STATISTICAL ANALYSIS PLAN

Clinical Trial

A multicenter, multinational, randomized, controlled, assessor blinded study, performed in subjects with thermal burns, to evaluate the efficacy and safety of NexoBrid compared to Gel Vehicle and compared to Standard of Care (DETECT)

Sponsor:
MediWound, Ltd.
42 Hayarkon Street
North Industrial Area
Yavne, Israel 8122745

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Competence Center for Clinical Trials Bremen - Biometry

Author: Dr. Martin Scharpenberg

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1. Table of Contents

2. Introduction

2.1. Background

Optimal treatment of burn injuries requires understanding of and response to the profound local and systemic reactions following thermal injury.

The treatment strategy should be planned and executed on the basis of a timely (early) assessment of the extent of tissue damage and remaining viable tissue available for the healing process. Only when the eschar is removed and the wound bed revealed, can the true damage be observed and assessed, and the appropriate wound closure modality prescribed [1] [2] [3].

As soon as the burn eschar is formed, it induces, within hours, a cascade of local and systemic pathologic changes. Dead eschar becomes heavily contaminated in two to three days, creating a source for local and systemic infection and sepsis [1] [4] [5] [2] [6]. Local inflammatory responses may lead to additional destruction of healthy surrounding tissue, extending the original damage. If not otherwise removed, inflammatory and autolytic processes slowly decompose and destroy the eschar, which will slough in approximately two weeks. The long inflammation based spontaneous sloughing period allows the simultaneous formation of advanced granulation tissue, later evolving into a heavy and deforming scar.

Delaying the removal of the eschar increases the incidence and severity of systemic and local complications, especially in more extensive burns [1] [2] [4] [5] [6] [7] [8] [9] [10]. The decomposing eschar is a source of strong local and (if the burn is extensive) systemic mediators that induce inflammatory reaction and that may cause burn wound progression into potentially viable tissue [2] [5] [7] [9] [11].

Moreover, the eschar is non-resilient and forms a cocoon-like constricting shell around circular body parts. With increasing tissue edema, this increases the interstitial/compartment pressure, seriously compromising the viability of the still barely surviving skin by stretching it and compressing blood supply to other vital structures. An early diagnosis and response to these events releasing surgical escharotomy, is important and should be executed emergently. In this case too, delay in diagnosis and reluctance to perform surgical escharotomy may delay

this procedure especially in cases where experienced burn surgeons are not readily and immediately available [4] [12].

To prevent eschar-related complications and to initiate the healing process, it is imperative to remove all of the offending eschar at the earliest possible opportunity. Eschar removal, “Debridement”, is the first stage of the comprehensive wound care process. There is no benefit in the presence of the eschar on the burn. Thus, the decision is not whether or not to remove an eschar but when and how. Therefore, delay or avoidance of eschar removal should have a specific reason.

Estimation of burn size and depth is essential for a diagnosis-based treatment strategy, but cannot be accurately performed in most instances unless and until the opaque eschar has been completely removed.

Immediate (within 24 hours post-burn) eschar removal may improve the potential for survival of the Zone of Stasis, reverse the Zone of Hyperemia and may attenuate, or avoid many of the systemic reactions [1] [9] [5] [2] [6] [13]. Delayed eschar removal (within the first three days) may not provide all the benefits of immunological and inflammatory modulation, but will reduce the burn bacterial load and its outcomes [4] [12] [14] [15]. Delaying eschar removal up to the seventh day will forfeit the local and systemic benefits, but may still prevent healing by secondary intention potentiating better long-term results (especially in terms of scarring) [2] [5] [6] [16].

In current standard of care, removal of the eschar may be accomplished by surgery. Usually with tangential excision followed by split thickness autografting (TE&STSG) of the acute exposed debrided wound, or by non-surgical topical care that includes daily dressing changes, bathing, scraping, wet to dry dressing, macerating activity of bacteria, etc., until the eschar sloughs, leaving a clean wound bed for subsequent non-surgical or surgical wound closure treatment. This combination of debridement followed by surgical or non-surgical wound closure treatment is the basis of all burn wound care modalities.

The choice of debridement method (non-surgical or surgical) depends on many variables such as the burn depth, anatomical site and size (expressed as % TBSA of the burn wound), the patient’s general condition, available donor site for autograft harvesting as well as the availability of surgical facilities and staff. Tangential excision should be carried down into the healthy intact tissue to make sure that no trace of the eschar remains. It is estimated that up

to 30–50% of healthy tissue (mainly dermis), which is essential for the spontaneous epithelialization potential of the wound, may be sacrificed in this procedure, thus usually necessitating autografting. These surgical procedures are traumatic, often long and difficult, require skilled personnel and sophisticated medical resources and are usually accompanied by bleeding [1] [2] [12] [16] [17] and heat loss [18]. The surgically excised raw wound bed should be covered immediately, i.e., in most cases autografted. Thus, the excision is followed by harvesting of autografts from intact, non-injured skin areas, skin graft transplantation, and care of the donor sites in addition to the original burn injury. These surgical procedures are performed under general anesthesia that requires pre- and post-surgical fasting and recovery. In most centers, caution and post surgery intensive care dictate that only a limited percentage of the affected areas (~15%TBSA) will be surgically debrided at one time in order to limit inflicting excessive stress on the patient. Thus, the decision to surgically debride a burn is not taken lightly. Reluctance, hesitation and doubts due to uncertain diagnosis delay the decision to perform surgery, extending the time to start and finish of the debridement from the optimal few hours to several days.

In partial, mixed thickness, and intermediate depth wounds, and with difficult to diagnose depth “indeterminate” wounds, early surgical debridement will usually be avoided and a more conservative debridement approach using topical medications will be pursued. Such conservative non-surgical debridement lasts for days until the eschar decomposes or until a decision is taken to excise the offending, remaining eschar. The non-surgical debridement process involves the combined activity of topical medications, contaminant microorganisms, autolytic tissue processes and the inflammatory process with frequent dressing changes, showering, mechanical scraping of loose debris etc. The infectious-inflammatory processes are slow (lasting between 10-14 days) and may involve significant systemic and local complications. Using topical anti-bacterial or anti-inflammatory medicaments may reduce the infectious-inflammatory processes but will also delay eschar separation (sloughing). Locally, all these processes prolong the removal of the eschar and lead to additional tissue damage due to the death of the Zones of Stasis and Hyperemia which deepen, transforming partial thickness damage into a full thickness injury. The long debridement time with sustained inflammatory processes can lead to the formation of granulation tissue that will develop into heavy scars.

This excerpt of literature emphasizes that timely eschar removal is, for the last 40 years, the goal of burn wound care initiation. Physicians today are forced to choose between or combine two sub optimal alternatives of eschar removal; (i) the fast and efficient surgery (TE&STSG) with its diagnosis-dependent often prolonged initiation, non-selective, traumatic and demanding results or (ii) the inefficient non-surgical prolonged debridement means with their associated sequelae. The lack of fast and effective non-surgical eschar removal means has left surgery as the available SOC for deep burns.

An optimal treatment solution would be a non-surgical, selective means for removing exclusively the eschar without harming the non-injured tissues immediately or nearly immediately after patients' admission and stabilization, independently of the burn depth diagnosis. Such early removal of the eschar will enable the physician accurate visual diagnosis of the exposed burn wound bed. The clean exposed bed can be treated by the most appropriate wound closure strategies tailored for each patient and wound area in order to achieve optimal long-term results with minimal trauma and cost to the patient.

The development of NexoBrid as a product for enzymatic debridement of burns started several decades ago.

The safety and efficacy of NexoBrid (for burn eschar debridement) has been extensively investigated in 7 clinical studies [19], including a phase 3 pivotal trial (MW 2004-11-02) [20]. Efficacy data generated during NexoBrid clinical program demonstrated the following direct beneficial effects of removal of burn eschar by NexoBrid:

1. **Effective eschar removal by NexoBrid:** NexoBrid removes the necrotic tissue without surgery, which is an imperative first step in burn care. Non-surgical effective debridement facilitates debridement in children, aged and of difficult skin areas (hands, feet, joints etc.)
2. **Selective eschar removal:** NexoBrid removes the eschar in a minimally invasive manner that leaves the surrounding uninjured and viable tissues unharmed and thus allows maximization of spontaneous healing and reduces the incidence and extent of skin grafting [21].
3. **Early and fast debridement:** NexoBrid allows earlier initiation and successful completion of the eschar removal process compared to SOC, enabling preservation of the underlying tissue from secondary ischemia, and allowing the healthcare provider to

directly visually assess the burn wound bed earlier to prescribe proper subsequent treatment.

4. **Avoiding escharotomy surgery and its sequelae:** Early application of NexoBrid may reduce the elevated interstitial pressure in circumferential burns and alleviate burn-induced compartment syndrome, thus reducing the need for eschar incision.

Indirect beneficial effects:

1. Reduction of incidence and extent of surgery (excision and grafting) by NexoBrid eschar removal treatment.
2. Autografting can be reduced by selective debridement with increased preservation of uninjured dermis and potential for spontaneous epithelialization. In choosing the approach of epithelialization over preserved dermis, autografting is reserved to close full thickness wounds. In addition to the benefit of reduced surgery, decreased autografting reduces sacrifice of the subject's healthy uninjured skin and accompanying morbidity.
3. Blood loss is minimized by reduced excisional and autografting surgery.

2.2. Study Objective

1. To demonstrate the efficacy of enzymatic eschar removal with NexoBrid by providing complete eschar removal as compared with Gel Vehicle,

2. To demonstrate the efficacy of enzymatic eschar removal with NexoBrid by providing earlier complete eschar removal, reduction in patients' surgical burden and its related blood loss as compared to SOC,

3. To assess the safety of NexoBrid compared to SOC, including demonstration that treatment with NexoBrid does not cause an unacceptable level of harm on wound closure outcome and long-term outcomes of cosmesis and function

2.3. References to Study Protocol

This Statistical Analysis plan refers to the study protocol version 11 of 23 June 2016. The trial protocol code is MW2010-03-02.

2.4. Amendments

The study was initiated under the trial protocol version 08. Since then, there have been two amendments (to version 9 and version 11). Details of the Amendments are provided in Appendix 13.5.

3. Study Administrative Structure

3.1. Sponsor

Sponsor: MediWound, Ltd
42 Hayarkon Street
North Industrial Area
Yavne, Israel 8122745

Represented by: Prof. Lior Rosenberg
Chief Medical Officer
MediWound Ltd.

Keren David Zarbiv
Director Clinical Affairs
MediWound Ltd.

Nimrod Leuw
Director of QA/QC
MediWound Ltd.

3.2. Study Conduct

Study Conduct (CRO): Dr. Marco Schwarzer
GCP-Service International Ltd. & Co. KG
Anne-Conway-Str. 2
28359 Bremen

3.3. Statistics

Statisticians: Prof. Dr. Dr. h.c. Jürgen Timm
Dr. Martin Scharpenberg
Competence Center for Clinical Trials Bremen
University of Bremen
Linzer Str. 4
28359 Bremen
Germany

4. Signatures

Keren David Zarbiv
Sponsor Representative
MediWound Ltd.

Keren David — Signature — 15/5/18 — Date

Dr. Marco Schwarzer
Study Conduct (CRO)
GCP-Service International Ltd. & Co. KG

ppa. [signature] — Signature — 15/05/2018 — Date

Prof. Dr. Dr. h.c. Jürgen Timm
Principal Statistician
Competence Center for Clinical Trials Bremen,
University of Bremen

[signature] — Signature — 15.5.18 — Date

Dr. Martin Scharpenberg
Statistician/Author
Competence Center for Clinical Trials Bremen,
University of Bremen

M. Scharpenberg — Signature — 15.05.18 — Date

5. List of Abbreviations

abbreviation	meaning
ABA	American Burn Association
ABL	Actual Blood loss
AE	Adverse Event
AFT	Accelerated Failure Time
ATC	Anatomical Therapeutical Chemical Classification
BICS	Burn-induced Compartment Syndrome
BMI	Body Mass Index
BSHS-B	Burn Specific Health Scale - Brief
CS	Clinically Significant
DDD	Defined Daily Dose
DPT	Deep Partial Thickness
DSMB	Data Safety Monitoring Board
EAP	Efficacy Assessment Period
EAR	Extremities at Risk
EBV	Estimated Blood Volume
ECG	Electrocardiogram
eCRF	electronic Case Report Form
EMA	European Medicines Agency
ER	Eschar Removal
FAS	Full Analysis Set
FDA	Food and Drug Agency
FT	Full Thickness

abbreviation	meaning
FU	Follow up
Hb	Hemoglobin
HD	Hospital Discharge
HEENT	Head, Eyes, Ears, Nose and Throat
HR	Hazard Ratio
ICF	Informed Consent Form
INR	International Normalized Ratio
ITT	Intention to Treat
KKSB	Competence Center for Clinical Trials Bremen
LEFS	Lower Extremity Function Scale
LOCF	Last Observation Carried Forward
LTFU24	24 months Long-Term follow up period
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
MVSS	Modified Vancouver Scar Scale
NCS	Not Clinically Significant
PDF	Portable Document Format
POSAS	Patient Observer Scar Assessment Scale
PP	Per Protocol
PRBC	Packed Red Blood Cells
PTT	Prothrombin Time

abbreviation	meaning
ROM	Range of Motion
RTF	Rich Text Format
SAE	Serious Adverse Event
SOC	Standard of Care
SOP	Standard Operating Procedure
SPT	Superficial Partial Thickness
SSD	Silver Sulphadiazine
STFU12	12 months Short-Term Follow up period
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBSA	Total Body Surface Area
TW	Target Wound
VAS	Visual Analogue Scale
WBC	White Blood Cell (Count)

6. General Definitions

6.1. Hardware and software used

The statistical analysis will be performed using SAS® version 9.4. The statistical report will be created with Microsoft Office running on hardware of Competence Center for Clinical Trials Bremen (KKSB).

6.2. Reporting standards and language

The language of the statistical report is English. This statistical analysis report is written as specified in the standard operating procedure (SOP) *SR01 StatisticalReport* of the KKSB and in accordance with the ICH E3 guideline (structure and content of clinical study reports).

6.3. General format and content of tables, figures and patient data listings.

Tables, figures and listings of subjects will be created in SAS® version 9.4 and be converted in rich text format (RTF) or portable document format (PDF). Appendix 13 contains a detailed description of contents and layout of the result representation.

6.4. Quality control

The statistical analysis plan follows the SOPs ST01 to ST06 of the KKSB. The statistical analysis must follow the statistical analysis plan.

The implementation of the analysis for the primary endpoint, the secondary endpoints, the safety endpoint “time to wound closure” as well as the 12 months and 24 months MVSS assessment will be repeated by a second statistician independent from the implementation of the main statistician. Possible differences will be discussed together with the quality control and the responsible statistician (see Section 3.3).

7. Details of Trial Design

7.1. General Information of Trial Design

This is a multi-center, multi-national, randomized, controlled, assessor blinded, three-arm study aiming to demonstrate superiority of NexoBrid treatment over Gel vehicle control and over SOC treatment in thermal burn subjects with burns.

One hundred and seventy five subjects (175 subjects) are planned to be randomized to the study in a 3:3:1 ratio (NexoBrid: SOC: Gel Vehicle). The study will be conducted at sites in the US and out of the US.

Following the enrollment of a patient to the study and prior to randomization, physicians will identify one or more TWs per patient according to the TW definitions described in Section 1.1 of the protocol.

All subjects' DPT and FT burns that comply with the specified entrance criteria under Section 7.1.3 are intended to receive study treatment per the randomized study arm and therefore, must be designated as TWs. This will further allow an evaluation of the subject systemic safety by allowing treatment of the subject's entire deep burns as per the randomized study arm. After reviewing the entrance criteria, prior to randomization, eligible subjects will be stratified into different groups in order to standardize and minimize bias between study groups in terms of efficacy and safety outcomes that are expected to be correlated to the patient total burn area and its depth. Please refer to Section 13.7.1 of the protocol for full description of the randomization procedure.

