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PROTOCOL

CLINICAL INVESTIGATION OF MEDICAL DEVICE

Title of the Study:	Single-center prospective randomized controlled study on the efficacy of Blue Light in the therapy of neuro-ischemic foot lesions in patients with type I or II diabetes in a combined Hospital – Territory treatment regimen (H.E.R.M.E.S. – blue light photobiomodulation therapy on neuroischemic patients)
Reference number or identification code of the clinical investigation:	*EmoLED_DF_001*
Protocol version number:	*2.0*
Issue date:	*24/02/2021*
Sponsor:	*EMOLED Srl* *Via de' Bardi, 36 – 50125 Firenze (FI)*
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APPROVAL OF THE PROTOCOL

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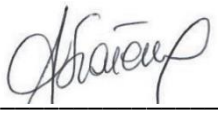
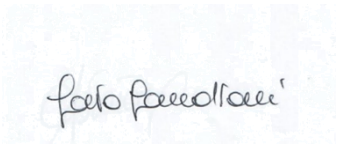
The Investigators involved:

- approve this protocol
- declare that the study will be conducted according to GCP, UNI EN ISO 14155:2012 and in accordance to this protocol.

Prof. Alberto Piaggesi  Data: 26/11/2020

Dott.essa Alessia Scatena  Data: 27/11/2020

Dott.essa Sara Sandroni  Data: 02/12/2020

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Information about the medical device/s

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 shield 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 years 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 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 micro-USB 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/cm^2) 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

Proper wound management and dressing after surgery, trauma or disease is an important part of the healing process, not only to prevent infections or other complications, but also to accelerate wound healing with as little scarring as possible.

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 (2, 3). 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 (4). 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.

Evaluating the scenario of the patient suffering from diabetic pathology and focusing only on one of its complications, our country has a unique organization for the management of the Diabetic Foot, based on 176 dedicated Units divided into 3 levels of care:

• 1° Level: territory general practitioner: screening and treatment of uncomplicated injury;

• 2° level: diabetic foot care center: prevention, diagnosis and treatment of non-ischemic ulcers (complicated lesion);

• 3° level: operating unit specifically dedicated to diabetic foot treatment (critical injury and/or recurrence).

Despite a dedicated organization, in 55-66% of cases the duration of diabetic foot ulcers (DFU) is not known, or the diagnosis is delayed by more than 3 weeks from the appearance of the ulcer. The delayed sending of acute DFUs carries a high risk of amputation (about 50% of total amputations affect diabetic patients). Guidelines and protocols are often not recognized and followed: 30-40% of general practitioners consider the management of DFUs unclear for the absence of Standards of Care and specific paths.

The availability of podological clinics in the Anti-diabetes Centers in Italy is still not homogenous and most of the Centers does not present the professional figure of the podiatrist within the multidisciplinary team.

This clinical trial will be a prospective, single-center, randomized controlled parallel group, superiority study with the commercial objective of evaluating the clinical efficacy of a battery-powered portable device that uses blue LEDs. The study aims, over the planned observation weeks, to clinically compare two groups of patients with neuro-ischemic foot lesions in patients with type I or II diabetes. The aim is to determine any differences in outcome between the two groups that are considered and whether the therapy of the treatment group is a valid alternative to current therapy in terms of percentage of patients healed, speed of healing, quality of life and economic impact.

Group 1, the control group, will follow the standard treatment provided by the facility protocol with wound cleaning and adequate dressing. Group 2, the treated group, will follow the standard treatment provided by the hospital protocol with wound cleaning and adequate dressing, adding the treatment with the EmoLED device, which is set between the cleansing and the application of the dressing. This treatment consists in irradiating with the blue light emitted by the device, for two minutes, each circular area with a diameter of 5 cm of the lesion, twice a week for twenty weeks.

The measurement of the difference in efficacy of the device between the two groups will be evaluated in terms of percentage of patients healed, intended as a 100% re-epithelialization of the lesion. Therefore, this clinical trial aims to investigate whether the group of patients undergoing standard therapy with, in addition, the EmoLED treatment reaches a percentage of healed patients and / or a healing speed higher than the control group. If confirmed, this result would imply significant gains in terms of reduction of outpatient visits / home visits and therefore also in terms of reduction of public health costs, thus also increasing the quality of life of patients suffering from neuro-ischemic diabetic foot ulcer.

The population subject of this trial is widely representative of the target population and the center involved is a structure of excellence in the treatment of diabetic foot lesions under study.

The EmoLED device, CE marked (certificate n. G1 18 02 99242 002), has been used in its prototype version in three different clinical studies. The first two studies analyzed the efficacy of EmoLED on (acute) surgical lesions, the third, on the basis of some spontaneous observations from professionals in the sector, evaluated the efficacy of continuous therapy on chronic lesions.

A fourth study, currently under way, aims to assess, in addition to clinical efficacy and safety, also the impact of treatment with Emoled device on the quality of life of patients and socio-economic aspects related to chronic leg ulcers.

