal use does not necessarily have to be reported by the manufacturer to the Ministry of Health according to the reporting procedures. Abnormal use should be managed by the health facility. If a manufacturer becomes aware of abnormal use cases, he can bring them to the attention of other appropriate organizations and health facility personnel.

Safety follow-up of subjects withdrawn from the Study or who have completed the same

Each morbid condition derived from each patient's accident will be monitored throughout the whole study period. The subjects that withdrew from the experimentation after the treatment with the device under study for any reason, will undergo a continued monitoring of the effects of the accident until the results from the tests related to the adverse event, and required by the SOC, will recover to baseline or until the investigator will determine that those events are not clinically relevant anymore.

All the subject who still present ongoing effects of an accident upon completion of the study will be monitored until the test results are not back to baseline, or is no longer expected from them to

change, or until the investigator does not determine that those results are no longer clinically relevant.

Amendments to the clinical evaluation plan

If, during the study, a modification to the protocol is deemed necessary, the investigator communicates this need to the sponsor, who convenes the investigator, the study director and any other experts in the field, and discusses the proposed variations and, where appropriate, submits a request for amendment to the Ethics Committees to approve the changes deemed necessary.
If a substantive amendment request is necessary, the clinical trial will be suspended until approval has been received by the relevant ethics committees.

Early termination and suspension of clinical evaluation

In the event of suspension or early termination of the study by the promoter, taken as a safety measure due to events related to the conduct of the study that may affect the health of the patients recruited, the promoter is required to notify the Ministry of Health and the Ethics Committees involved within 90 days from the recruitment of the last patient or the early closure. In case of suspension or early termination of the study not for safety reasons, the sponsor shall justify and explain their decision. The suspended clinical trial cannot be reactivated unless a substantive amendment and the positive opinion of the Ministry of Health and the Ethics Committees have been submitted.

Statistics

Calculation of the sample number
Lack of reliable data in the scientific literature on average recovery times of decubitus ulcers (PUs), the absence of clinical experience combining blue light treatments with the therapy of this type of lesion, and the lack of reliability about any inference based on clinical studies that associate this type of treatment with other types of skin lesions, due to the different aetiologies and types of patients, do not make possible to perform a reliable calculation of the sample number. The predefined sample size was therefore set on the basis of considerations concerning the recruitment capacity of the centre involved in the project. Based on an estimate of the historical clinical activity data at the Cardiorespiratory Rehabilitation Units, at the Neuro-Motor Rehabilitation Unit and at the Acquired Severe Brain Injury Unit (UGCA), where patients will be enrolled, the percentage of patients with decubitus ulcers at the entrance is about 20%, an information that implies that compared to an average of about 426 patients/ year, in 10 months of enrollment it is possible to reach a number of 40 patients compared to a percentage of drop-out/ waste equal to about 25%. A total of 40 patients will be recruited, 20 patients with second stage PUs and 20 patients with third stage PUs. Thus, this study is intended as a "proof of concept", the results of which may be used as preliminary information to define the sample size in later studies.

Management of missing data
Since it is a "proof of concept" study based on a limited number of patients, we will include in the study also patients who do not conclude the study according to the principle "intention to treat" and the resulting missing data will be processed through the imputation technique of the missing data *last observation carried forward - LOCF*. If the patient does not leave the study but skips one or more visits, the averages of the two adjacent visits or the last available data (LOCF) will be taken into account.

Statistical analysis
Demographic and clinical data will be presented in tables and graphs in the form of frequencies and percentages for categorical variables, mean, standard deviation, median and interquartile range for continuous variables, differentiating by reference population and treatment group.
At a first analysis a qualitative comparison will be carried out between the treatment groups (improvement or not improvement), both general and differentiated for lesion of 2° and 3° stage, through the $\chi 2$ test or Fisher's exact test for counts of less than 5. The results will be presented in contingency tables and bar charts.
A quantitative comparative statistic between the treatment groups will then be performed by comparing the outcome variables (percentage reduction of the lesion area and reduction of the PUSH index) through the non parametric test of Mann-Whitney, one of the most powerful nonparametric tests used to verify whether two independent groups belong to the same population. The Mann-Whitney test is usually applied when the assumptions to perform the parametric test are not met, which means when the distribution of the variable under study is not normal and/or the sample sizes are reduced (n 30 cases in at least one sample).

In this case, the choice to perform a non parametric analysis lies in the low sample size and therefore it is preferred to have more conservative meaningfulness values. The results will be presented in tables, with median and interquartile interval in the two groups with the relative p. value, and graphically, through box plot.
To analyse the internal and between groups time-related differences, Friedman's non-parametric test for the outcome variables will be performed.
Generalized linear models (GLM) will be used to investigate whether differences between the two groups are influenced in a confounding way by demographic and clinical covariates, pre-treatment measurement, and to measure their impact that will allow us to understand which variables affect the outcome and will also allow us to analyse the differences between groups being equal for these covariates.
A descriptive statistic of adverse events, if present, will also be made through tables with frequencies and percentages, and bar charts.
All significance tests will have a statistically significant p. value <0.05. The analysis will be carried out with the open source software R Commander (v. 2.2-0).

croData publication policy

At the end of the clinical study and of the related analysis about the obtained results, these will be made available anonymously and aggregated in full respect of the protection of the right to privacy, in accordance with Regulation no. 2016/679 (GDPR, General Data Protection Regulation), for publication in national or international journals, or for presentation by investigators at scientific conferences and in medical journals. For the writing of any publications concerning the study in question, may be used professional medical writers who will write manuscripts on behalf of the company and for which the company undertakes to ensure analogous compliance with current legislation.

After the conclusion of the study, the sponsor will be responsible for drawing up the final report of the clinical trial in the manner and timing prescribed in the current legislation regarding clinical investigations; external consultants of the sponsor, experts in this field, may be involved in the preparation of the final report and for which the sponsor ensure the same compliance with current legislation.

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