Following the stratification, patients will be randomized as per their stratification group (see Section 11.1.10) in a 3:3:1 ratio (NexoBrid: SOC: Gel Vehicle).

Patients will be treated in the same way in all study arms (NexoBrid, SOC or Gel vehicle) except for the eschar removal stage which will be performed as per the randomization study arm.

Prior to initiation of eschar removal treatment subjects will be medicated with appropriate analgesia (please refer to Appendix 2- Pain Management Procedures of the protocol) and undergo wound cleansing and dressing of all wounds with antibacterial solutions (as specified

in Section 8.1 of the protocol). Following wound cleansing and antibacterial treatments, subjects will undergo the eschar removal process as per treatment assignment (NexoBrid, SOC or Gel Vehicle, following randomization) as further described in *APPENDIX* 16- Treatment Diagram of the protocol.

Subsequent to complete eschar removal, all wounds will be assessed and treated in accordance with post-eschar removal wound care strategy. Post eschar removal, subjects will undergo daily vital signs and pain assessments, until hospital discharge (HD) and weekly assessments of wound progress, until complete wounds and donor sites closure. Following wound closure confirmation visit, subjects will be followed up at 1, 3, 6, 12, 18 and 24 months post last wound closure confirmation for long-term outcomes evaluation.

7.1.1. Study medication

7.1.1.1. Topical arms

7.1.1.1.1. NexoBrid

NexoBrid is presented as lyophilised Bromelain powder and gel vehicle for preparation of a gel for cutaneous use, including concentrate of proteolytic enzymes enriched in Bromelain as the active component. Following mixing of the powder with the gel vehicle, each gram of the prepared product contains 0.09 g partially purified Bromelain. Partially purified Bromelain is a mixture of enzymes extracted from the stem of *Ananas comosus* (pineapple plant).

7.1.1.1.2. Gel Vehicle

The same Gel Vehicle used in the preparation of NexoBrid will be used as negative control.

7.1.1.2. Standard of Care

SOC arm will include surgical and/or non-surgical eschar removal procedures.

Surgical procedures will include tangential/ minor/ avulsion/ Versajet/ dermabrasion excisions. Non-surgical procedures will include the application of collagenase ointment (e.g. Santyl), antimicrobial solutions (e.g. Dakin’s Solution, Sulfa-Nystatin Solution), ointments/creams (e.g. Bacitracin, Polysporin, Silvadene) and/or Silver dressings (e.g. Mepilex Ag, Aquacel Ag, Acticoat).

The need of either non-surgical or surgical procedures will be determined by the burn specialists and can be repeated as needed until complete debridement is achieved.

7.1.2. Intended dosage and duration of treatment

7.1.2.1. NexoBrid

Two grams or five grams of NexoBrid sterile powder are mixed in 20 grams or 50 grams of sterile Gel Vehicle (ratio of 1:10), respectively to obtain sterile NexoBrid Gel. NexoBrid Gel is applied to the burn wound at a dose of 2 g NexoBrid sterile powder mixed with 20g sterile Gel Vehicle per 1% of TBSA (~ surface of an adult palm) for four hours (or 5 g NexoBrid sterile powder mixed with 50g sterile Gel Vehicle per 2.5% of TBSA). The NexoBrid powder and the Gel Vehicle are to be mixed at the patient bedside ≤ 15 min prior to use.

NexoBrid should not be applied to more than 15% TBSA in one session.

NexoBrid may be applied for a second time to the same burn area, if eschar removal from the target wound after the first application is not complete but at least 50% of the eschar was removed during the first application. NexoBrid may only be applied twice to the same burn wound area.

7.1.2.2. Gel Vehicle

Twenty (20) grams or 50 grams of sterile Gel Vehicle will be applied on the burn skin. Gel Vehicle is applied to the burn wound at a dose of 20 grams sterile Gel per 1% of TBSA (~ surface of an adult palm) or 50 grams per 2.5% TBSA for four hours.

Gel Vehicle should not be applied to more than 15% TBSA in one session.

Gel Vehicle may be applied for a second time to the same burn area, if eschar removal from the target wound after the first application is not complete but at least 50% of the eschar was removed during the first application. The Gel Vehicle may only be applied twice to the same burn wound area.

7.1.2.3. Standard of Care

No more information on intended dosage or duration of treatment was specified in the protocol for the Standard of Care arm than that already given in Section 7.1.1.2.

7.1.3. Study population and choice of study invitee

7.1.3.1. Inclusion Criteria - Patient level

1. Males and females; ≥ 18 years of age,
2. Thermal burns caused by fire/flame, scalds or contact,
3. Patient total burns area ≥ 3% DPT and / or FT,
4. Patient total burns area should be ≤ 30% TBSA; SPT, DPT and/or FT in depth,
5. Informed consent can be obtained within 84 hours of the burn injury,
6. Patients who are willing and able to sign a written consent form.

7.1.3.2. Inclusion Criteria - Wound level

1. At least one wound (a continuous burn area) that is ≥0.5% TBSA (DPT and/or FT) (this minimal wound size should not include face, perineal or genital),[1]
   All planned target wounds (TWs) should meet the following criteria:
2. SPT areas that cannot be demarcated from DPT and FT areas should be less than 50% of the % TBSA of the TW
3. Wound's blisters can be removed/unroofed, as judged by the investigator.

7.1.3.3. Exclusion Criteria - Patient level

1. Patients who are unable to follow study procedures and follow up period,
2. Modified Baux index[2] ≥ 80,
3. Patients with burned charred fingers, 3rd degree in depth and possibly devoid of circulation,
4. Patients with abraded wound/s that cannot be treated by an enzymatic debrider application (NexoBrid) will be excluded from the study,
5. Patients with electrical or chemical burns,
6. Patients with circumferential (≥ 80% of the limb circumference) DPT and/or FT burns defined as Extremities at Risk (EAR) as described in section 11.8 of the protocol,

[1] All these wounds which are in line with this criteria should be defined as target wounds (TWs) and treated per randomization

[2] Modified Baux score will be calculated based on the %TBSA affected by burns, age of the patient and the presence of smoke inhalation injury; Age + Percent Burn + 17 * (Inhalation Injury, 1 = yes, 0 = no).

7. The following pre-enrollment dressings: a. Flammacerium, b. Silver Nitrate ($AgNO_3$),
8. Patients with pre-enrollment wounds which are covered by eschar heavily saturated with iodine or by SSD pseudoeschar (e.g. pseudoeschar as a result of >12 h SSD treatment),
9. Patients with pre-enrollment escharotomy,
10. Patients with diagnosed infections as described in section 11.9 of study protocol,
11. Diagnosis of smoke inhalation injury,
12. Pregnant women (positive pregnancy test) or nursing mothers,
13. Poorly controlled diabetes mellitus (HbA_{1c}>11%) in patients with known diabetes as captured in the medical history,
14. BMI greater than 39.0 kg/m^2 in patients with burns area of up to 15% TBSA or BMI greater than 34.0 kg/m^2 in patients with burns area of more than 15% %TBSA,
15. ASA greater than 2 (Appendix 13- ASA classification system of study protocol),
16. Cardio-pulmonary disease (MI within 6 months prior to injury, severe pulmonary hypertension, severe COPD or pre-existing oxygen-dependent pulmonary diseases, severe broncho-pneumonia within 1 month prior to injury, steroid dependent asthma or uncontrolled asthma),
17. Pre-existing diseases which interfere with circulation (severe peripheral vascular disease, severe edema and/or lymphedema, regional lymph nodes dissection, significant varicose veins),
18. Any conditions that would preclude safe participation in the study or adding further risk to the basic acute burn trauma (such as severe immuno-compromising diseases, life threatening trauma, severe pre-existing coagulation disorder, severe cardiovascular disorder, significant pulmonary disorder, significant liver disorder including post alcoholic abuse impaired function or neoplastic disease, blast injury),
19. Chronic systemic steroid intake,
20. History of allergy and/or known sensitivity to pineapples, papaya, Bromelain or papain,
21. Current (within 12 months prior to screening) suicide attempt,
22. Mentally incapacitated adults who are incapable of giving legal consent Enrollment in any investigational drug trial within 4 weeks prior to screening,
23. Current (within 12 months prior to screening) severe alcohol or drug use disorder (see definition in section 1.1 of study protocol),

24. Prisoners and incarcerated,
25. Patients who might depend on the clinical study site or investigator.

7.1.4. Drop-out criteria

After screening and prior to treatment any changes in the patient's medical condition should be assessed. The patient should be excluded from treatment if these changes represent deterioration in his/her condition which may affect the patient's safety and/or suitability for the study (e.g. exclusion criteria).

Throughout the course of a subject's participation in the study, he/she may be removed from the study as follows:

- Whenever it is necessary to safeguard his/her welfare as judged by the Principle Investigator,
- Participation in another investigational drug trial,
- Subjects who express a desire to withdraw from the study,
- Subjects with EAR identified post randomization but prior to treatment.

The reason for any subject withdrawal from the study should be recorded in the eCRFs and the occurrence should be reported to the sponsor. Early termination visit should include final assessments as in the Hospital Discharge visit, as applicable (please refer to Section 8.7 of the study protocol).

Discontinued subjects will not be replaced.

When a subject is removed from the study treatment, he/she will receive treatment according to the standard of care as appropriate in the investigative site and will be followed up according to the study schedule, in accordance with patient’s availability.

7.1.4.1. Stopping rules (apply for NexoBrid or Gel Vehicle arms only)

Treatment (eschar removal) should be stopped following a safety concerns raised by the treating physician or if a patient expresses his desire to withdraw from the study. In addition, in the following situations:

Burn Induced Compartment Syndrome (BICS) Monitoring and Diagnosis

All circumferential wounds should be closely monitored during treatment for signs of improvement or deterioration that may require escharotomy. If clinical assessment and SpO_2 monitoring are possible, they will be done continuously and recorded in the eCRFs every 2 hours. If such assessment is not practical, direct pressure will be measured every 2 hours from start of the eschar removal. The eschar removal process shall be discontinued (escharotomy may be performed at the discretion of the physician in any of the following cases:

- Increasing interstitial/compartment pressure >30 mmHg
- Difference of >20 mmHg of diastolic pressure between the opposite uninjured extremity
- Decreased SpO2 reading during treatment with difference of >6% compared to a non injured extremity or deterioration of clinical signs
- Deterioration of any of the 5 “P” signs: Pain, Paralysis, Pulselessness, Pallor, Paraesthesia

Since in such cases the eschar removal procedure will not be completed as planned, such patients (if any) will still be part of the full analysis set (FAS, see Definition in Appendix 13.1.1) and any missing data usually collected during eschar removal (e.g. time to end of eschar removal, etc.) will be handled as missing data according to the protocol.

In case of 3 consecutive cases where there is a need to disrupt the dressing and perform a surgical escharotomy, enrollment will be halted and a DSMB shall be convened to:

- assess the severity of the phenomenon in a closed session
- Recommend on appropriate corrective measures, as applicable to prevent such reoccurrences

As discussed above, increased interstitial/compartment pressure is a common medical condition in circumferential deep burns and its detection and resolution is part of daily standard of care. The only difference in the treatment of the patients in the study is during the eschar removal stage as dictated by randomization.

Coagulopathy during or within 24 hours of eschar removal

It is evident that during the first 48 hours post injury there is deterioration in coagulation parameters in burn patients [22]. In addition, some burn patients are treated with

anticoagulants after admission (in some burn centers all patients are treated with anticoagulants). Due to these facts, any stopping rule concerning coagulopathy after topical debriding agent application must take these factors into consideration.

Enrollment will be halted and a DSMB will convene if 3 subjects develop the following signs which have been defined as life threatening coagulopathy in trauma patients:

INR>2 or a PTT>2 times the normal range [23], in cases where the patient is not receiving anticoagulants that can explain the coagulopathy.

A DSMB shall be convened to assess in a closed session the following:

- The relationship between coagulopathy and the treatment arm
- The severity of the phenomenon
- Recommend on appropriate corrective measures, as applicable to prevent such reoccurrences

DSMB investigation will consider patients' medical history, including any anticoagulants given to the patient prior to start of treatment.

Post treatment diagnosed infection (until complete wound closure)

Following the eschar removal treatment, in case 3 subjects develop infections listed in Section 11.9 of the study protocol and are assessed as related to the treatment arm (possibly, probably or related as defined in section 12.1 of the study protocol), enrollment will be halted and a DSMB will convene in close session to investigate cases reported and their relatedness and severity.

In all the above 3 described cases, enrollment may continue in accordance with DSMB conclusions.

7.1.5. Concomitant medication and treatment

Concomitant medications will be recorded throughout the study, starting from signing on ICF and will include those prescribed for common burn management practice. Any record should include dose, frequency, route, start and stop date and indication.

Unit of blood transfused (whole blood or blood packed cells) with the appropriate volume will be captured for the entire patient's duration in the study and recorded in the concomitant medications form. Any analgesia and anesthesia administered per subject will be recorded in the eCRFs. Incidence & number of procedures for wound care requiring anesthesia will be analyzed.

7.2. Trial schedule

The healing and care of the burn wound may be divided into the three distinctive phases:

1. Eschar removal treatment phase (until complete eschar removal)

2. Wound management (until complete wound closure)

3. Scar modulation and maturation

These phases are common to the care of all wounds and are well defined in acute wounds such as burns.

In this study, these phases and their treatment strategy are equal for all arms, NexoBrid, SOC, and Gel Vehicle, following the ABA white paper recommendations [24].

The total duration of the study treatment and follow up period of each participating subject is expected to be 25 months: following the Eschar removal procedure, each subject will be followed up weekly (Weeks 1, 2, 3 and 4 etc.) until complete wound closure is achieved and 2 weeks later for wound closure confirmation, for each of the subject's TWs. Long-term follow-up visits will be performed at 1, 3, 6, 12, 18 and 24 months post last wound closure confirmation visit (for details see Table 1).

Table 1: Treatment flow chart - all arms[3]

#- If applicable
* Weekly FU assessments will be performed every 7± 2 days from start of treatment until complete wound closure. Long-term FU will be performed 1,3,6,12,18 and 24 M after last wound closure confirmation.
**First treatment session will include any procedure for eschar removal: the NexoBrid/ /Gel Vehicle/Surgical or non-surgical procedure, as per randomization arm
** Procedure can be either surgical or non-surgical. A whole period of continuous dressing changes (from first coverage application to last coverage application) is considered as one non-surgical procedure, assessments should not be repeated for each dressing change; Pre procedures assessments refer only to surgical procedures. Post procedures assessments refer to any surgical procedures and for non-surgical procedure only if it is the last procedure

Parameter	Screening tests	Baseline within 1 h Pre First treatment session - All arms	Topical arms (NexoBrid or Gel Vehicle): (refer to first and second application). S=Soaking, R=Removal	SOC procedures** (including additional procedures in the topical arms)	Daily assessments	Wound Management & Re-admissions[4]- surgical procedures assessment	HD or premature discontinuation visit	Weekly FU*	Long-Term FU (1, 3, 6,12,18 & 24 months)*
Inform Consent signing	X								
Medical history	X								
Burn History[5]	X								
ConMeds & AEs	X	X	X	X	X		X	X	X
Physical examination[6]	X						X		
Demographic data	X								
Vital Signs	X		Post S	Post	X				
Pain assessment	X	X	4h post R	Pre and 4h post	X				
Photograph of TWs	X[7]	X	Post S	Pre & Post		Pre & Post		X	X

[3] This table differs from the corresponding table in the protocol. Minor corrections were performed.

[4]Wound Management will include any, post complete eschar removal additional excisions, Autografts, and donor site data if relevant.

[5] Data captured will include burn etiology, mechanism of injury, place of injury, treatments prior to admission and general description of the burn wound (anatomical locations and description of the eschar (such as: white, moist, dry, gray, charcoal, etc.), % TBSA assessments and depth

[6] Physical examination will include height and weight. Height will be taken only once, at screening.