The clinical safety of the device was therefore evaluated in previous studies which, having a comparison, demonstrated the superiority of treatment with EmoLED compared to other advanced therapies.

In the context of this study, neuro-ischemic foot injuries (grade IC or IIC according to the University of Texas Wound Classification) will be considered in patients with type I or II diabetes in "mixed" Hospital - Territory treatment. The details of the application of the treatment will be explained later; in any case, however, the treatment with EmoLED consists in irradiating the lesion for two minutes with the light emitted by the device.

This treatment does not interfere with other systemic therapies that may be in place, nor does involve any additional risk compared to standard treatment; the potential benefit is imputable not only to a reduced healing time and a greater probability of healing, but also to the reduction of pain and the improvement of the quality of life, with the consequent indirect benefits.

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 (6) and psoriasis (7) and its effectiveness in accelerating the process of healing of an induced wound on animal models (8-10).

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 (13-15).

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) (16,17). 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 (18,19) 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 for each circular area of 5 cm diameter that needs to be treated.

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. On (11) page 78 there is an attached table showing the physiological phenomena induced by temperature:

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

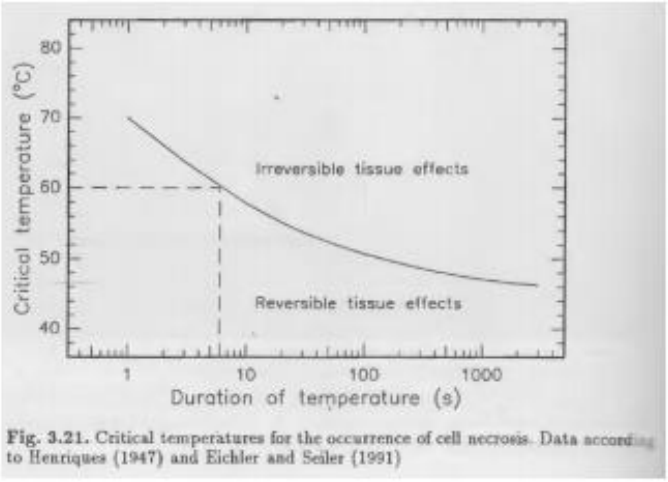

Fig. 3.21. Critical temperatures for the occurrence of cell necrosis. Data according to Henriques (1947) and Eichler and Seiler (1991)

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 (14) 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 analyzes 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 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 are compared. 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 EmoLED treatment. Even in clinical practice, since 2018, no adverse events or side effects have occurred in facilities that use the device, and its efficacy in different types of ulcers has been confirmed (20, 21).

Study Objectives

This is a pilot case / control clinical study on a group of outpatients with diabetic foot lesions aiming to verify the efficacy and safety of the Blue light photobiomodulation therapy with EmoLED medical device, in addition to standard therapy compared to the standard therapy alone, evaluating the percentage of healed lesions (which have reached complete and lasting re-epithelialization), the assessment of the healing time and reduction of the ulcerated area, the perception of pain and the quality of life of the enrolled patients.

The aim of this study is therefore to determine any differences in outcome between the two considered groups and, in particular, if the therapy of the EmoLED group is more effective than the standard therapy in terms of healing rate of lesions, healing time and reduction of the ulcerated area, pain perception and quality of life, and if is at least as safe in terms of occurrence of adverse events. Furthermore, another objective is to assess the economic impact of the two treatment options in the two groups.

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_2011 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 various centers involved before having received the favorable written approval of the Ethics Committee of the coordinating center and the respective Ethics Committees. The medical assistance provided to the subject is responsibility of adequately qualified doctors, as all the centers involved in this

study are specialized in the treatment of the pathology object of the clinical trial (Diabetology units or Diabetic Foot clinics).

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 each center is responsible for an adequate management of the study of their own center and for the coordination of the personnel involved. The PI must be responsible for all medical decisions regarding the study and ensure that adequate medical assistance is provided to the subject in case of any adverse event. The investigator should 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 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 risk, 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 legal tutor of the information provided. If the patient manifests their interest to participate in the study, they, or if the subject is unable to provide informed consent, their legal representative, signs the Informed Consent and decides whether to inform their attending physician of their participation in the study. At the same time, the patient gives his availability, after any discharge, to return to the facility for the established observation time and for the assessment of the progress of healing and scarring. A copy of the Informed Consent, subscribed by the patient 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 main 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 main 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 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 legal representative, receives a copy of the documentation or registration.

Pre-clinical tests and previous clinical experience

Preclinical animal model study observations

Preclinical observations aimed to 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-epithelialization 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

This clinical study will be a prospective, randomized controlled, double-blind single-center study with the aim of evaluating the clinical efficacy and safety of use of a portable, battery-powered light-emitting device that uses LEDs that emit blue light. The clinical study aims to compare the existing standard treatment for grade IC or IIC neuro-ischemic diabetic foot lesions according to the University of Texas Wound Classification in patients with type I or II diabetes, with a protocol that provides for the administration of the treatment with EmoLED for 20 consecutive weeks in addition to conventional therapy.