[7] Photos should be taken after cleansing

Parameter	Screening tests	Baseline within 1 h Pre First treatment session - All arms	Topical arms (NexoBrid or Gel Vehicle): (refer to first and second application). S=Soaking, R=Removal	SOC procedures** (including additional procedures in the topical arms)	Daily assessments	Wound Management & Re-admissions[4]- surgical procedures assessment	HD or premature discontinuation visit	Weekly FU*	Long-Term FU (1, 3, 6,12,18 & 24 months)*
Clinical assessment of the burn[8]	X	X	Post S	Post		Post		X	
Local lab - HbA1C, WBC, βhCG	X								
Central lab hematology/ biochemistry tests & Urinalysis		X	4 h Post R	Pre & 4 h Post					
Central lab Hematology						1h pre & 4 h post			
Cleansing including at least 2h soaking)	X	#							
Fluid balance[9]	X	X	X	X	X				
Randomization	X								
Local lab Wound culture		X	Post S	Pre &Post					
Local lab Blood culture		X	4h Post R	4 h Post					
12-Lead ECG (if feasible)		60, 40, 20 min pre treatment	During: 0.5, 2,4h from start of first application only	During: 0.5, 2, 4h from start of first treatment only	12, 24, 48h			week 1	

[8]Clinical assessments will include % TBSA per depth, % eschar removed, % excised, % autografted, % wound epithelialization (TW and donor site, if relevant), % graft take as relevant.

[9] Daily fluid balance will be monitored for 7 days from injury or until hospital discharge, whichever comes first for patients with catheter.

Parameter	Screening tests	Baseline within 1 h Pre First treatment session - All arms	Topical arms (NexoBrid or Gel Vehicle): (refer to first and second application). S=Soaking, R=Removal	SOC procedures** (including additional procedures in the topical arms)	Daily assessments	Wound Management & Re-admissions[4]- surgical procedures assessment	HD or premature discontinuation visit	Weekly FU*	Long-Term FU (1, 3, 6,12,18 & 24 months)*
PK (for a subset of 32 NexoBrid Pts)		X	During:0.5, 2, 4h from start of first application		12,24,48, 72h				
Immunogenicity assessment (NexoBrid patients only)		X						weeks 4,8[10]	Long-term FU: 6, 24
Local lab PTT & INR		X	4 h post R - first application only	4 h post- first treatment only	24,48h				
Circumferential extremity wounds monitoring[11]	X	X	during and post R (4 hours from start)	1h pre & post					
Eschar Removal treatment			X	X					
Coverage				Start and end date , type of primary dressing		Type of primary dressing& frequency			

[10] In case that 8 weekly visit is not performed, this assessment should be taken on the relevant weekly/first visit as close as possible to 8 weeks.

[11] The Extremities At Risk (EAR) burns prone to develop BICS will be identified at screening and excluded. Monitoring for the development of EAR in circumferential extremity wounds at the above specified timelines will be done. During each topical application (NexoBrid or gel Vehicle), clinical assessment and SpO2 monitoring will be done continuously. If such assessment is not practical, direct pressure will be measured every 2 hours from start of eschar removal. Direct pressure will be measured before and after each eschar removal procedure and additional surgical procedures

Parameter	Screening tests	Baseline within 1 h Pre First treatment session - All arms	Topical arms (NexoBrid or Gel Vehicle): (refer to first and second application). S=Soaking, R=Removal	SOC procedures** (including additional procedures in the topical arms)	Daily assessments	Wound Management & Re-admissions[4]- surgical procedures assessment	HD or premature discontinuation visit	Weekly FU*	Long-Term FU (1, 3, 6,12,18 & 24 months)*
Wound closure confirmation[12]								X	Long-term FU: 1, 3
Cosmesis, Function and Quality Of Life[13]									X
Scar-modulating procedures and scar						#			#
Blood transfusion (units & volume)	#	#	#	#	#	#	#	#	#
Histological biopsy	#	#	#	#	#		#	#	#

[12] Wound closure should be confirmed 2weeks after the initial wound closure assessment for each TW.
[13] Cosmesis will be evaluated in donor site scars as well

7.3. Criteria for evaluation

7.3.1. Demographic data and other baseline characteristics

The following demographic data will be documented at screening/baseline:

- Date of birth
- Gender
- Ethnicity
- Race
- Serum Pregnancy Test (βHCG)

Furthermore, at screening the inclusion and exclusion criteria will be collected.

At baseline the following parameters will be documented.

Burn history / description:

- Date and time of injury
- Etiology of injury (Fire/Flame, Scald, Contact)
- Place of injury (Outdoors, Indoors, Car, Other)
- Information whether patient was transferred from another facility
- Date and time of primary admission
- Date and time of current admission

General wound description:

- Wound number
- Anatomical location
- %TBSA of 2° SPT Burns
- %TBSA of 2° DPT Burns
- %TBSA of 3° FT Burns
- Information whether the respective wound will be designated as TW
- Information whether a wounded extremity is circumferential
- Extremities at risk

- Information whether eschartomy had been performed prior to randomization
- Eschar description
- Information whether cleansing was performed
- Information whether all superficial keratin (blister) were removed from wound

Target wound identification:

- Target wound number
- Anatomical location
- %TBSA of 2° SPT Burns
- %TBSA of 2° DPT Burns
- %TBSA of 3° FT Burns
- Information whether the wound was treated prior to enrollment

Information needed for randomization:

- Total wounds TBSA % per patient
- TWs DPT total area (% TBSA)
- TWs FT total area (% TBSA)
- Overall TWs depth per patient
- Overall TW area (SPT + DPT + FT)
- Randomization result (treatment)
- Date of randomization

Circumferential extremity wounds:

- Information whether any treated TWs were circumferential extremity wounds
- Target wound number
- Information whether pressure measurement was done
- Interstitial pressure (mmHg)
- Date and time of measurement

Physical examination:

- Height (cm or inch)
- Weight (kg or lb)
- BMI

Furthermore, for each of the following body systems 4 categories are documented (normal, abnormal-NCS [not clinically significant], abnormal-CS [clinically significant], not done). In case of abnormal findings, they have to be documented. If abnormality is present for another body system, this has to be documented, too. Reasons for the category "not done" have to be documented.

- Head, eyes, ears, nose and throat (HEENT)
- Respiratory system
- Cardiovascular system
- Abdomen
- Musculoskeletal / Extremities
- Neurological
- Lymph Nodes

Vital signs:

- Temperature (incl. unit and method of measurement)
- Heart rate (beats/min)
- Respiratory rate (breaths/min)
- Systolic and diastolic blood pressure (mmHg)
- Assessment of vital signs (normal, abnormal-NCS, abnormal-CS)

Pain assessment:

- Pain measured via visual analogue scale (VAS, mm from the left point of the scale)
- Assessment of pain (normal, abnormal-NCS, abnormal-CS)

Local laboratory:

- Leukocyte count (WBC)
- Assessment of leukocyte count (normal, abnormal-NCS, abnormal-CS)
- Information whether patient is diabetic
- HbA1c (%)

Information on infections as well as the medical history will be recorded.

7.3.2. Efficacy evaluation

7.3.2.1. Primary target variable

The following primary endpoint will be evaluated at a 'per patient' level i.e., on the full set of target wounds treated for each patient (please refer to protocol Section 1.1 for target wound definition) in accordance with the study protocol:

Incidence of complete eschar removal (see definition in Appendix 13.1.1) in the topical arms (NexoBrid and Gel Vehicle): Incidence of complete eschar removal (ER) will be based on the assessment of the blinded assessor at the end of the topical agent soaking period. In cases where 2 applications are performed, complete ER will be based on the assessment at the end of the topical agent soaking period following the 2nd application.

7.3.2.2. Secondary target variables

The following secondary endpoints will be evaluated in this study:

Reduction in surgical needs

Reduction of surgical need for excisional eschar removal is measured by an analysis of incidence of surgical eschar removal (tangential/ minor/ avulsion/ Versajet and/or dermabrasion excision).

Earlier eschar removal

The time when complete eschar removal has been achieved will be assessed for each patient on the entire eschar removal period. For definition of complete eschar removal, see Appendix 13.1.1.

Blood loss

Blood loss incurred during the eschar removal procedures will be evaluated. Actual blood loss will be measured by considering the estimated blood loss, changes in Hemoglobin during the eschar removal procedures and the amount of units of blood transfused. Blood loss (Actual Blood Loss, ABL) will be calculated using the following formula [25]:

$$ABL = \frac{EBV * \left(Hb_{before} - Hb_{after}\right)}{\left(Hb_{before} + Hb_{after}\right)/2} + V_{WB} + \frac{5}{3}V_{PC}$$

EBV= Estimated blood volume is assumed 70 cm^3/Kg

(Hb_{before}- Hb_{after})= Changes in Hb following each eschar removal procedure

V_{WB}= Volume [mL] of whole blood transfused

V_{PC}= Volume [mL] of packed red blood cells transfused.

The ABL will be summed over all procedures carried out to remove eschar, with the Hb measured immediately before each procedure and 4 hours after its completion. The amount of blood transfused will be summed over all transfusions carried out during all debridement procedures and 4 hrs later i.e. the before-after periods that are spanned by these Hb measurements.

Additionally, ABL will also be computed using the formula above, but over the whole eschar removal period (not procedure wise) i.e. the eschar removal process will be considered as one continuous procedure. Therefore, only the Hemoglobin value before the first eschar removal procedure and after the last procedure will be used and all blood transfusions performed between these measurements will be included.

Analyses with both blood loss values will be conducted. However, some missing values are expected. The analysis with less missing values will be regarded as more reliable and is therefore considered the main analysis. The other analysis is considered supportive.

7.3.3. Safety evaluation

7.3.3.1. Time to complete wound closure

Time to reach complete wound closure (definition see Appendix 13.1.1) as assessed by the blinded assessor and marked in the eCRFs will be assessed in days, starting from randomization date.

7.3.3.2. Cosmesis and function at 12 months from wound closure confirmation

Cosmesis and Function assessment will be performed based on the Modified Vancouver Scar Scale (MVSS) at 1, 3, 6, 12, 18 and 24 months after the wound closure confirmation visit by a

burn wound specialist who is masked to randomized wound treatment and to the other outcome assessments of the same wound. Table 2 gives an overview of the MVSS.

Table 2: Modified Vancouver Scar Scale (MVSS)

Pigmentation	***Pliability***	***Height***	***Vascularity***	***Pain***	***Pruritus***
(0) Normal	(0) Normal	(0) Flat	(0) Normal	(0) None	(0) None
(1) Hypopigmentation	(1) Supple-flexible with minimal resistance	(1) <2mm	(1) Pink	(1) Occasional	(1) Occasional
(2) Mixed	(2) Yielding- giving way to pressure	(2) 2-5 mm	(2) Red	(2) Requiring medication	(2) Requiring medication
(3) Hyperpigmentation	(3) Firm- inflexible, not easily moved, resistance to manual pressure	(3) >5mm	(3) Purple		
	(4) Banding-rope-like tissue that blanches with extension of the scar				
	(5) Contracutre-perment shortening of scar, producing deformity or distortion				

7.3.3.3. Cosmesis and function at 24 months from wound closure confirmation

See section 7.3.3.2.

7.3.3.4. Adverse Events

For each AE the following information will be determined:

- AE description
- AE is an infection (yes, no, unknown)
- onset date and time
- outcome date and time
- outcome (resolved, resolved with sequelae, not resolved, death)
- intensity (mild, moderate, severe)
- action taken regarding study drug (yes, no)
- relation to study drug (not related, remotely related, possibly related, probably related, related)
- treatment required (none, medication, surgery, hospitalization, other)
- seriousness (serious, not serious)

The following definitions to assess the relationship between an adverse event and the study drug is used:

- Not Related: The event is clearly related to other factors such as a patient's clinical state, therapeutic interventions or concomitant medications.
- Remotely Related: The event was most likely produced by other factors such as a patient's clinical state, therapeutic interventions or concomitant medications and does not follow a known response pattern to the study drug.
- Possibly Related: The event has a reasonable temporal relationship to study Drug administration and follows a known response pattern to the study Drug. However, a potential alternate etiology may be responsible for the event. The effect of Drug withdrawal is unclear. Rechallenge information is unclear or lacking.
- Probably Related: The event follows a reasonable temporal sequence from the time of Drug administration and follows a known response pattern to the study Drug and cannot be reasonably explained by other factors. There is a reasonable response to withdrawal of the Drug. Re-challenge information is not available or advisable.
- Related: The event follows a temporal sequence from the time of Drug administration and follows a known response pattern to the study Drug and either occurs immediately

following study Drug administration, or improves on stopping the Drug, or reappears on repeat exposure.

For the assessment of the intensity of the AE the following definitions should be used:

- Mild Adverse Events: A mild adverse event is one that the symptoms are barely noticeable to a patient. It does not influence performance or prevent a patient from carrying on with normal life activities.
- Moderate Adverse Events: A moderate adverse event is one that the symptoms make a patient uncomfortable and causes some impairment to normal life activities. Treatment for symptom(s) may be required.
- Severe Adverse Events: A severe adverse event is one that the symptoms cause severe discomfort to a patient and severely limits the patient's normal daily activities. Treatment for symptom(s) is given. Note that serious and severe are not synonymous. A serious adverse event must fulfill the requirements listed in the definition below.

A serious adverse event is an adverse event that is:

- fatal
- life-threatening
- results in persistent or significant disability/incapacity
- requires or prolongs inpatient unexpected hospitalization
- is a congenital anomaly or birth defect or
- is a medically important event.

7.3.3.5. Laboratory assessments and vital signs

The following tests described below are performed. Any abnormal findings must be assessed for clinical significance – Abnormal NCS or Abnormal CS:

Serum chemistry, hematology and urinalysis testing will be performed at a central laboratory during the eschar removal phase. Any additional procedures during the wound management phase require hematology tests, as described in the protocol, and will be performed using Central lab. Pregnancy testing, HbA1c, leukocyte count (at Screening, in addition to central lab hematology), wound cultures, blood cultures, PTT/INR and biopsies will be performed locally on-site.

The following tests are performed in special laboratories: Pharmacokinetics test, Immunogenicity evaluation and evaluation of QT prolongation (using multiple ECG tests).

Serum Chemistry:

Urea, Creatinine, Glucose, Total Bilirubin, Cholesterol, Triglycerides, Uric Acid, Total Protein, Albumin, Globulin, Calcium (Ca++), Phosphorous, Sodium (Na+), Potassium (K+), Chloride (Cl-), Alkaline Phosphatase, SGOT (ASAT), SGPT (ALAT), LDH.

Hematology:

Hemoglobin, Hematocrit, RBC, MCV, MCH, MCHC, Platelets, Leukocyte count (WBC) with differential count (absolute numbers).

HbA_{1c} will be assessed at a local lab at Screening for diabetic patients as captured during the medical history.

PTT and INR will be tested at a local lab pre treatment and 4 hours after start of eschar removal.

Pregnancy Testing:

A serum pregnancy (βHCG) will be performed at a local lab for women of child bearing potential.

Urinalysis:

pH, Ketone, Specific gravity, Protein, Glucose, Bilirubin, Microscopic examination.

Qualitative Wound Culture:

Test burn wound for microbial flora (swab).

Blood Culture:

Test blood for microbial flora.

Diagnosis of Invasive Burn Wound Infection is based on Quantitative (Histological) Wound Culture Biopsy:

Infection should be assessed clinically by symptoms and signs that include local burn wound signs purulent drainage, erythema, warmth, exudation, odor, pain, and systemic signs that may or may not be related to the burn wound, such as fever, and leukocytosis as well as wound size and time to wound healing. Histological Biopsy may be performed to rule out suspicion of a clinical invasive burn wound infection (please refer to Appendix 8- Biopsy & Histologic Assessment Method) [26].

Clinical Invasive Burn Wound Infection will be defined when an Invasive Burn Wound Infection is diagnosed by quantitative bacterial count of >105 organisms per gram of tissue and histological signs of bacterial invasion of healthy tissues will define a positive invasive burn

wound infection. At least one of the following histological signs of invasive burn wound infection is required:

- Microorganisms presence in viable unburned tissue
- Small vessel thrombosis and ischemic necrosis of unburned tissue
- Inflammation evident in unburned tissue
- Hemorrhage in uninjured tissue
- Evidence of microbial proliferation
- Variable migration of microorganisms along hair follicle and sweat glands
- Dense growth of nonviable-viable tissue interface (subeschar space)
- Intracellular viral inclusion
- Light microscopy
- Type A Cowdry bodies: herpes simplex virus-1
- Owl's eye inclusion bodies: cytomegalovirus
- Electron microscopy
- Intracellular virions

Vital signs (temperature, pulse, blood pressure and respiration rate) will be completed 24 hours prior to start of treatment and once daily from start of eschar removal until hospital discharge.

7.3.3.6. Additional safety endpoints

Additional safety endpoints to be evaluated in this study are:

- pain assessment (using VAS and as reported as AEs)
- units (and volume) of blood transfusion given during hospitalization
- Immunogenicity evaluation for NexoBrid patients
- Incidence of increased interstitial/compartment syndrome
- Incidence of QT prolongation
- Extent of analgesia, anaesthesia and antibiotic use
- Rates of hospital readmission
- Change in INR/PTT and incidence of change to > upper limit of normal (after treatment)
- Change in blood glucose and incidence of change to above upper limit of normal (after treatment)
- Long-term functionality evaluation of the extremities using the ‘Lower Extremity Functional Scale’
- 'QuickDASH' questionnaires

- 'Range Of Motion' measurements
- Long-term Quality of Life using EQ5D and Burn Specific Health Scale- Brief (BSHS-B)

7.3.4. Exploratory analyses

1. Reduction in surgical needs as measured by an analysis of % wound area surgically excised for eschar removal (and % TBSA excised of the treated TW) (tangential/ minor/ avulsion/ Versajet and/or dermabrasion excision),
2. Additional Cosmesis and Function evaluation will be performed using the Patient and Observer Scar Assessment Scale (POSAS) version 2.0 at 1, 3, 6, 12, 18 and 24 months after the wound closure confirmation visit by a burn wound specialist who is masked to randomized wound treatment and to the other outcome assessments of the same wound. POSAS version 2.0 was published in 2005 in Plast. Reconstr. Surg. 116: 514-522 by van de Kar et al. The POSAS consists of two parts: a Patient Scale and an Observer Scale. Both scales contain six items that are scored numerically and make up a 'Total Score' of the Patient and Observer Scale. The POSAS has been developed for all types of scars and has been tested in linear scars, burn scars and keloids and was found to be validated and reliable [27].

   Each item of both scales has a 10-point score, with 10 indicating the worst imaginable scar or sensation. The lowest score is '1', and corresponds to the situation of normal skin (normal pigmentation, no itching etc). The score goes up to the worst imaginable. The Total Score can be simply calculated by summing up the scores of each of the six items. The Total Score will therefore range from 6 to 60 (observer and patient) and 12-120 in total.

   POSAS will be evaluated for all measurement times mentioned above,
3. Additional Cosmesis and Function evaluation will be performed using the Modified Vancouver Scar Assessment Scale (MVSS) measurements obtained at 1, 3, 6 and 18 months,
4. Incidence of surgical Escharotomy procedures on circumferential extremities target wounds,
5. Incidence of reduction in interstitial/compartment pressure in circumferential extremity wounds (measured immediately following eschar removal),
6. Reduction in surgical need as measured by analysis of incidence of surgically harvested donor sites wounds,
7. Reduction in surgical need as measured by analysis of % area of surgically harvested donor site wounds,

8. Blood loss following eschar removal procedures using changes in Hematocrit following eschar removal procedures,
9. Cosmesis MVSS and POSAS will be used to assess the quality of the donor sites scars,
10. PK evaluation for a subset of NexoBrid patients (as described in section 11.21 of the protocol)
11. Autograft related parameters.
    - 11.1. Efficacy and safety analyses for early and late grafted wounds (for definition see Appendix 13.1.1)
    - 11.2. Total number of target wound grafting procedures
    - 11.3. Incidence of repeated/additional grafting procedures
    - 11.4. Area of repeat grafting
12. Duration of hospitalization

8. Handling of protocol violations

Before database lock and statistical analysis, possible protocol violations will be discussed by data management, sponsor, PI and his/her proxy and the statisticians and classified as minor or major violations. The list with all protocol violations will be prepared by the data management.

A protocol violation will be classified as major if

- the treatment differs from the planned treatment (randomization arm), or
- the documentation of a primary observation was not done or is different from the study protocol: i.e. assessment was not done by a blinded assessor (assessment by blinded assessor from photos is acceptable), or
- there was drug misuse (this may include application time deviated in 25% or more, deviation of more than 20% in the TBSA area treated in one session at a time, if relevant, error in drug preparation (e.g. 2g mixed in 50g), storage temperature below -2°C - 8°C or >24h at room temperature and used without any stability confirmation by sponsor), or
- the first blinded assessor was unblinded, or
- treatment started more than 5 days from injury, or
- one of the following inclusion criteria is violated:
    - Males and females; ≥ 18 years of age,
    - Thermal burns caused by fire/flame, scalds or contact,
    - Patient total burns area ≥ 3% DPT and / or FT,
    - Patient total burns area should be ≤ 30% TBSA; SPT, DPT and/or FT in depth,
    - Informed consent can be obtained within 84 hours of the burn injury
    - At least one wound (a continuous burn area) that is ≥0.5% TBSA (DPT and/or FT) (this minimal wound size should not include face, perineal or genital)
    - Wound's blisters can be removed/unroofed, as judged by the investigator
- one of the following exclusion criteria is met:
    - Patients with burned charred fingers, 3rd degree in depth and possibly devoid of circulation
    - Patients with abraded wound/s that cannot be treated by an enzymatic debrider application (NexoBrid)
    - Patients with electrical or chemical burns
    - The following pre-enrollment dressings: a. Flammacerium, b. Silver Nitrate ($AgNO_3$)

- Patients with pre-enrolment wounds which are covered by eschar heavily saturated with iodine or by SSD pseudoeschar (e.g. pseudoeschar as a result of >12 h SSD treatment),
- Patients with pre-enrollment escharotomy
- Enrollment in any investigational drug trial within 4 weeks prior to screening

In all other cases protocol violations will be classified as minor.

9. Populations Analyzed

The following sample size calculation is taken from the protocol.

9.1. Sample Size

The following sample size calculations are based on the consideration of the primary and two secondary endpoints for this study.

Estimated effects of NexoBrid from study MW2004-11-02 were used to design the study with adequate statistical power using the following steps.

(a) Sample size calculations were based on study MW2004-11-02;

(b) The effect of NexoBrid versus standard of care was estimated together with the standard error of the estimate; the effect of NexoBrid versus gel was estimated based on study MW2002-04-01, previous phase II study, which included 35 patients treated with gel vehicle.

The size of the effect to be detected in this study, MW2010-03-02, was taken as the previous estimate decreased by half its standard error, in order to use a conservative value.

9.1.1. Primary Endpoint: Incidence of complete eschar removal (NexoBrid vs. Gel)

Estimated proportion achieving complete eschar removal (for further definition and explanation see section 7.3.2.1) in all TWs from study MW2004-11-02 (analyzed as post hoc): NexoBrid 0.595

Standard error = 0.057

Anticipated proportion achieving complete eschar removal using NexoBrid: 0.595-0.057/2 = 0.5665

Estimated proportion achieving complete eschar removal using Gel vehicle in a previous Phase II study, MW2002-04-01: 0 (0 out of 35 patients).

Anticipated proportion achieving complete eschar removal using gel: 0.0 or 0.10. (0.0 is the point estimate; 0.10 is the 97.5 upper confidence limit).

Because numbers of complete eschar removals are anticipated to be low in the gel group, we calculate sample size using Fisher's exact test. We used the computer program nQuery Advisor version 7.0 to calculate power for a two-sided significance level of 5%, and thereby

found the following sample size combination that led to approximately 90% power for detecting a statistically significant difference at the 5% level, under the assumption of a 10% rate in the Gel Vehicle group (see above).

For 90% overall power, number in NexoBrid group = 65 and in Gel vehicle group 13. Total sample size = 78.

9.2. Secondary Endpoint: Incidence of surgical excision

Estimated proportions having surgical excision from study MW2004-11-02: NexoBrid 0.22; SOC 0.62

Standard errors: NexoBrid 0.048; SOC 0.054

Difference = 0.40; Standard error of difference = 0.072

Anticipated difference: 0.40-0.072/2 ≈ 0.36

We therefore take the anticipated proportion with complete excision to be: NexoBrid 0.24 and SOC 0.60.

Using Fisher's exact test, the sample size needed to achieve 90% power using a two-sided significance level of 5% is **86**: 43 in the NexoBrid group and 43 in the SOC group.

9.3. Secondary Endpoint: Time to complete eschar removal

The logrank comparison between NexoBrid and SOC in the previous trial yielded an estimated log hazard ratio of -1.37 (hazard ratio of 0.254) with a standard error of 0.28.

Therefore, target log hazard ratio (log HR) is -1.37 +0.28/2 = -1.23 (hazard ratio = 0.29).

The formula for the number of events (successful complete eschar removals) is:

$$d = \frac{4\left(z_\alpha + z_\beta\right)^2}{(\log HR)^2}$$

where z_α = 1.96 two-sided significance level of 5%; and z_β = 1.28 for 90% power; For 90% overall power, number of events = 28.

Proportions of events: p_1 = 0.595 in NexoBrid and p_2 = 0.728 in SOC.

Total number of patients required $n = \frac{2d}{p_1+p_2} = 44$ (to the nearest even number), 22 in each of the NexoBrid and SOC groups.

9.3.1. Summary of sample size

Although the maximum sample size from the above calculations is a total of 121 patients (65 in the NexoBrid group, 13 in the Gel vehicle group and 43 in the SOC group (to match NexoBrid numbers)), we propose entering larger numbers to provide an extra margin of assurance in achieving positive results on the efficacy outcomes, and adequate information on safety outcomes to allow better benefit vs. risk assessment.

Therefore, we plan a study with total sample size of 175 patients; 75 (NexoBrid) + 75 (SOC) + 25 (Gel Vehicle).

9.4. Safety Set

The safety set includes all patients who received a treatment and analysis is focused on the treatment actually performed.

9.5. Full analysis Set (Intent-to-Treat population)

The full analysis set (FAS) includes all patients who are randomized into the trial. The analysis is focused on the planned treatment.

9.6. Per Protocol Set

The per protocol (PP) set includes all subjects who do not have major protocol violations (cf. section 8). Analysis of the PP population is for supportive purposes, whereas the analysis of the ITT population (FAS) provides the primary and secondary efficacy and the comparative safety analyses. The PP analysis will include patients in the treatment group according to treatment received.

9.7. Enrolled population

The enrolled population consists of all subjects who passed through the screening processes and signed inform consent.

10. Data Handling

10.1. Handling of Missing Data, Outliers and Implausible Data

Missing data in the primary and secondary outcomes is the main concern.

10.1.1. Primary Endpoint: Incidence of complete eschar removal

Little or no missing data are expected for the primary endpoint, since the outcome is assessed only several hours after the start of treatment for both NexoBrid and Gel Vehicle groups. The main analysis will be a complete-case analysis. In each treatment group, only patients with documented total eschar removal (at the end of the eschar removal soaking period) will be counted and this number divided by the total number of complete cases.

Three sensitivity analyses will be conducted for this endpoint. A first analysis will count all patients with missing data (for this endpoint) as positive (i.e. complete eschar removal) and a second analysis will count all patients with missing data as negative (i.e. no complete eschar removal). The third sensitivity analysis will be a tipping point analysis, in which the effect of missing data on the conclusion will be investigated. This analysis will be conducted as follows:

1. Impute the entries of the incomplete data sets m = 100 times from Bernoulli distributions. For the imputations of the Gel Vehicle group, the event probability p is chosen as the observed incidence of complete eschar removal in the Gel group (without missing data). For the NexoBrid group the event probability will be $p = p_{obs} - s$, where s is a shift parameter (see below). The random seeds for the imputations will be 14983,…,15082.
2. For each of the 100 imputed data sets compute Fishers exact test and calculate the proportion of tests that reject the null-hypothesis.
3. Repeat steps 1. and 2. for $s = 0, 0.05, ...$ as long as $p_{obs} - s$ is still non-negative.

The analysis outlined here allows investigating for which assumed success probability for the patients with missing data in the NexoBrid group, the conclusion of the analysis remains stable.

Secondary Endpoints

10.1.1.1. Secondary Endpoint: Incidence of surgical excision

The same procedure as for the primary endpoint will be applied for this endpoint, i.e. the main analysis will be a complete-case analysis. In each treatment group, only patients with documented surgical excision will be counted and this number divided by the total number of randomized subjects with non-missing endpoint. Two sensitivity analyses will be conducted for this endpoint. A first analysis will count all patients with missing data (for this endpoint) as positive (i.e. no surgical excision performed) and a second analysis will count all patients with missing data as negative (i.e. surgical excision performed).

10.1.1.2. Secondary Endpoint: Time to complete eschar removal

The analysis of the secondary endpoint time to eschar removal will compute missing values as censored at the date of the last non-missing eschar removal assessment (typically last debridement procedure).

10.1.1.3. Secondary Endpoint: Blood loss

Missing blood loss values for some eschar removal procedures may occur within a certain patient. A regression model containing main prognostic factors (wound area, depth of wound and type of procedure) for blood loss of eschar removal procedures will be computed on a procedure level treating all procedures as independent. On this basis a multiple imputation will be calculated. The procedure can be described as follows:

1. Test for normality: The Shapiro-Wilk test will be performed on the dataset of non-missing values in each treatment group. The result of these tests will determine the type of the analysis on the imputed data sets (step 3). If the normal distribution hypothesis is not rejected at the 5% significance level in either group, then differences in distribution between NexoBrid and SOC will be tested using a t-test. If the normal distribution hypothesis is rejected either in the NexoBrid group or in the SOC group, then the differences in distribution between the treatment groups will be tested using a Mann-Whitney test.
2. Imputation: Fit a linear regression model with target variable blood loss on a procedure level (as computed in Section 10.3.3.3) and dependent variables size of wounds treated in this procedure (wound area), proportion of area SPT of wounds treated in this procedure (as defined in Section 10.3.3.3.2), proportion of area DPT of wounds treated in this procedure (as defined in Section 10.3.3.3.3), proportion of area FT of wounds treated in this procedure (as defined in Section 10.3.3.3.4) and type of

procedure (surgical/non-surgical) to the subset containing patients with complete data in those variables for both treatment groups separately. Impute the missing entries of the incomplete data sets m = 25 times as follows: Draw a set of regression coefficient from their sampling distribution obtained from fitting the respective linear models outlined above (a different distribution for the regression coefficients will be obtained in the different treatment groups). With these sampled coefficients calculate the predicted blood loss values for the procedures with missing blood loss using the covariate information. To these predicted blood loss values add the realizations of a normally distributed random variable with zero mean and standard deviation equal to the residual standard deviation from the linear model fitted to the complete data set. The random seeds for these 25 imputations will be (11468, 11469, 11470, ..., 11492). On the imputed datasets calculate the blood loss on a patient level.

3. Analysis: Analyze each of the m complete data sets with a t-test or the Mann-Whitney test according to the test result in step 1. This step results in m estimates of the effect and m estimates of the standard deviation.
4. Pooling: Integrate the m analysis results into a final result with the SAS procedure PROC MIANALYZE according to Rubin's combination rules for multiple imputation to obtain the final estimates of mean and variance [28].