Enrolled patients have to be managed in Home Care Nursing Service (by nurses of the “Rete Assistenziale Lesioni Cutanee”), after discharge, twice a week, and have to go to the referral outpatient center participating in the trial once a month for follow-up visits, or in case of adverse events.

Primary and secondary objectives

As better detailed in the paragraph on statistical analysis, we expect to record, in the observation time, a difference in efficacy of the arm of the patients of the Treated Group (treated with EmoLED for 20 weeks) compared to the Control arm in terms of percentage of wounds healed in 24 continuous weeks (20 weeks of treatment plus 4 additional weeks of observation), but also a difference in healing time, wound surface regression, quality of life, perception of pain, acceptance of therapy, safety of treatment and costs related to the two therapies.

Endpoints

Study primary endpoint

The primary endpoint of the study will be the percentage of lesions considered healed (meaning as having a lasting and complete re-epithelialization) in the two groups at the twenty-fourth week of observation.

Healing must be confirmed by the Principal Investigator or their staff during an outpatient visit at the reference center participating in the study; the date of the recovery will in any case be indicated as the one of the first response, if it had occurred before the last visit to the reference center.

Study secondary endpoints

1. Healing time (in which healing is represented by a lasting and complete re-epithelialization);

2. Reduction of the lesion surface in both arms, during the 20 weeks, measured in absolute terms (square centimeters) and as % of the initial size;

3. Quality of life (QoL), evaluated both with specific questionnaires (EuroQoL-5D) and generic questionnaires (Wound-QoL);

4. Perception of pain measured with a subjective analogue rating scale (VAS);

5. Assessment of the costs related to the two arms in the perspective of the National Health System, NHS (considering only direct health costs) and in the perspective of the Society (considering beyond direct health costs, direct non-health costs and indirect costs);

6. Patient's satisfaction with the EmoLED therapy, assessed by administering specific questionnaires;

7. Appreciation, complexity and usefulness of the EmoLED therapy by the operators assessed through the administration of specific questionnaires;

8. Security of treatment (number of Adverse Events in the two groups);

9. Evaluation of the cost-effectiveness of using EmoLED for the treatment of ulcers.

Study design

The study is a double-blind pilot trial on outpatient patients, followed in an integrated way at a specialized center for the care of diabetic foot and Home Care as territorial home nursing service, articulated over a total period of 24 weeks (20 weeks of treatment plus 4 weeks of observation). Therefore, after an initial characterization and treatment setting by the specialist center, the patients will be followed biweekly by the nurses of Home Care, to then be re-evaluated at the center on a monthly basis.

The assessment of the evolution of the lesions will be made at the specialist center, in which the evaluating physician will not be aware of the type of home treatment performed by the patient and its allocation group and the execution of treatment with EmoLED will be performed ensuring the blindness also to the patient. This also ensures an adequate level of masking to ensure that the study is double-blind.

Both for the treatment group and for the control group, the patient will not be aware of the group to which he has been assigned; the operator will make the patient wear glasses (distributed to each patient at the beginning of the study) totally obscuring and will take out of the room any family members or other people before performing/simulating the treatment with the EmoLED device. In case the patient has been assigned to the control group, the operator will simulate to perform the treatment, turning on the device but pointing the light beam away from the patient (e.g. illuminating the floor).

Once the inclusion criteria are verified and the patient has given their consent to participate in the study, a two-week pre-enrollment period will be observed, in which the patient will receive standard treatment (as

indicated below); at the end of the two weeks of pre-enrollment, the patient will be re-evaluated to see if the lesion has not reduced by 50% with standard treatment alone.

If so, the patient will leave the study while, if the lesion has not reached this percentage of re-epithelialization, the patient will be randomized into the two study groups: the control group will continue with only the standard treatment as described above, while the treatment will receive in EmoLED treatment in addition to the standard treatment.

Patients will be randomized into the two arms of the study according to a list of random numbers generated by special IT tools and communicated by the scientific supervisor to the Care Provider via e-mail.

It is a 1: 1, single-block randomization of 40 patients distributed in the two groups (treated and control). After the two-week enrollment observation period, the Investigator at the outpatient center will ascertains the conditions for the study prosecution and he will notify the Head of the Care Providers to take charge of the patient included in the study and to proceed with the assignment to one of the groups. In this way, the Investigator at the outpatient center will be blinded to the treatment performed at home by the patient.