For the additional analysis of blood loss which treats the whole eschar removal process as one continuous procedure (see section 7.3.2.2) the multiple imputation will be performed on a patient level. The procedure can be described as follows:

1. Test for normality: The Shapiro-Wilk test will be performed on the dataset of non-missing values in each treatment group. The result of these tests will determine the type of the analysis on the imputed data sets (step 3.). If the normal distribution hypothesis is not rejected at the 5% significance level in either group, then differences in distribution between NexoBrid and SOC will be tested using a t-test. If the normal distribution hypothesis is rejected either in the NexoBrid group or in the SOC group, then the differences in distribution between the treatment groups will be tested using a Mann-Whitney test.
2. Imputation: Fit a linear regression model with target variable blood loss on a patient level (as computed in Section 10.3.3.3) and dependent variables wound area, depth of wound (categorized as described in Section 11.1.10) and course of debridement procedure (as defined in Section 10.3.3.3.5) to the subset containing patients with complete data in those variables for both treatment groups separately. Impute the missing entries of the incomplete data sets m = 25 times as follows: Draw a set of

regression coefficient from their sampling distribution obtained from fitting the respective linear models outlined above (a different distribution for the regression coefficients will be obtained in the different treatment groups). With these sampled coefficients calculate the predicted blood loss values for the procedures with missing blood loss using the covariate information. To this predicted blood loss values add the realization of a normally distributed random variable with zero mean and standard deviation equal to the residual standard deviation from the linear model fitted to the complete data set. The random seeds for these 25 imputations will be (12467, 12468, 12469, ..., 12491). On the imputed datasets calculate the blood loss on a patient level.

3. Analysis: Analyze each of the m complete data sets with a t-test or the Mann-Whitney test according to the test result in step 1. This step results in m estimates of the effect and m estimates of the standard deviation.
4. Pooling: Integrate the m analysis results into a final result with the SAS procedure PROC MIANALYZE according to Rubin's combination rules for multiple imputation to obtain the final estimates of mean and variance [28].

10.1.2. Safety Endpoints

10.1.2.1. Safety Endpoint: Time to reach complete wound closure

The analysis of the safety endpoint time to complete wound closure will compute missing values as censored at the date of the last non-missing wound closure assessment.

10.1.2.2. Safety Endpoint: Cosmesis and function at 12 months from wound closure

A higher ratio of missing values is expected in the case of later MVSS values, especially for 24 months. It is expected that the 1 months MVSS value exists. If this is not the case the missing MVSS value will be replaced by the mean MVSS value of all patients who belong to the same treatment group and the same stratum (see Section 11.1.10) at that time point. For missing MVSS values at later time points (3, 6, 12, 18 and 24 months) the following imputation method will be used:

For each time point (1, 3, 6 and 12 months) and each treatment arm separately, a linear regression model with target variable MVSS at 12 months and explanatory variables MVSS at the respective time point and all stratification variables (see Section 11.1.10) is fitted to the data of patients having both MVSS values. Missing MVSS values at 12 months are then

imputed with the prediction of the linear regression model, which has the last non-missing MVSS value before the 12 months measurement for the individual as explanatory variable (i.e. for patients who have the 6 months MVSS measurement but not the 12 months measurement, the 12 months measurement is imputed from the 6 months measurements; for those who have the 3 months measurement but not the 6 months or 12 months measurement the 12 months measurement is imputed from the 3 months measurement; and for those who have the MVSS measurement at 1 month but not at 3 months, 6 months or 12 months the 12 months measurement will be imputed from the 1 month measurement). To this prediction, the realization of a normally distributed random variable with zero mean and standard deviation equal to the residual standard deviation from the linear model is added. The random seed for this imputation is 45318.

Similarly, for each time point (1, 3, 6, 12 and 18 months) and each treatment arm separately, a linear regression model with target variable MVSS at 24 months and explanatory variables MVSS at the respective time point and all stratification variables (see Section 11.1.10) is fitted to the data of patients having both MVSS values. Missing MVSS values at 24 months are then imputed with the prediction of the linear regression model, which has the last non-missing MVSS value before the 24 months measurement for the individual as explanatory variable. To this prediction, the realization of a normally distributed random variable with zero mean and standard deviation equal to the residual standard deviation from the linear model is added. The random seed for this imputation is 45448.

Using this imputation method deviates from the handling of missing data specified in the protocol. There, simple LOCF was intended. However, LOCF is not considered a suitable imputation method for the MVSS values as early MVSS values are not considered representative for late MVSS measurements in the same individual. Furthermore, LOCF is heavily criticized in the statistics community since it may lead to biased results even when the missing occurs completely at random (see [29], [30]).

Additionally, best case – worst case imputations will be used as a supporting sensitivity analysis. One using the worst case MVSS value of 9 (observed maximum MVSS value in the SOC group in the previous study MW2004-11-02) for all missing MVSS data and one using the best case value of 0 (observed minimum MVSS value in the SOC group in the previous study MW2004-11-02).

10.1.2.3. Safety Endpoint: Cosmesis and function at 24 months from wound closure

See 10.1.2.2.

10.2. Handling of Withdrawals and Dropouts

Withdrawn subjects will be evaluated in the FAS and safety set. Missing values are imputed as mentioned in Section 10.1.

10.3. Data Transformation

For all continuous variables, mean, standard deviation, minimum, maximum, median, 25% quantile and 75% quantile will be calculated per treatment group and overall.

For all categorical variables counts and frequencies of categories will be calculated per treatment group and overall.

10.3.1. Demographic data and other baseline characteristics

10.3.1.1. Age

Age will be calculated as the difference in years (rounded down) between date of birth and the date of the baseline physical examination.

10.3.1.2. Height

A patient's height is either given in cm or inch. Height in inch will be transformed to height in cm and vice versa using the formulae

$$height(cm) = 2.54 \times height(inch)$$

$$height(inch) = 0.3937 \times height(cm)$$

Hence, height data for each patient will be available in inch as well as in cm.

10.3.1.3. Weight

A patient's weight is either given in kg or lb. Weight in lb will be transformed to weight in kg and vice versa using the formulae

$$weight(kg) = 0.4536 \times weight(lb)$$

$$weight(lb) = 2.2046 \times weight(kg)$$

Hence, weight data for each patient will be available in lb as well as in kg.

10.3.1.4. Time since injury

The time since injury will be computed as the time in hours between injury and first treatment.

10.3.1.5. Early grafted wounds

For each target wound, a binary (yes/no) variable will be computed indicating whether it was early autografted (for definition see Appendix 13.1.1).

For each patient a binary (yes/no) variable will be computed indicating whether the patient had at least one early grafted target wound.

10.3.1.1. Late grafted wounds

For each target wound, a binary (yes/no) variable will be computed indicating whether it was late autografted (for definition see Appendix 13.1.1).

For each patient a binary (yes/no) variable will be computed indicating whether the patient had at least one late grafted target wound.

10.3.1.2. Wounds at least partially found in the anatomical area of the hand

For each target wound, a binary (yes/no) variable will be computed indicating whether it was at least partially found in the anatomical area of the hand.

For each patient a binary (yes/no) variable will be computed indicating whether the patient had at least one target wound which was at least partially found in the anatomical area of the hand.

10.3.2. Primary Endpoint: Incidence of complete eschar removal in the topical arms

Information on whether complete eschar removal was obtained after the application of NexoBrid or Gel vehicle per target wound will be combined to a single binary variable (yes/no) indicating whether complete eschar removal was achieved for all target wounds of a patient at the end of the eschar removal treatment (after the 2 hrs soaking). The eschar removal treatment may contain one or two topical removal procedures. Complete eschar removal is judged after the last topical procedure in a patient.

Furthermore, for all patients with at least one early grafted target wound (for definition see Appendix 13.1.1), a binary (yes/no) variable will be computed indicating whether complete eschar removal has been achieved in all early grafted wounds of the patient at the end of the eschar removal treatment (after the 2 hrs soaking).

Additionally, for all patients with at least one late grafted target wound (for definition see Appendix 13.1.1), a binary (yes/no) variable will be computed indicating whether complete eschar removal has been achieved in all late grafted wounds of the patient at the end of the eschar removal treatment (after the 2 hrs soaking).

Furthermore, for patients who had at least one target wound that is found, at least partially, in the anatomical area of the hand, a binary (yes/no) variable will be computed, indicating whether complete eschar removal has been achieved in all target wound that are found, at least partially, in the anatomical area of the hand.

10.3.3. Secondary Endpoints

10.3.3.1. Secondary Endpoint: Incidence of surgical excision

For each patient a binary (yes/no) variable will be computed, indicating whether this patient needed surgical excision for eschar removal.

Furthermore, for patients with at least one early grafted target wound, a binary (yes/no) variable will be computed, indicating whether this patient needed surgical excision in an early grafted target wound. Similarly, for patients with at least one late grafted target wound, a binary (yes/no) variable will be computed, indicating whether this patient needed surgical excision in a late grafted target wound.

Additionally, for patients who had at least one target wound that is found, at least partially, in the anatomical area of the hand, a binary (yes/no) variable will be computed, indicating whether this patient needed surgical excision in a target wound that is found, at least partially, in the anatomical area of the hand.

For each patient the number of TWs will be computed and used as an explanatory variable in the analysis of this secondary endpoint.

10.3.3.2. Secondary Endpoint: Time to complete eschar removal

Information about date will be transformed to time in hours from randomization. Time until complete eschar removal will be calculated as the time (in hours) until complete eschar removal has been achieved for a TW. From these times, time to reach complete eschar removal at a patient level, i.e. for all TW's of an individual patient will be calculated. For wounds/patients that do not reach complete eschar removal, their time will be censored at the last non-missing eschar removal assessment (typically the last debridement procedure).

For further analysis, for patients with at least one early grafted target wound, time to complete eschar removal will also be calculated as above, but taking into account only early grafted wounds. Additionally, for patients with at least one late grafted target wound, time to complete eschar removal will also be calculated as above, but taking into account only late grafted wounds.

Additionally, for patients who had at least one target wound that is found, at least partially, in the anatomical area of the hand, time to complete eschar removal will also be calculated as above, but taking into account only target wounds that are found, at least partially, in the anatomical area of the hand.

For an additional analysis, time to complete eschar removal will be computed as above but in hours from injury.

Furthermore, time to complete eschar removal will be computed as above but in hours from ICF.

The patients' age will be categorized into two categories: "younger than 35 years" and "35 years or older". The number 35 years is found to be the median age of the adult population in the previous study MW2004-11-02.

10.3.3.3. Secondary Endpoint: Blood loss

10.3.3.3.1. Blood loss for main analysis

For each patient and each procedure carried out to remove eschar the estimated blood volume (EBV) will be calculated as

$$EBV = 70\frac{cm^3}{kg} * weight(kg)$$

Furthermore, the total volume of whole blood and packed red blood cells (PRBC) transfused (in milliliters) will be calculated per procedure (transfusions given during the procedure and 4 hours ± 15 minutes later) and denoted by V_{WB} and V_{PC} in the following.

From these quantities, the blood loss (Actual Blood Loss, ABL) will be calculated using the following formula [25]:

$$ABL = \frac{EBV * \left(Hb_{before} - Hb_{after}\right)}{\left(Hb_{before} + Hb_{after}\right)/2} + V_{WB} + \frac{5}{3}V_{PC}$$

The factor of 5/3 is derived from chapter 5 of Transfusion Medicine, 4th Edition [31] and compensates for the fact that equal volumes of whole blood and packed red blood cells are not comparable.

The ABL will be summed over all procedures carried out to remove eschar, with the Hb measured immediately before each procedure and 4 hours after its completion. The amount of blood transfused will be summed over all transfusions carried out during the debridement procedure and 4 hrs later i.e. the before-after periods that are spanned by these Hb measurements.

Additionally, blood loss will be computed considering the whole debridement process as one continuous procedure. For this value, the formula for ABL given above will also be used. However, the ABL will be computed using the Hb measured immediately before the first procedure and 4 hours after completion of the last procedures. The amount of whole blood and packed red blood cells (PRBC) transfused will be calculated as before, but incorporating all such transfusions in the time frame spanned by the Hb measurements used for the calculation (pre first procedure to 4 hrs post last procedure (in which complete debridement was achieved).

Analyses with both blood loss values will be conducted. However, some missing values are expected. The analysis with less missing values will be regarded as more reliable and is therefore considered the main analysis. The other analysis is considered supportive.

10.3.3.3.2. Proportion of SPT area of wounds treated per procedure

For the multiple imputation outlined in Section 10.1.1.3 information on the proportion of the SPT area of wounds treated per procedure will be computed using the following formula:

$$Prop(SPT) = \frac{\sum_i \%SPT(W_i) * \%TBSA(W_i)}{\sum_i \%TBSA(W_i)},$$

where both sums range over all target wounds treated in a debridement procedure, $\%SPT(W_i)$ is the percent area of SPT of target wound i and $\%TBSA(W_i)$ is the area of the target wound i. $Prop(SPT)$ will be computed for each procedure in each patient separately.

10.3.3.3.3. Proportion of DPT area of wounds treated per procedure

For the multiple imputation outlined in Section 10.1.1.3 information on the proportion of the DPT area of wounds treated per procedure will be computed using the following formula:

$$Prop(DPT) = \frac{\sum_i \%DPT(W_i) * \%TBSA(W_i)}{\sum_i \%TBSA(W_i)},$$

where both sums range over all target wounds treated in a debridement procedure, $\%DPT(W_i)$ is the percent area of DPT of target wound i and $\%TBSA(W_i)$ is the area of the target wound i. $Prop(DPT)$ will be computed for each procedure in each patient separately.

10.3.3.3.4. Proportion of FT area of wounds treated per procedure

For the multiple imputation outlined in Section 10.1.1.3 information on the proportion of the FT area of wounds treated per procedure will be computed using the following formula:

$$Prop(FT) = \frac{\sum_i \%DPT(W_i) * \%TBSA(W_i)}{\sum_i \%TBSA(W_i)},$$

where both sums range over all target wounds treated in a debridement procedure, $\%FT(W_i)$ is the percent area of FT of target wound i and $\%TBSA(W_i)$ is the area of the target wound i. $Prop(FT)$ will be computed for each procedure in each patient separately.

10.3.3.3.5. Course of debridement procedure

For the multiple imputation outlined in Section 10.1.1.3 for each patient a categorical variable describing the course of the debridement process will be calculated. The different levels of the variable are defined as:

- Only non-surgical SOC debridement
- Non-surgical SOC debridement followed by surgical debridement
- Only surgical debridement
- Only NexoBrid debridement
- NexoBrid debridement followed by non-surgical SOC debridement followed by surgical debridement
- NexoBrid debridement follow by surgical debridement
- Only Gel Vehicle debridement
- Gel Vehicle debridement followed by non-surgical SOC debridement followed by surgical debridement
- Gel Vehicle debridement followed by surgical debridement

10.3.4. Safety Endpoints

10.3.4.1. Safety Endpoint: Time to reach complete wound closure

Information about date will be transformed to time in days from randomization. Time until complete wound closure (for definition see Appendix 13.1.1), will be defined as the time (in days) until complete wound closure has been achieved at a wound level. For wounds, which do not reach complete wound closure, the time will be censored at last date of wound closure assessment.

10.3.4.2. Safety Endpoint: Cosmesis and function at 12 months from wound closure

MVSS sub scores at 12 months for pigmentation, pliability, height, vascularity, pain and pruritus will be added up to obtain the overall MVSS score (range 0 to 18) at 12 months per target wound. The patient MVSS score will be the average of the MVSS over all TWs. Additionally, the average MVSS score for donor site scars will be calculated per patient.

10.3.4.3. Safety Endpoint: Cosmesis and function at 24 months from wound closure

MVSS sub scores at 24 months for pigmentation, pliability, height, vascularity, pain and pruritus will be added up to obtain the overall MVSS score (range 0 to 18) at 24 months per target wound. The patient MVSS score will be the average of the MVSS over all TWs. Additionally, the average MVSS score for donor site scars will be calculated per patient.

10.3.4.4. General Parameters of safety

Volume of blood transfusions given

The volume of blood transfusions given during hospitalization will be computed for each patient as $V_{WB} + \frac{5}{3}V_{PC}$, where V_{WB} and V_{PC} are the volume (in milliliters) of whole blood and packed red blood cells transfused. As in the calculation of blood loss in Section 10.3.3.3.1, the factor of 5/3 is derived from chapter 5 of Transfusion Medicine, 4th Edition [31] and compensates for the fact that equal volumes of whole blood and packed red blood cells are not comparable.

The volume of blood transfused will be calculated overall, as well as separately for volumes transfused before start of treatment, during the ER period, within 1 week after the ER period and later than 1 week after the ER period for each patient.

Extent of analgesia, anesthesia and antibiotic use

The numbers and percentages of patients per each level of sedation (see definitions in Appendix 13.1.2) per procedure (in topical arms: 1st and second topical application, surgical rescue procedures and non-surgical rescue procedures; in SOC arms: surgical procedures and non-surgical procedures) will be calculated.

The number of days of exposure to antibiotic drugs will be calculated for each patient. This will be done overall, for antibiotic use due to AE involvement (as indicated in the eCRF) and for prophylactic antibiotic use (as indicated in the eCRF) separately.

Incidence of increased interstitial/compartment syndrome

For each patient in the topical arms a binary (yes/no) variable is indicating whether the patient developed a burn-induced compartment syndrome (BICS) during the eschar removal procedure is given in the data.

Incidence of QT prolongation

Incidence of QT prolongation will be analyzed separately. The analysis and all needed transformations are described in a separate document.

Rates of hospital readmission

For each hospital readmission, a binary (yes/no) variable will be computed indicating whether this readmission was planned or not. A readmission will be considered as being planned when the reason for readmission was indicated as “planned wound management” or “scar modulation” in the eCRF. Readmissions for which the reason was given as “AE” in the eCRF are considered as being unplanned. Hospital readmission for which the reason is given as “other” will be reviewed by blinded personnel of the sponsor in order to decide whether they are to be classified as planned or unplanned.

For each patient a binary (yes/no) variable will be computed indicating whether this patient had a hospital readmission or not. Furthermore, a variable giving the number of planned hospital readmissions and a variable giving the number of unplanned readmissions will be computed for each patient.

Relative and absolute frequencies of hospital readmission will be calculated per treatment arm.

Change in PTT/INR

Change in PTT/INR will be computed as change from pre Eschar Removal procedure to 4h ± 15 min post first application (Topical arms) respectively to 4h post first SOC treatment (SOC arm) for each patient. Additionally, change from baseline PTT/INR will be computed for the first and second daily assessment post treatment (24h & 48h after start of debridement).

The proportion of patients with a change to > upper limit of normal range PTT/INR (patients with normal PTT/INR at baseline and PTT/INR > upper limit of normal range at the respective time point) will be computed per treatment arm and time point of measurement (4h post treatment, 24 h, 48h). Reference count is the number of patients with normal PTT/INR values at baseline. For the classification of normal and abnormal PTT/INR values, the reference limits of the local labs will be used.

Change in blood glucose

Change in blood glucose will be computed as change in blood glucose value from pre first topical procedure to 4h post first topical procedure (4h after removal of topical) and from

post first topical to post second topical (if available) and from pre surgical procedure to post surgical procedure for each patient. In case non-surgical procedure was the only one performed for a SOC patient, the change in blood glucose value will be computed as change from pre to post procedure. Furthermore, a binary (yes/no) variable will be computed indicating whether there was a change to above upper limit of normal (the upper reference limit of the central lab) from baseline to 4h post treatment for each debridement procedure. This is the case if the blood glucose level at baseline was below the upper limit of the normal range as given in the data base and was above the upper limit at 4h post treatment. From this for each patient a binary variable will be computed indicating whether there was a change in blood glucose to > upper limit of normal range at any of the debridement procedures.

Lower Extremity Function Scale (LEFS)

The LEFS consists of 20 items which can each be answered on a scale of 0 to 4. In the database the answers will be coded as 1 to 5. Therefore, the total LEFS for a patient will be computed as

$$LEFS = \sum_{i=1}^{20} a_i - 20,$$

where a_i is the answer to item i (coded 1 to 5).

Disabilities of the Arm, Shoulder and Hand (QuickDASH)

The QuickDASH consists of 11 items which are scored 1-5. At least 10 of the 11 items must be completed for a score to be calculated. The assigned values for all completed responses are simply summed and averaged, producing a score out of five. This value is then transformed to a score out of 100 by subtracting one and multiplying by 25. Hence, the QuickDASH score for a single patient is calculated by:

$$QuickDASH = \left(\frac{sum\ of\ n\ reponses}{n} - 1\right) * 25.$$

Range of Motion (ROM)

For each visit (12 months FU and 24 months FU), a binary (yes/no) variable is given in the eCRF, indicating whether the range of motion assessment for that patient was abnormal or not. Furthermore, for each visit (12 months FU and 24 months FU) a binary (yes/no) variable

will be computed, indicating whether there was at least one abnormal (NCS) ROM assessment for that patient. Finally, for each visit (12 months FU and 24 months FU) a binary (yes/no) variable will be computed, indicating whether there was at least one abnormal (CS) ROM assessment for that patient.

EQ-5D (Quality of Life)

EQ-5D consists of 5 items with 3 possible answers each. It will be evaluated per the EQ-5D-3L user guide [32].

For each item relative and absolute frequencies of the answers will be computed in total and per age group defined in the EQ-5D-3L user guide per treatment arm and overall. Furthermore, summary statistics of the EQ VAS will be computed in total and per age group defined in the EQ-5D-3L user guide per treatment arm and overall.

The EQ-5D states will be converted to a single summary index as described in the EQ-5D-3L user guide.

Burn Specific Health Scale – Brief (BSHS-B)

The Burn Specific Health Scale – Brief (BSHS-B) consists of 40 item which can each be scored with 0 – 4. In the dataset the scores will be coded as 1- 5. The 40 items can be aggregated to 9 domains. The domains are:

- Heat sensitivity (questions 28-32 of the eCRF)
- Affect (questions 10-16 of the eCRF)
- Hand Function (questions 4-8 of the eCRF)
- Treatment Regimens (questions 33-37 of the eCRF)
- Work (questions 9, 38-40 of the eCRF)
- Sexuality (questions 21-23 of the eCRF)
- Interpersonal Relationships (questions 17-20 of the eCRF)
- Simple Abilities (questions 1-3 of the eCRF)
- Body Image (questions 24-27 of the eCRF)

For each domain a domain specific sub score is computed using the following formula:

$$Score(Domain) = \frac{1}{n}\sum_{i=1}^{n}(Score_i - 1),$$

where n is the number of items of the domain and $Score_i$ is the score of the ith item as coded in the data set (i.e. the domain score is the mean of the item scores as coded in the BSHS-B).

An overall BSHS-B score is computed as the mean of the domain specific sub scores.

Immunogenicity evaluation

The immunogenicity evaluation will be done in a separate analysis and is described in a separate document.

Pyrexia and hypothermia

Pyrexia (and related terms) and hypothermia reported as AEs will be presented in the AEs tables.

Systemic Infection

For each patient a binary (yes/no) variable will be computed indicating whether the patient developed a systemic infection during the study. For data captured with the eCRF version from 13 Oct 2016 the information, whether an AE is a systemic infection, is directly available in the AE description. For data captured before that, a medical person appointed by the sponsor will review all MedDRA Preferred Terms belonging to the MedDRA SOC of “Infections and Infestations”, as well as all other Preferred Terms reported in the study, and all reported terms of AEs including the word “infection”, to decide which AEs meet the definition of systemic infection.

Systemic adverse events

Counts and frequencies of systemic adverse events (marked as “general” in the eCRF) per treatment will be computed. This will be done separately by:

- System organ class
- Preferred term
- Severity (mild / moderate / severe AEs)
- Relatedness (not related / remotely related / possibly related / probably related / related AEs)

- Time of onset (before treatment / during the treatment session / during the first week after treatment / during week 2 to week 4 after treatment / during week 5 to week 8 after treatment / more than 8 weeks after treatment)

For each of the tree stages of analysis (see Section 11 for details), the safety data gathered in that stage will be analyzed separately. That is, in the acute stage analysis, all AEs reported until the 3 months follow-up will be analyzed. In the second stage analysis (12 months follow-up), all AEs reported between the 3 months follow-up and the 12 months follow-up (including AEs from first stage that are still ongoing) will be analyzed. Analogously, in the third stage analysis (24 months follow-up), all AEs reported between 12 months follow-up and the 24 months follow-up (including AEs from the first or second stage that are still ongoing) will be analyzed.

Local adverse events

Local adverse events are classified as being local – TW related or local – not TW related in the eCRF. Per each of these categories, counts and frequencies of adverse events per treatment group will be computed. This will be done separately by:

- System organ class
- Preferred term
- Severity (mild / moderate / severe AEs)
- Relatedness (not related / remotely related / possibly related / probably related / related AEs)
- Time of onset (before treatment / during the treatment session / during the first week after treatment / during week 2 to week 4 after treatment / during week 5 to week 8 after treatment / more than 8 weeks after treatment)

For each of the tree stages of analysis (see Section 11 for details), the safety data gathered in that stage will be analyzed separately. That is, in the acute stage analysis, all AEs reported until the 3 months follow-up will be analyzed. In the second stage analysis (12 months follow-up), all AEs reported between the 3 months follow-up and the 12 months follow-up (including AEs from the first stage that are still ongoing) will be analyzed. Analogously, in the third stage analysis (24 months follow-up), all AEs reported between 12 months follow-up and the 24 months follow-up (including AEs from the first or second stage that are still ongoing) will be analyzed.

Vital signs

Body temperatures given in °F will be transformed to °C using the following formula:

$$Temperature[°C] = \frac{5}{9}(Temperature[°F] - 32)$$

Differences in the vital signs assessments (body temperature, heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure) between daily visits will be calculated by

$$Diff_d = VS_{d-1} - VS_d$$

where VS_d is the vital sign assessment at day *d* (daily follow-up) and d is varied from 2 to 7. Furthermore, pre-post differences for vital signs assessments per procedure will be calculated in the same way, using the screening assessment as pre first procedure and the post first procedure measurement as pre second procedure measurement if needed.

Pain assessment

For each eschar removal procedure and each daily follow-up pain assessment the change in pain from baseline will be calculated as

$$Diff_d = Pain_{Base} - Pain_d$$

where $Pain_d$ is the pain assessment at the respective time point. For the calculation of the difference in pain for the eschar removal procedures the pain assessment post that procedure will be used.

10.3.5. Exploratory Analyses

10.3.5.1. %SPT area

For each patient the % SPT area (the area of SPT as a percentage of the total area of all TWs) will be calculated. From this a binary variable will be computed indicating whether % SPT area is < 25% or ≥ 25%. Cumulative and absolute frequencies for each category will be computed.

10.3.5.2. %TBSA

A binary variable indicating whether total %TBSA of all TWs is ≤ 15% or > 15% will be calculated per patient. For this variable cumulative and absolute frequencies for each category will be computed.

10.3.5.3. Application data

For the patients of the NexoBrid and the gel vehicle arm the number of applications (0, 1, 2) will be determined. Cumulative and absolute frequencies will be computed for each category. Furthermore, for each patient the amount of NexoBrid gel each patient in the NexoBrid treatment arm has been exposed to, will be calculated.

10.3.5.4. Maintenance of complete wound closure

For each follow-up visit the information of complete wound closure for each TW will be aggregated to a binary variable indicating maintenance of complete wound closure on a patient level (i.e. complete wound closure for each TWs of a patient). Cumulative and absolute frequencies will be computed for each category.

10.3.5.5. % wound area surgically excised for eschar removal

% of wound area surgically excised for eschar removal, A_{total}, will be calculated using the following formula:

$$A_{total} = \frac{\sum_i (a_i * \%TBSA_i)}{\sum_i \%TBSA_i}$$

where $\%TBSA_i$ is the area of TW i (in % TBSA) and a_i denotes the % of wound area surgically excised for eschar removal of TW i.

10.3.5.6. Incidence of reduction in interstitial/compartment pressure in circumferential extremities target wounds

Interstitial/compartment pressure in circumferential extremities target wounds is measured within 1 hour (+30 min) prior to start of each eschar removal procedure and post-eschar removal. For each circumferential target wound and each eschar removal procedure a binary (yes/no) variable will be computed indicating whether reduction in interstitial/compartment pressure in that wound was present.

10.3.5.7. POSAS

The Patient Observer Scar Assessment Scale (POSAS) consists of two parts: a patient scale and an observer scale. Both scales contain six items that are scored numerically with 1-10. For each wound of a patient the total score of the patient scale will be computed as:

$$POSAS_P = \sum_{i=1}^{6} p_i,$$

where p_i is the score the patient assigned to item i. The total score of the observer scale will be computed analogously as:

$$POSAS_O = \sum_{i=1}^{6} o_i,$$

where o_i is the score the observer assigned to item i.

A total score for the POSAS is then obtained by summing the patient scale score and the observer scale score:

$$POSAS_{tot} = POSAS_P + POSAS_O.$$

For each patient the mean of $POSAS_{tot}$ over all TWs will be computed. Analogously the mean of $POSAS_{tot}$ over all donor site scars will be computed.

Additional to the quantitative scoring of the items they are also score qualitatively (for each item different categories are available). Absolute and relative frequencies of the categories for each item will be computed over all patients per treatment arm.

The POSAS will be computed for TWs and donor site scars at the long-term follow up visits.

10.3.5.8. Incidence of surgical Escharotomy procedures on circumferential extremities target wounds

For each circumferential extremities target wound a binary (yes/no) variable will be computed, indicating whether this wound was escharotomized. Furthermore, a binary (yes/no) variable for each patient will be computed indicating whether this patient had at least one circumferential extremities target wound which was escharotomized.

10.3.5.9. Incidence of reduction in interstitial/compartment pressure in circumferential extremity wounds

For circumferential extremity wounds the reduction in interstitial/compartment pressure will be computed as

$$Press_{diff} = Press_{base} - Press_{post}$$

where $Press_{base}$ is the interstitial/compartment pressure at baseline and $Press_{post}$ is the interstitial/compartment pressure after eschar removal. This will be done for each topical eschar removal procedure. Furthermore, for each circumferential extremity wound and each topical eschar removal procedure a binary (yes/no) variable will be computed indicating whether interstitial/compartment was reduced during the procedure.

10.3.5.10. Incidence of surgically harvested donor sites wounds

For each patient, the number of donor sites wounds will be calculated.

10.3.5.11. % area of surgically harvested donor site wounds

For each autograft procedure the % TBSA of the donor site is captured. These values will be summed up over all autograft procedures for each patient giving the % area of surgically harvested donor site wounds.

10.3.5.12. Blood loss using changes in hematocrit following eschar removal procedures

For each debridement procedure, the change in hematocrit will be calculated as the difference of the hematocrit value before the procedure and the hematocrit value 4 hours post the procedure. This difference in hematocrit values will be summed for each patient.

Furthermore, for each patient, the difference of the hematocrit value prior to the first debridement procedure and the hematocrit value 4 hours post the last debridement procedure will be calculated.

10.3.5.13. Blood loss using changes in hemoglobin following eschar removal procedures

For each debridement procedure, the change in hemoglobin will be calculated as the difference of the hemoglobin value before the procedure and the hemoglobin value 4 hours post the procedure. This difference in hemoglobin values will be summed for each patient.

Furthermore, for each patient, the difference of the hemoglobin value prior to the first debridement procedure and the hemoglobin value 4 hours post the last debridement procedure will be calculated.

10.3.5.14. Blood loss comparing NexoBrid procedures to surgical procedures

For a comparison of blood loss between NexoBrid procedures and surgical procedures, the blood loss per eschar removal procedure will be calculated as outlined in Section 10.3.3.3.1.