Patient selection

All consecutive patients treated at the specialist outpatient center for diabetic foot care who meet the 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 suffering from neuro-ischemic diabetic foot ulcer grade IC or IIC according to the University of Texas Wound Classification (22); If the patient has two or more lesions, which may fall within the inclusion criteria, the most serious lesion (index lesion) will be selected and followed for the entire duration of the study;*
- *Patients who, following hospital discharge or after the first outpatient assessments, are followed by Home Care Nursing Service and are always monitored at the same referral center;*
- *Patients with type I or II diabetes, with glycated haemoglobin≤ 10%;*
- *Patients willing to constantly wear the offload devices prescribed by the reference center;*
- *Patients with ulcer localized on ttoes, on the plantar side, on the margins or on the dorsumof the foot wider than 1 sqcm;*
- *Patients with peripheral neuropathy confirmedwith Monofilament (Semmes-Weinstein 5.07 / 10g);*
- *Patients with ABPI of 0.7 - 0.9, ankle PA> 70mmHg, TcPO2 between 36 - 50 mmHg;*
- *Patients with lesions lasting from 1-24 months;*
- *Patients who understand the purpose of the Clinical Study and provide their informed consent in writing.*

Exclusion Criteria:

- *Patients who have participated in a clinical study with a drug or medical device for less than a month;*

- *Patients who are unable to understand the aims and objectives of the study;*
- *Patients who are bedridden or unable to walk or Patients with neoplasms;*
- *Patients with pressure ulcers;*
- *Patients who have infectious signs according to IDSA criteria;*
- *Patients who underwent revascularization in the previous two months;*
- *Patients who have presented an acute ischemic event within the previous 3 months;*
- *Patients with heel injuries;*
- *Patients with nephropathy undergoing dialysis;*
- *Patients with osteomyelitis;*
- *Patients with Charcot's neuro-arthropathy:*
- *Patient on high dose corticosteroid therapy (≥ 40 mg/day);*
- *Patients with a history of self-harm who can voluntarily alter the course of healing;*
- *Patients with psychiatric disorders;*
- *Women who are pregnant or breastfeeding (the state of pregnancy or breastfeeding will be certified on the basis of the patient's declaration);*
- *Patients with pathologies or under treatment with drugs that induce photosensitization of the skin;*
- *Patients with a life expectancy of less than one year.*

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.

Sample Size

Since there is no reference in literature to the efficacy of the treatment under study, as this is a pilot trial, it is not possible to establish a sample size that guarantees adequate significance to the results.

Furthermore, the number of eligible patients cannot be determined a priori because, after two weeks of pre-screening, the enrolled patient could conclude the study without producing data for the endpoint analysis. At the facility involved in this clinical study, will therefore be recruited a total of 40 consecutive patients (20 per arm) who are randomized.

The estimated time for enrollment is approximately 18 months.

Medical-surgical procedures and study follow up

Standard treatment, according to the most recent version of the IWGDF guidelines (IWGDF – International Working Group on Diabetic Foot < https://iwgdfguidelines.org/wp-content/uploads/2019/05/IWGDF-Guidelines-2019.pdf>), includes debridement of the lesion, offloading with a device up to the calf, non-

removable (except for contraindications), dressing of the wound with advanced dressings in use at the trial center and at the home care according to SoC, to be renewed biweekly.

For the purposes of the study, in order to standardize the standard treatment as much as possible, all patients, except for contraindications, will adopt the same brace, made irremovable [Optima Diab, Molliter, Civitanova Marche (23-24)]

The treatment with EmoLED, in addition to the standard treatment, will be performed twice a week in correspondence with the dressing change of the lesion by the Home Care Nursing Service, blinded to the doctors of the specialist center, for twenty consecutive weeks, for a total of 40 treatments.

Treatment with the EmoLED device consists of irradiating each area of 5 cm in diameter of the lesion for 2 minutes; if the lesion is wider than 5 cm in diameter, the applications will be repeated to cover the entire area of interest. EmoLED treatment is additional to standard patient therapy.

During the twenty weeks of treatment with the EmoLED device and until healing, treatments with other types of phototherapies (including photodynamic therapies), topical negative pressure therapy, or other therapeutic interventions in addition to standard therapy will not be allowed.

In the event of clinical signs of infection of the wound, in the case of mild, moderate or severe infection according to I.D.S.A. (25), the lesion will be treated with antiseptic medication and, if deemed necessary by the investigator, with systemic anti-infective therapy, identified by medical personnel. The onset of infection will be reported in the appropriate section of the Data Collection Form and eventually reported to the promoter in case of serious infection (Serious Adverse Event).

In case, therefore, the lesion presents signs of infection as described above, the patient will interrupt their presence in the study and will be treated adequately. The patient can continue the study only if there is a remission of the symptoms of infection. The event will be reported in the appropriate section of the Data Collection Form.

The patient will be invited to a scheduled check-up at the referral center to assess the progress of the lesion, once every 30 days, or as needed.

The patient, if the wound healing occurs while being managed at home, will be invited to return for a check-up visit at the referral center within a week, to confirm the healing.

If at the conclusion of the participation of the patient in the clinical investigation, any additional treatments are required to those normally provided for the clinical condition considered in this study, the sponsor will cover the costs incurred for such treatments.

Measurable parameters

Following enrollment and signature of the informed consent, during the first visit / treatment, at the reference outpatient center, the salient data and photographic images of the lesion of each patient will be collected in the appropriate data collection form (see Annex).