10.3.5.15. Autograft related parameters

The total number of grafting procedures per patient and per target wound will be computed. Procedure will be based on the date in the eCRFs per patient and per wound.

Furthermore, a binary (yes/no) variable will be computed indicating whether more than one autografting procedure was performed on a wound level as well as on a patient level.

The area of repeat autografting for each wound will be calculated as follows:

$$Area_{repeat} = \sum_{i=2}^{n} Area_i,$$

where n is the total number of autografting procedures for the specific wound and $Area_i$ is the area autograft in the i-th grafting procedure for that wound.

The area of repeat autografting on a patient level is calculated as the sum over the areas of repeat grafting over all TWs of that patient.

10.3.5.16. Duration of hospitalization

Duration of acute hospitalization will be computed as the difference in days between hospital admission and hospital discharge (initial hospitalization only).

10.3.5.17. Time to reach 100% wound closure

Information about date will be transformed to time in days from randomization. Time until 100% wound closure will be defined as the time (in days) until 100% wound closure has been achieved at a wound level. For wounds that do not reach 100% wound closure, the time will be censored at last date of wound closure assessment. In case one or more wounds do not reach complete wound closure, the time to wound closure at a patient level will be censored at the minimum of all wound wise censoring times.

10.3.6. Laboratory values

The following transformations will be applied to the central laboratory data obtained pre and post the eschar removal procedures:

Laboratory findings that are given in various units will be transformed to standard units. Laboratory values will be categorized as “abnormal (low)”, “normal” and “abnormal (high)” based on the reference limits of the labs.

Pre-post differences of laboratory values for each eschar removal procedure will be calculated.

Absolute and relative frequencies of “abnormal (low)”, “normal” and “abnormal (high)” findings will be calculated per laboratory value, eschar removal procedure and treatment arm.

10.4. Multiple visits per time point

Study visit time-points are scheduled at screening, pre eschar removal, eschar removal (treatment), post-treatment, hospital discharge, follow-up (weekly until wound closure, wound closure confirmation, three monthly visits post wound closure), long-term follow-up at 6, 12, 18

months and 24 months post wound closure confirmation and re-admission to hospital/day care (if done). One visit is expected for each time-point. The following approach is used if there are multiple visits per patient per time-point.

For all endpoints, if a subject has more than one visit with data at a time-point, the later non-missing evaluation will be used for analyses.

11. Statistical Analysis of Target Variables

The analysis is planned to be carried out in three stages, as described below.

1. The first analysis will be performed at the end of the Efficacy Assessment Period (EAP). This analysis will be the only inductive analysis of the trial and will include statistical tests for the primary and secondary endpoints as described below, as well for short-term safety endpoints (including time to wound closure). The analysis will be conducted, when 3 months had passed from the last patient reaching complete wound closure, in accordance with FDA guidance for industry *Chronic Cutaneous Ulcer and Burn Wounds — Developing Products for Treatment "Trial subjects generally should remain in the study for follow-up evaluation at least 3 months following complete wound closure. The purpose for this follow-up period is to help distinguish actual wound healing from transient wound coverage"*. At this time-point, data will be available for all patients on the primary end-point (proportion with complete eschar removal), the three secondary endpoints (proportion undergoing surgery, time to complete eschar removal, and blood loss) and short-term safety endpoints (including time to wound closure). Missing values for early drop outs etc. will be handled as described in Sections 10.1 and 10.2. The complete data set documented so far will be locked and analyzed as described above. In the analysis of adverse events and concomitant medication, for each patient only the data until the 3 months follow up will be analyzed in this stage. Data obtained after the 3 months follow up will be analyzed in the second and third stage of analysis. MVSS data, although captured in the eCRF for a few of the subjects at the stage of EAP, will not be included, revealed or analyzed during the EAP analyses.

2. The second analysis will be performed for the 12 months follow up period (STFU12) and will be started after the last patient has reached the 12m assessment. At this analysis, all accumulated safety data at the 12m follow-up will be analyzed, particularly the 12m MVSS assessment for cosmesis and function. The complete data set documented so far will be locked and analyzed as described below. In the analysis of adverse events for each patient only the data obtained after the 3 months follow up and until the 12 months follow up (including AEs and concomitant medications from the first stage that are ongoing) will be analyzed in this stage.

3. The third and final analysis covers the data of the long-term safety follow up (LTSFU24). It will be conducted after the last patient has reached the 24 months

assessment at last 12 months after (STFU12) analysis (stage 2). At this analysis, all accumulated safety endpoints at the 24m follow-up will be analyzed, particularly the 24m MVSS assessment for cosmesis and function. The complete data set documented during the trial will be locked and analyzed as described below (hard lock). In the analysis of adverse events for each patient only the data obtained after the 12 months follow up (including AEs and concomitant medications from the first or second stage that are still ongoing) will be analyzed in this stage.

Two “blinded assessors” are appointed in each clinical site during the site initiation visit. The assessor signs a “blinded assessor memo” which describes the procedures required to maintain the investigator blinded from the treatment arm. The first blinded assessor is responsible for the eschar removal assessment of the topical arms and is not involved with product application. All patients will complete the eschar removal treatment by the time of the first planned analyses of the acute stage and thus, there is no risk of unblinding of the first blinded assessor. The second blinded assessor is responsible for the wound closure and long-term assessments and is not involved with any eschar removal procedures. The second blinded assessor will remain blinded throughout the whole duration of the trial including after the acute and the first long-term (12 months) analysis results are available. To assure that patients’ treatment codes will not be revealed during the EAP analyses, all patients’ identification numbers will be Pseudonyms by data management before analysis is being performed and presented.

The data sets for analysis will be 100% SDVd and signed by the site investigator. Therefore, data changes are prevented during the further conduct of the study. Data management quality control actions will ensure that data used in the analysis is not changed afterwards see the Data Management Plan for further details.

For a better overview of this SAP, the statistical analysis of target variables described below will be displayed separately for these 3 stages of analyses.

All variables not mentioned explicitly in the following will be analyzed descriptively (as part of the case wise listing).

Unless otherwise specified, all statistical tests will be conducted against a two-sided alternative hypothesis, employing a significance level of 0.05.

11.1. First stage analysis – end of efficacy assessment period (EAP)

11.1.1. Demographics and Other Baseline Characteristics

For all considerations regarding the demographics and other baseline characteristics the full analysis set will be used.

The demographic and baseline information concerning

- Age
- Gender
- Ethnicity
- Race
- Height (cm)
- Weight (kg)
- BMI
- Physical examinations (categorized as normal, abnormal-NCS, abnormal-CS, not done):
    - Head, eyes, ears, nose and throat (HEENT)
    - Respiratory system
    - Cardiovascular system
    - Abdomen
    - Musculoskeletal / Extremities
    - Neurological
    - Lymph Nodes
- Vital signs:
    - Temperature
    - Heart rate
    - Respiratory rate
    - Systolic and diastolic blood pressure (mmHg)
    - Assessment of vital signs (normal, abnormal-NCS, abnormal-CS)
- Pain assessment:
    - VAS (mm)
    - Assessment of pain (normal, abnormal-NCS, abnormal-CS)
- Local laboratory:
    - Leukocyte count (WBC)

    - Assessment of leukocyte count (normal, abnormal-NCS, abnormal-CS)
    - Diabetes status (yes/no)
    - HbA1c (%)
- Infections (yes/no for infections in last week before baseline)
- Relevant medical history (regarded as categorical variable)
- Burn history (on a patient level):
    - Time since injury
    - Etiology of injury (Fire/Flame, Scald, Contact)
    - Place of injury (Outdoors, Indoors, Car, Other)
    - Information whether patient was transferred from another facility
- General wound description (on a wound level):
    - Anatomical location (face, head, neck, etc.)
    - %TBSA of 2° SPT Burns
    - %TBSA of 2° DPT Burns
    - %TBSA of 3° FT Burns
    - Indicator (yes/no) of circumferential extremity involvement
    - Extremities at risk (not relevant, pain, paralysis, etc.)
    - Information whether eschartomy had been performed prior to randomization
    - Eschar description (white, moist, dry, etc.)
    - Information whether cleansing was performed
    - Information whether all superficial keratin (blister) were removed from wound
- Target wound description:
    - General wound description (see above; on a wound level; for target wounds only)
    - Information whether the wound was treated prior to enrollment (TWs)
    - Treatment of TWs prior to enrollment (SSD, Iodine, etc.)
- Target wound description (on a patient level):
    - Total wounds %TBSA
    - TWs DPT total area (%TBSA)
    - TWs FT total area (%TBSA)
    - Over TWs depth per patient (all full thickness, mixed, all deep partial thickness)
    - Overall TW area (SPT+DPT+FT)
    - %SPT area classified as <25% or ≥25% of TWs area
- Number of circumferential extremity wounds per patient

- Interstitial pressure (mmHg) for circumferential extremity wounds (on a wound level)
- Criteria for inclusion (met/not met)
- Counts of patients/wound per strata (%TBSA cut point 15%, depth cases 1-3, center)

will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles in case of numeric values and counts and percentages in case of categorical items to describe the study population. The analysis will be done by treatment group and in total.

The descriptive results for all variables will be tabulated. Exemplary tables are presented in Section 13.2. Histograms will be produced for each continuous variable, bar plots for categorical variables. Figures can be seen in Section 13.3.

If any of the baseline factors, gender, age, percent SPT area (the area of SPT as a percentage of the total area of all TWs, classified as <25% or ≥25%), time from injury to randomization or number of TWs (1, 2, ≥3) are found to be significantly different between the treatment groups (Chi-squared tests (or in case of small estimated cell counts Fisher's exact test) for categorical variables and one-way analysis of variance for continuous variables) then the factor will be included as an extra adjusting covariate in the supportive analysis models for the primary and secondary efficacy endpoints and the comparative analyses of safety endpoints.

11.1.2. Primary Efficacy variable

The primary efficacy variable will be analyzed using the FAS.

The proportions of patients who reached complete eschar removal at the end of the topical agent soaking period will be compared using logistic regression. The primary analysis will be based on the binary variable (yes/no): 'has complete eschar removal been achieved in all TWs' and will compare NexoBrid with the Gel Vehicle (for further definition and explanation of complete eschar removal see Section 1.1 of the protocol and Section 10.3.2 of this SAP). For handling of missing data see Section 10.1.1. The statistical test will be based on Fisher's exact test because of the small numbers expected in the Gel vehicle group. The odds ratio of achieving complete eschar removal for NexoBrid versus Gel Vehicle and its 95% confidence interval will be estimated using exact distribution methods and will be displayed as shown in Table 6. Bar plots will be shown for the proportions of patients who reached complete eschar removal.

If numerically possible, the comparison will supportively be adjusted for the stratification factor wound depth (all TWs full thickness, mixed TWs, all TWs deep partial thickness) by including

the factor in a logistic regression model together with the treatment variable (NexoBrid vs. Gel Vehicle). Similarly, if convergence of the estimation procedure can be obtained, then, in two other supportive analyses, the factor “Total %TBSA per patient” and the factor treatment center group (see Section 13.8.5 of the protocol as well as Section 11.1.7 of this SAP) will be included in a logistic regression model with the treatment variable. Statistical inference will be based upon exact distribution methods using the “exact” statement in the SAS procedure PROC LOGISTIC. The estimated regression coefficients of the regression analyses will be presented as displayed in Table 7.

The odds ratio of achieving complete eschar removal for NexoBrid versus Gel Vehicle will be estimated from these supportive models, as well as 95% confidence intervals and the level of statistical significance. The results will be displayed as shown in Table 6.

The primary efficacy analysis will be repeated on a wound level as an additional analysis using a mixed logistic regression model with a random effect for patient.

Furthermore, as an additional sensitivity analysis, the primary analysis will be repeated using the PP as analysis population.

If any of the baseline factors, gender, age, percent SPT area (the area of SPT as a percentage of the total area of all TWs, classified as <25% or ≥25%), time from injury to randomization or number of TWs (1, 2, ≥3) are found to be significantly different between the treatment groups, then, if possible, the factor will be included as an extra adjusting covariate in a supportive analysis of the primary endpoint.

11.1.3. Secondary Efficacy variables

The secondary endpoints will be analyzed as described below and will compare efficacy in the NexoBrid group with the SOC group. Missing data will be handled by the methods described in Section 10.1. All secondary endpoints will be analyzed using the FAS. Sensitivity analyses will include analyzing the endpoints using the PP as analysis population.

11.1.3.1. Incidence of surgical excision

This is a binary yes/no variable and the proportion of patients who need excision for eschar removal will be compared using logistic regression. The explanatory variables in the model will include treatment, and the following variables: overall TW depth (all TWs full thickness, mixed TWs, all TWs deep partial thickness), “Total %TBSA per patient” and number of TWs (1, 2, ≥3). The odds ratio of requiring surgery for NexoBrid versus SOC will be estimated from the model, as well as 95% confidence intervals and the level of statistical significance. The results

will be displayed as shown in Table 6 and Table 7. Bar plots will be shown for the proportions of patients who needed excision for eschar removal.

If numerically possible, as a supportive analysis, the variable treatment center will be added into the logistic regression model and the analysis repeated.

As a sensitivity analysis, the logistic regression outlined above will be repeated using the PP as analysis population.

If any of the baseline factors, gender, age, percent SPT area (the area of SPT as a percentage of the total area of all TWs, classified as <25% or ≥25%), time from injury to randomization or number of TWs (1, 2, ≥3) are found to be significantly different between the treatment groups, then the factor will be included as an extra adjusting covariate in the logistic regression model described above as additional analysis.

As supportive analysis, the incidence of surgical excision for eschar removal will be compared between NexoBrid and Gel Vehicle using the same explanatory variables as in the main analysis of this endpoint.

A further supportive analysis will repeat the analysis (NexoBrid versus SOC) as described above, but on a wound level. I.e. a mixed logistic regression model with explanatory variables treatment, TW depth, %TBSA per wound and number of TWs and a random effect for patient will be performed.

11.1.3.2. Time to complete eschar removal

Time until complete eschar removal will be defined as the time until complete eschar removal has been achieved at a patient level, i.e. for all TW's of an individual patient. This will be measured as time from the randomization date (hours). For patients who do not reach complete eschar removal, their time will be censored at the last non-missing eschar removal assessment (typically the last debridement procedure). Kaplan-Meier curves will be presented graphically (see Figure 3) and in a table (see Table 8) to display the distribution of time to complete eschar removal under the two treatments (NexoBrid vs. SOC). Median time to complete eschar removal will be estimated for each treatment group with a 95% confidence interval. Additionally, time to complete eschar removal will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The treatment groups will be compared using a Cox regression model. The comparison will be adjusted for the explanatory variables overall TW depth (all TWs full thickness, mixed TWs, all TWs deep partial thickness), "Total %TBSA per patient", center group (as defined in Section 11.1.10) and number of TWs (1, 2, ≥3), by

including each of them in the Cox regression model together with the treatment variable (NexoBrid vs. SOC). The results of the regression analysis will be displayed as in Table 7. The treatment groups will be compared by testing the null hypothesis of no difference, comparing the ratio of the estimated treatment coefficient to its standard error to a standard normal distribution.

The Cox Regression analysis outlined above will be performed if the proportional hazards assumption appears to hold. This will be checked by including in the regression model a variable representing the interaction between time since randomization and treatment group. If the coefficient for this time variable is non-significant at the 5% significance level, then the Cox Regression analysis will be adopted. If the time-treatment group interaction is significant, then a generalized Wilcoxon-Gehan test will be performed using the *Wilcoxon* option (also provided by default) in the SAS procedure PROC LIFETEST using the following SAS Code:

```
PROC LIFETEST DATA=burn_data;
TIME time*status(0);
STRATA depthgroup numtw tbsagroup centergroup / GROUP = treatment_group;
RUN;
```

Here burn_data is the data set containing all necessary information. The variable time is the time to complete eschar removal, status is a censoring variable and depthgroup, numtw, tbsagroup, and centergroup are the variables, which is adjusted for. Depthgroup, tbsagroup and centergroup are the stratification levels defined in Section 11.1.10 and numtw is the number of target wounds (1, 2, ≥3).