During the home visits, twice a week (concomitantly with the dressing change and with the treatment with EmoLED for the Treated Group, respectively), photographic images of the lesion of each patient, some clinical data and the perception of pain through VAS scale will be collected. These variables will be measured at each

visit or until healing. During the visits (to be scheduled at least once every 30 days) carried out at the reference center participating in the trial, the clinical data on the Data Collection Form (see Annex), the photographic images of the lesion of each patient, pain perception using the VAS scale and quality of life data will be collected.

Questionnaires relating to the Quality of Life Section will be administered at the first visit and at the last visit. In particular, standard tools such as the generic questionnaire EQ-5D and the specific questionnaire Wound-QoL will be administered, both available and validated in Italian.

The parameters used that will be found for the definition of the result in the study are:

• Re-epithelialization of the lesion / reduction of the lesion area through data collection form and photographic images of the lesions treated with EmoLED and control lesions (Annexes B, C);

• Intensity of pain perceived by the patient through the VAS scale (Annexes B, C);

• presence of infection (Annexes B, C);

• Level of quality of life perceived by the patient through a questionnaire on the quality of life (EuroQoL and Wound-QoL) (Annexes D, E);

• Appreciation of the therapy (patient) through a specific questionnaire to be filled in by the patient (Annex F);

• Satisfaction, complexity and usefulness of using the device (operator) through a specific questionnaire to be filled in by the operator (Annex G);

• Direct and indirect costs (Annexes H, I);

• Detection of Adverse Events (Annexes B, C).

Registration of clinical data

The data related to the evaluation of the study endpoints are acquired, for both groups, during the visits.

The parameters of the Data Collection Form, the questionnaires about quality of life and the questionnaire about the acceptability of the therapy are acquired during the outpatient visit at the reference centre and kept on paper for the clinical trial length, and conserved in both paper and digital form; the pictures of the lesion are acquired and kept in digital form, during the length of the clinical trial and afterwards.

The storage coding follows the patient recruitment code and acquisition timing.

During each visit, a picture of the lesion will be taken and analyzed so that the process of healing can be valued in a quantitative and qualitative way.

At each visit, data on the patient's perception of pain will also be collected.

The Quality of Life related questionnaires will be administered at the first visit and at the last visit, scheduled after 24 weeks, at the reference center. In particular, standard tools such as the generic EQ-5D questionnaire, validated in Italian, will be administered. Similarly, the questionnaire for the patient acceptance of the therapy (generic for both the group treated with EmoLED and the control group) will be administered at the

last visit, scheduled after 24 weeks, or on the date of recovery. The operator satisfaction questionnaire (to be administered exclusively to staff belonging to Home Care) will be administered at the end of the study.

Data collection form (screening period) (Annex A)

The collection of data relating to the Clinical Section includes the patient's identification code, date of birth, sex and characteristics of the lesion in question. There is also a space for any concomitant pathologies, any drug therapy (to be registered in terms of active substances). The last section is intended for recording the size of the wound.

Data collection form (Research Center) (Annex B)

The collection of data relating to the Clinical Section includes the patient identification code, date of birth, sex and characteristics of the lesion to be collected in the initial phase. There is also a space for any concomitant pathologies, any drug therapy (to be registered in terms of active substances), any notes.

The second part of the form is organized as a matrix where the row represents the measurable parameters for each time of observation (reported in column).

At each visit the investigator marks with an “X” the chosen value for each parameter and the most suitable description. The investigator will also fill a check list, for each visit, to ensure that the picture of the lesion has been taken, and there will also be a checkbox in case of presence of infection in the wound bed.

Regarding the measurement of the size of the lesions, with “length” and “width” it is meant the longest diameter of the lesion and the largest diameter orthogonal to the first.

There is also a section dedicated to recording any additional visits, the date of recovery and the observation period following the end of the treatment. On the last page there is a table for reporting adverse events that will occur during the trial. In this table must be reported all types of adverse events (even serious). In this table will be reported the date of onset of the event, the type of event (there are already some entries of common adverse events that may occur on the wound or in the perilesional tissue), the solution implemented (additional therapy, hospitalization, etc.), the date of resolution of the event, the way of resolution and correlation of the event with topical therapy or treatment with EmoLED. The onset of any adverse event (even not serious or unrelated) must be reported to the sponsor, as well as included in the table above. In case of suspected or established correlation of the adverse event with treatment with EmoLED, the event will be declared as an "incident" and the principal investigator will be obliged to report it to the competent authorities as described in paragraph “Incidents and their reporting”.

Data collection form (Home Care) (Annex C)

During the home visit phase by the home care staff, a short data collection form will be filled out (twice a week) where in addition to the patient's identification code and belonging to the Treated or Control group, the same parameters of the data collection form will be annotated weekly for the Experimental Center. On the last page there is a table for reporting adverse events (see Annex B).

Data collection form on Quality of Life (Annexes D, E)

At the first visit and at the last visit to the experimental center, the enrolled patient will be given a form for the collection of data on quality of life, in which the subject is asked to answer some questions (about autonomy, pain, daily discomfort, etc.) also related to the presence of the chronic lesion under examination.

Questionnaires relating to the Quality of Life Section will be administered at the first visit and at the last visit, planned after 24 weeks. In particular, standard tools such as the generic questionnaire EQ-5D and the specific questionnaire Wound-QoL will be administered, both available and validated in Italian.

Therapy satisfaction questionnaire (Patient) (Annex F)

The questionnaire on the patient's satisfaction about the therapy (generic for both the group treated with EmoLED and the control group) will be administered at the last visit, scheduled after 24 weeks, or at the control visit 4 weeks from the date of recovery. In this questionnaire, the patient is asked questions aimed at understanding whether the therapy (standard or with the addition of treatment with EmoLED) has caused any kind of discomfort or has been well tolerated.

Satisfaction, complexity and usefulness questionnaires (Operator) (Annex G)

The operator satisfaction questionnaire (to be administered exclusively to staff belonging to Home Care) will be administered at the end of the study. It includes questions for the evaluation of the satisfaction of the healthcare personnel who used the device in use in this trial, with regard to its handling, functionality, effectiveness.

Direct and indirect cost questionnaires (Patient, Caregiver and Operator) (Annexes H, I)

Cost-specific questionnaires will be administered to patients and the main caregiver (if present) at the last visit with the aim of identifying the resources absorbed by the patients (and their caregivers) treated in the two approaches.

The consumption of resources will be assessed by collecting information aimed at quantifying direct health costs (visits, hospitalizations, access to the emergency room, use of medicines, etc.), direct non-health costs (informal care, transport costs and accommodation per visit, etc.) and indirect costs (loss of productivity of the patient and of the caregiver/s) and considering both the costs associated with adherence to treatment taking the last visit as "standard event", and the costs associated with complications and events relating the ulcer, that determined the need of treatments/examinations and/or medical evaluations. The principal investigator will also be given a questionnaire to determine the costs incurred by their structure for the management of patients with leg ulcers.

Image acquisition

A photographic image of the lesion will be collected during the 24 weeks of observation, or until healing and, eventually, the checkup to confirm the healing, to measure the area.

The photographic images will be acquired through the WoundViewer device (Omnidermal Biomedics srl – Torino IT), already present at the experimental centre.

The staff of the center will be trained in the use of this device and this training will be certified by the Omnidermal Biomedics srl company.

The WoundViewer device is able to store the photographic images per patient and also to calculate the wound area through a suitable analysis algorithm.

The value of the lesion area will be reported in the Image Collection Form (Annexes A, B, C) at the time of the visit.

Bias - systematic errors

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. Although the sponsor does not participate in any way in the clinical trial, he will always remain at the team's disposal for any clarification regarding the correct management and use of the device under study.

Number of experimental medical devices expected to be used

Each investigator centre involved in the treatment will be provided with 5 EmoLED medical devices, distributed between the Diabetic Clinic and the Home Care.

Timeframe of the study

The study lasts 26 weeks (considering pre-screening, treatment period and final observation) with an approximate enrollment period of 18 months. However, the study will continue until the number of patients planned by this protocol is reached. For this reason, the overall duration of the study, from the enrollment of the first patient to the completion of the last, is estimated at 24 months

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.

Discontinuity of patients regarding adherence to plantar offloading can affect recovery times. Before recruiting a patient, the doctor ensures that the patient is available to follow the prescribed therapy and to go to the clinic when required.

In case the patient misses a check-up visit, this can be regained within two days following the scheduled date. If this is not possible, the visit will not be carried out and the patient will be evaluated at the next visit. If the patient misses more than one visit, they will be excluded from the study (drop out).

Other reasons why patients will have to leave the study early are the lack of treatment, with EmoLED or with standard therapy, for more than four consecutive times, or for the lack of a quarter of home visits in total (unless non-treatment is provided for in this Protocol, such as in the case of suspension due to infection). Patients in which it is not possible to establish the exact date of recovery for various reasons, and patients who, in the opinion of the investigator, show a poor compliance with the therapeutic standards required by the study protocol, will also be excluded.

In such cases, the investigator will exclude patients from the study, recording on the data collection form, in addition to the date of exclusion, also the reasons why the patient was excluded.

The patient may, however, withdraw from the study at any time without having to give any explanation to the investigator (withdrawal of consent).

Following the exclusion or abandonment of the study by the patient, the data collection form is identified, scanned and stored.

If a patient withdraws from the study, the Principal Investigator records the event in the identification register and on the data collection form and stores it all.

Clinical investigation monitoring plan

In order to verify that the rights, safety and well-being of enlisted patients are protected, that the reported 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 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 that the Informed Consent was obtained and registered for each subject before the beginning of the study.

Researchers will be contacted on a regular basis throughout the whole duration of the study to check and verify the various documents (data collection 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 monitoring staff will carry out an opening visit of the center (SIV), a minimum of 3 visits during the study (MOV) - to be scheduled approximately one after 1/2 months after the SIV based on the number of patients enlisted, one to 2/3 enrollment, and another at the conclusion of the observations of 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 2000 euros per patient, divided into the visits foreseen 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 data collection 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 two 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 (numerical progressives), starting from 01. The patient code is reported in the data collection 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 company as an authorized person to be treated in accordance with Article 29 of the GDPR, as a person designated in accordance with Article 2 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 controller informs the holder without undue delay after becoming aware of the breach. The holder 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

data 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 remedy 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 persons, the controller 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 data collection 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 committees to approve the changes.

Incidents 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.

Definitions

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

- «**Incident**»: 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 incident**»: any incident 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 incidents 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 incident 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 incidents 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 incidents:

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 “Incident

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 incident, 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 incidents with medical devices for the manufacturer or their authorized representative, in particular the immediate communication to the competent authority of all the incidents 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 incident 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 incident and an EMOLED brand device.

The timing is as follows:

a) Manufacturers report *any serious incident* immediately after establishing the causal link, even if only reasonably possible, between the incident and their device, and *no later than 15 days* after becoming aware of the incident;

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 incident and in any case *within 10 days* after the date on which the manufacturer becomes aware of the serious incident.

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 incident, 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 Incident 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.

b. Unexpected adverse reaction or unexpected side effect

c. Interactions with other substances or products

d. Device degradation / destruction (e.g. fire)

e. Inappropriate therapy

f. 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 incident

In assessing the link between the device and the incident, the manufacturer must take into account:

- the opinion, based on available evidence, of health professionals;
- the results of the preliminary incident 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 incident.

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 incidents 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 incident associated with a device has occurred and that the incident was such that, if it happened again, it could lead to death or a serious deterioration in the state of health.

Condition 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 incident 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 for subjects withdrawn from the study or who have completed the study

Each morbid condition derived from each patient's incident 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 incident 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 incident 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 of the coordinator centre, 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 sponsor, 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

Sample number

Despite the presence in the scientific literature of data on average healing times of diabetic foot ulcers, the absence of clinical experience that associates blue light treatments with the therapy of this type of lesions and the lack of reliability of any inference based on clinical studies that associate this type of treatment with other types of skin lesions, due to the different etiologies and types of patients, it is not 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 historical clinical activity data at the Diabetology Unit, where patients will be enlisted, the number of patients with diabetic foot ulcers is about 500 patients/year of which about 35% is affected by neuroischemic ulcer.

With an average of 30% of these patients reaching a healing rate of more than 50% in the first two weeks, and a further percentage of patients being excluded at the time of recruitment based on inclusion/exclusion criteria, in 18 months of enlistment it is possible to reach a number of 40 patients compared to a percentage of drop-out/denial equal to about 25%. A total of 40 patients will be recruited, 20 patients per arm.

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

As a pilot study based on a limited number of patients, the study will also include patients who do not complete the study according to the principle of "intention to treat" and the resulting missing data will be processed through the imputation technique of the missing data last observation carried forward-LOFC.

In case the patient does not leave the study but skips one or more control visits, the averages of the two adjacent visits or the last available data (LOCF) will be taken into consideration.

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.

In the first analysis a qualitative comparison between the treatment groups will be carried out (healed/not healed) through the test x2 or the exact test of Fisher in the case of counts of less than 5. The results will be presented in contingency tables and bar charts.

A quantitative comparative statistic between treatment groups will then be performed by comparing the percentage reduction in the area of injury, the variation in quality of life level (QoL) and the variation in pain perception (VASS) through Mann-Whitney's nonparametric test.

The choice to perform a non-parametric analysis lies in the low sample size and therefore it is preferred to have more conservative significance values. The results will be presented in tables with median and interquartile interval in the two groups with its p.value and graphically through the box plot.

To analyze the internal and time-related differences between groups, 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 confusing way by demographic, clinical covariates, pre-treatment measurement, and to measure their impact which will allow us to understand which variables they include on the outcome and will also allow us to analyze the differences between the equal groups of these covariates.

For the comparison about recovery time, since the study is based on the principle of "intention to treat" and therefore there is no caesura, it will be performed by comparing the average recovery times through the non-parametric test of Mann-Whitney.

Then a descriptive statistic will be made through tables with frequencies and percentages, and bar charts of the treatment satisfaction indices in patients and operators. In addition, adverse events in both groups will be described, if present.

Due to the generally asymmetric nature of the cost distribution, the comparison of the costs associated with the two treatment arms, both from the perspective of the SSN and the Company, will be carried out using the non-parametric U test of Mann-Whitney.

Non-generalized linear regression models will be used to assess differences between groups, net of confounding factors.

The cost effectiveness of the two treatments in the time horizon of the study will be assessed by calculating the incremental cost effectiveness ratio (ICER) and expressed in terms of incremental cost for "quality adjusted life years" QALY.

Extrapolation of the results of the cost-effectiveness assessment by simulating longer time horizons will be carried out considering decision models (e.g. Markov models) and, where appropriate, integrating the data collected in the study with literature and/or evidence from other studies. All significance tests will have a statistically significant $p < 0.05$ value. The analysis will be carried out with the open source software R Commander (v. 2-2.0).

Data 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.

References

1. Greenhalgh, D. G. (2019). Management of Burns. New England Journal of Medicine, 380(24), 2349–2359.
2. Clark R (1985) Cutaneous tissue repair: basic biological considerations. J Am Acad Dermatol **13**: 701-725.
3. Mast BA, Schultz GS.(1996) Interactions of cytokines, growth factors, and proteases in acute and chronic wounds.Wound Repair & Regeneration **4**: 411-420.
4. Nwomeh BCM Yager DR, Cohen IK (1998) Physiology of the chronic wound. Clinics in Plastic Surgery 25 (3): 341
5. Hackam DJ, Ford HR (2002) Cellular, biochemical and clinical aspects of wound healing. Surgical Infections 3: S23-S35.
6. Calderhead (2007), The photobiological basis behind light-emetting diode (LED) phototherapy, Laser therapy.
7. Beani, Jeanmougin (2010) Narrow-band UVB therapy in psoriasis vulgaris: Good practice guideline andrecommendations of the French Society of Photodermatology, Annales de Dermatologie et de Venereologie.
8. Adamskaya et al. (2007), Light therapy by blue LED improves wound healing in an excision model in rats, Injury International Journal of the Care of the Injured.
9. Kleinpenning et al. (2010), Clinical and Histological effects of blue light on normal skin, Photodermatology, Photoimmunology & Photomedicine.
10. Cicchi et al (2016), Observation of an improved healing process in superficial skin wounds after irradiation with a blue-LED haemostatic device, Journal of Biophotonics.
11. Niemz MH (1996), Laser-tissue interactions: fundamentals and applications.
12. Rossi et al. (2010), A blue-LED-based device for selective photocoagulation of superficial abrasions: theoreticalmodeling and in vivo validation, Photonic Therapeutics and Diagnostics VI, Proceedings of SPIE Volume: 7548.
13. Zadeh MS, Kolb JP, Geromin D, D'Anna R, Boulmerka A, Marconi A, Dugas B, Marsac C, D'Alessio P. Regulation of ICAM-1/CD54 expression on human endothelial cells by hydrogen peroxide involves inducible NO synthase. J Leukoc Biol. 2000 Mar;67(3):327-34.
14. Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. The journal of experimental Medicine. 2011;208(3):417-420.

doi:10.1084/jem.20110367.

15. Racz E, Prens EP. Phototherapy and photochemotherapy for psoriasis. Dermatol Clin. 2015 Jan;33(1):79- 89. doi:10.1016/j.det.2014.09.007.
16. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol. 2000 Jul;85(1):9-18; quiz 18, 21.
17. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. Cellular and Molecular Life Sciences. 2016;73(20):3861-3885. doi:10.1007/s00018-016-2268-0.
18. Mistry RK, Brewer AC. Redox regulation of gasotransmission in the vascular system: A focus on angiogenesis. Free Radical Biology & Medicine. 2017; 108:500-516. doi: 10.1016/j.freeradbiomed.2017.04.025.
19. Hong WX, Hu MS, Esquivel M, et al. The Role of Hypoxia-Inducible Factor in Wound Healing. Advances in Wound Care. 2014;3(5):390-399. doi:10.1089/wound.2013.0520.
20. Mosti G, Gasperini S. Observations made on three patients suffering from ulcers of the lower limbs treated with Blue Light. Chronic Wound Care Management and Research 2018:5: 23-28; doi:10.2147/CWCMR.S172060.
21. Marchelli M, Perniciaro G, Granara D, Bedin F, Di Leo L, Viaggi C, Orlanno G, Gagliano C, Bottaro LG. Photobiomodulation with Blue Light in non healing wounds: case series evaluation. *Wounds International 2019:10(3):63-66.*
22. Lavery LA, Armstrong DG, Harkless LB: Classification of diabetic foot wounds. J Foot Ankle Surg 35:528–531, 1996.
23. Piaggesi A, Macchiarini S, Rizzo L, Palumbo F, Tedeschi A, Nobili LA, Leporati E, Scire V, Teobaldi I, Del Prato S. An off-the-shelf instant contact casting device for the management of diabetic foot ulcers: a randomized prospective trial versus traditional fiberglass cast. Diabetes Care. 2007 Mar;30(3):586-90.
24. Piaggesi A, Goretti C, Iacopi E, Clerici G, Romagnoli F, Toscanella F, Vermigli C. Comparison of Removable and Irremovable Walking Boot to Total Contact Casting in Offloading the Neuropathic Diabetic Foot Ulceration. Foot Ankle Int. 2016 Aug;37(8):855-61.
25. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, Pinzur MS, Senneville E; Infectious Diseases Society of America. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012 Jun;54(12):e132-73.