As sensitivity analysis the procedure outlined above will be repeated using the PP instead of the FAS as analysis population.

Supportive analyses will include adjustment for other baseline variables that are imbalanced (i.e. gender, age, percent SPT area [the area of SPT as a percentage of the total area of all TWs, classified as <25% or ≥25%], time from injury to randomization or number of TWs (1, 2, ≥3)), and investigating interactions between those variables and the treatment groups. In case the proportional hazards assumption could not be rejected, this will be done by including each of the imbalanced baseline variable as well as the interaction between that variable and the treatment group in a separate cox regression analysis. The results of these analyses will be presented as in Table 7. In case the proportional hazards assumption does not hold, the adjustment for the baseline variables will be done using the following SAS code:

```
PROC LIFETEST DATA=burn_data;
TIME time*status(0);
STRATA depthgroup numtw tbsagroup centergroup gender agegroup sptgroup /
GROUP = treatment_group;
RUN;
```

Here burn_data is the data set containing all necessary information. The variable time is the time to complete eschar removal, status is a censoring variable and depthgroup, numtw, tbsagroup, centergroup, gender, agegroup and sptgroup are the variables, which is adjusted for. Agegroup is the categorical age variable computed in Section 10.1.1.2, depthgroup, tbsagroup and centergroup are the stratification levels defined in Section 11.1.10 and numtw is the number of target wounds (1, 2, ≥3).

Another supportive analysis will be the comparison of treatments based on the time of complete eschar removal measured from the date of ICF using the same methods as for the time measured from randomization.

Another supportive analysis will be the comparison of treatments based on the time of complete eschar removal measured from the date of injury using the same methods as for the time measured from randomization.

As an additional analysis, time to complete eschar removal will be compared between NexoBrid and Gel Vehicle using the same explanatory variables as for the main analysis of this endpoint.

11.1.3.3. Blood loss

In Section 10.3.3.3 two blood loss estimates are defined. Analyses with both blood loss estimates will be conducted. However, some missing values are expected. The analysis with less missing values will be regarded as more reliable and is therefore considered the main analysis. The other analysis is considered supportive. Both analyses will be conducted as follows:

The measure of blood loss defined in Section 10.3.3.3 will be computed for each patient, and the distribution in the NexoBrid group will be compared with that in the SOC group. Means, standard deviations, medians, and interquartile ranges will be calculated and presented as in Table 4. Furthermore, the blood loss will be described graphically by histograms. The normality of the data will be tested on each treatment group using the Shapiro-Wilk test. If the normal distribution hypothesis is not rejected at the 5% significance level in either group, then differences in distribution between NexoBrid and SOC will be tested using a t-test. If the normal distribution hypothesis is rejected either in the NexoBrid group or in the SOC group, then the

differences in distribution between the treatment groups will be tested using a Mann-Whitney test. The test results will be presented as in Table 7.

As sensitivity analysis, the procedure outlined above will be repeated using the PP instead of the FAS as analysis population.

Supportive analyses will include adjustments for the covariates overall TW depth (all TWs full thickness, mixed TWs, all TWs deep partial thickness), “Total %TBSA per patient”, center group (as defined in Section 11.1.10), number of TWs (1, 2, ≥3) and number of debridement procedures. This will be done by including these variables in a regression model for blood loss together with treatment group.

If any of the baseline factors, gender, age, or percent SPT area (the area of SPT as a percentage of the total area of all TWs, classified as <25% or ≥25%), time from injury to randomization are found to be significantly different between the treatment groups, then the factor will be included as an extra adjusting covariate in a supportive analysis of blood loss (as described in the preceding paragraph).

As an additional analysis, blood loss will be compared between NexoBrid and Gel Vehicle without covariate adjustment (similar to the main analysis of this endpoint).

11.1.4. Safety variables

All safety variables except for the first safety endpoint “time to wound closure” will be evaluated on the safety set.

11.1.4.1. Safety Endpoint: Time to wound closure

This safety endpoint will be analyzed using the FAS. A sensitivity analysis will be done by repeating the analysis outlined below with the PP as analysis population.

Time to reach complete wound closure will be compared between the NexoBrid and SOC at a wound level using a method of survival analysis with clustered data that is based on appropriate assumptions. By “clustered data”, we refer to the multiple target wounds that can occur in a patient. As stated in the protocol, a non-inferiority margin will be incorporated into the analysis that will represent a 7-day advantage to the SOC group. This will be done by adding 7 days to the wound closure times in the SOC group. After that, the proportional hazards assumption will be checked in the same way as in the analysis of the timely eschar removal endpoint. I.e. a marginal cox regression model with a robust sandwich estimator (see below for further details) will be performed including the variables treatment, TW depth (on a wound level), %TBSA (on a wound level) and number of TWs (1, 2, ≥3) as well as a variable

representing the interaction between time since randomization and treatment group. If the coefficient for this time variable is non-significant at the 5% significance level, then the proportional hazards assumption will be considered appropriate. In this case we will use a marginal Cox regression analysis with a robust sandwich estimator. If not, then we will use an accelerated failure time (AFT) model with shared frailty. Note that this deviates from the previously planned parametric frailty model implemented in the SAS procedure PHREG. The latter one assumes proportional hazards and is therefore not suitable here.

Either method can be implemented in SAS (see [33] for details). The marginal Cox regression model is implemented in the PHREG procedure and the robust variance option is implemented by specifying the COVS(AGGREGATE) option. This induces the use of the robust variance method of Binder et. al. [34]. The accelerated failure time model with shared frailty will be implemented using the following SAS-code:

```
PROC NLMIXED DATA=burn_data;
BOUNDS gamma>0;
linp = b0 + b1*treatment_group + b2*spt + b3*dpt + b4*ft + b5*tbsa +
b6*number_tw2+ b7*number_tw3 + b8*center2 + b9*center3 + b10*center4 +
b11*center5 + z;
alpha = exp(-linp);
G_t = 1/(1+alpha*time**gamma);
g = alpha*gamma*time**(gamma-1)/(1+alpha*time**gamma)**2;
ll = (censor=0)*log(g) + (censor=1)*log(G_t);
model time~general(ll);
RANDOM z ~ normal(0,sigma) subject=subject_id;
RUN;
```

where subject_ID is a unique identification number for each patient, time is the time to wound closure on a wound level. Censor is a censoring indicator, treatment_group gives the treatment group, spt gives the % area SPT (on a wound level), dpt gives the % area DPT (on a wound level), ft gives the % area FT (on a wound level), TBSA gives the %TBSA (on a wound level), number_tw2 is an indicator variable for the subject having 2 target wounds (patient level), number_tw3 is an indicator variable for the subject having ≥3 target wounds (patient level), center2,…,center5 are indicator variables for the individual being in center group 2,…,5 (patient level) and burn_data is a SAS data set containing the required information.

The SAS code presented above calculates the treatment effect in an accelerated failure time model with shared frailty assuming a log-logistic distribution of the data. The commonly used Weibull-distribution is not applicable in this case, because the AFT model is equivalent to a proportional hazards model.

The presented methods include information on all the target wounds of each patient and account for any within-patient correlation. Missing information due to incomplete follow-up is incorporated naturally into such analyses as censored observations.

Kaplan-Meier curves will be presented graphically (see Figure 3) and in a table (see Table 8) to display the distribution of time to complete wound closure under the two treatments.

If any of the baseline factors, gender, age, or percent SPT area (the area of SPT as a percentage of the total area of all TWs, classified as <25% or ≥25%) are found to be significantly different between the treatment groups, then the factor will be included as an extra adjusting covariate in a supportive analysis of time to complete wound closure.

Furthermore, time to reach 100% wound closure will be analyzed as outlined above as an additional analysis.

11.1.4.2. Safety Endpoint: Cosmesis and function at 12 months from wound closure

Not applicable for this stage of analysis since the relevant information for all patients is not available yet. This endpoint will be analyzed in the second stage analysis (see 11.2.4.2).

11.1.4.3. Safety Endpoint: Cosmesis and function at 24 months from wound closure

Not applicable for this stage of analysis since the relevant information for all patients is not available yet. This endpoint will be analyzed in the third stage analysis (see 11.3.4.3).

11.1.4.4. Adverse Events

In this analysis, only the adverse events occurring in the acute phase (until the 3 months follow-up for each patient) will be analyzed.

All adverse events will be listed (see Table 14).

Adverse events are classified as being general, local – TW related or local – not TW related in the eCRF. Per each of these categories, the AEs will be listed as given in Table 9 and Table 10. The listing will be presented separately by:

- System organ class
- Preferred term
- Severity (mild / moderate / severe AEs)
- Relatedness (not related / remotely related / possibly related / probably related / related AEs)

- Time of onset (before start of treatment / during the treatment session / during the first week after treatment / during week 2 to week 4 after treatment / during week 5 to week 8 after treatment / more than 8 weeks after treatment)

SAEs will be analyzed in the same way as AEs.

11.1.4.5. Vital Signs

All vital signs (body temperature, heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure) will be analyzed descriptively with number of subjects, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

The analyses will be done for the vital signs assessments at screening, post eschar removal procedures, at the first 7 daily assessments as well as for the pre-post differences per procedure and the pre-post differences of the daily vital signs assessments described in Section 10.3.4.4 separately.

Furthermore, for the vital signs assessment at screening, post eschar removal procedures and at the first 7 daily assessments the information whether the overall vital signs assessment was normal, abnormal (NCS) or abnormal (CS) is given in the eCRF. These classifications will be analyzed separately for each time point of vital signs assessment by displaying counts and percentages by treatment group (see Table 5). The proportion of normal / abnormal (NCS) and abnormal (CS) assessments will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts). Bar plots of the proportions of patients with normal / abnormal (NCS) and abnormal (CS) assessments will be shown.

11.1.4.6. Pain Assessment

The pain assessment score will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms. Furthermore, a plot of the mean pain (and the mean change in pain from baseline) over time will be presented.

The analyses will be done for the pain assessments at screening, pre and post eschar removal procedures, at the first 7 daily assessments as well as for the pre-post differences of the daily vital sings assessments (difference to baseline) described in Section 10.3.4.4 separately.

Furthermore, for the pain assessment at screening, pre and post eschar removal procedures and at the first 7 daily assessments the information whether the pain assessment was normal, abnormal (NCS) or abnormal (CS) is given in the eCRF. These classifications will be analyzed separately for each time point of vital signs assessment by displaying counts and percentages by treatment group (see Table 5). The proportion of normal / abnormal (NCS) and abnormal (CS) assessments will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

11.1.4.7. Extend of exposure to study drug

Counts and percentages of patients having one respectively two applications will be presented as shown in Table 5 for the NexoBrid and the Gel Vehicle arm. A chi-squared test (or in case of small estimated cell counts Fisher's exact test) will be used to test for any differences in the application data between the two treatment groups. Bar plots of the extend of exposure will be shown.

Furthermore, numbers of patients in the NexoBrid arm being exposed to different grams of NexoBrid powder will be displayed.

11.1.4.8. Medical history and concomitant diseases

The concomitant diseases will be classified using the MedDRA dictionary. They will be tabulated with reference to system organ class and frequency of occurrence (see Table 9). A similar analysis will be conducted but on the level of preferred terms instead of system organ class. Concomitant diseases include all active conditions at start of trial (as documented in the medical history). The analysis of medical history conditions will be done and displayed separately for previous and ongoing diseases.

11.1.4.9. Concomitant medication

In this analysis, only the concomitant medication administered during the acute phase (until the 3 months follow-up for each patient) will be analyzed. The number of concomitant medication and patients involved will be tabulated with reference to ATC coding (see Table 11).

11.1.4.10. Central laboratory values

Laboratory measurements obtained prior and after each eschar removal procedure as well as pre-post differences in laboratory values for each eschar removal procedure will be analyzed descriptively with number of subjects, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4.

As described in Section 10.3.6, laboratory values will be categorized as “abnormal (low)”, “normal” and “abnormal (high)” based on the reference limits of the labs. This categorization will be analyzed by displaying counts and percentages of each category by treatment group and by procedure (see Table 5) for each laboratory value separately.

11.1.4.11. Blood transfusions

The volume of blood transfusions in milliliters (as calculated in Section 10.3.4.4) will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean volume of blood transfusions between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of the volume of blood transfusions in the treatment groups.

The volume of blood transfusions will be presented overall, as well as separately for volumes transfused before start of treatment, during the ER period, within 1 week after the ER period and later than 1 week after the ER period.

11.1.4.12. Maintenance of complete wound closure

This is a binary (yes/no) variable (on a wound level). Maintenance of complete wound closure will be analyzed by displaying counts and percentages of wounds maintaining complete wound closure by treatment group (see Table 5). The proportion of wounds maintaining complete wound closure will be compared between treatment groups using a chi square test (or Fisher’s exact test in case of small estimated cell counts). Bar plots of the proportions of wounds maintaining complete wound closure will be shown.

The analysis will be conducted for the information present at month 1 and month 3 after wound closure.

11.1.4.13. Incidence of QT prolongation

Incidence of QT prolongation will be analyzed separately. See separate document for the description of the analysis.

11.1.4.14. Analgesia, anesthesia and antibiotic use

For an analysis of the level of sedation, the number and percentage of patients per each level of sedation and each eschar removal procedure (in topical arms: 1st and second topical application, surgical rescue procedures and non-surgical rescue procedures; in SOC arms: surgical procedures and non-surgical procedures) will be tabulated.

The number of days of exposure to antibiotic drugs will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. This analysis will be conducted for overall antibiotic use, for antibiotic use due to AE involvement (as indicated in the eCRF) and for prophylactic antibiotic use (as indicated in the eCRF) separately.

11.1.4.15. Hospital readmission rates

Hospital admission rates will be analyzed on a patient level by displaying counts and percentages by treatment group (see Table 5). The proportion of patients needing readmission to a hospital will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts) and presented graphically using bar plots.

Furthermore, the number of planned hospital readmissions per patient will be analyzed by displaying counts and percentages by treatment group (see Table 5). The number of planned hospital readmissions per patient will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts) and presented graphically using bar plots.

Additionally, the number of unplanned hospital readmissions per patient will be analyzed by displaying counts and percentages by treatment group (see Table 5). The number of unplanned hospital readmissions per patient will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts) and presented graphically using bar plots.

Hospital readmission will also be analyzed on a readmission level. Counts and percentages of planned/unplanned readmissions will be displayed by treatment group (see Table 5). The proportion of planned/unplanned readmissions to hospitals will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts) and presented graphically using bar plots

11.1.4.16. INR/PTT change

Change in INR/PTT will be evaluated at 4h post first treatment as well as on the first and second daily assessment post treatment separately. This is a binary (yes/no) variable indicating whether the patient experienced a change of INR/PTT to > normal range (reference limits of the local laboratories). Rates of patients with this event will be analyzed by displaying counts and percentages by treatment group (see Table 5) in each time point separately (4h post, 24h and 48h). Reference count is the number of patients with normal PTT/INR values at

baseline. For the classification of normal and abnormal PTT/INR values, the reference limits of the local labs will be used. The proportion of patients with change of INR/PTT to > normal range will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

INR/PTT values will be classified as normal, abnormal (NCS) and abnormal (CS) based in the eCRF. These will be analyzed by displaying counts and percentages by treatment group (see Table 5) for each time point separately (4h post, 24h and 48h).

11.1.4.17. Blood glucose change

This is a binary (yes/no) variable indicating whether the patient experienced a change of blood glucose to > upper limit of normal range. Rates of patients with this event will be analyzed by displaying counts and percentages by treatment group (see Table 5) per eschar removal procedure. Furthermore, bar plots will be displayed. The proportion of patients with change of blood glucose to > upper limit of normal range will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

Furthermore, the change in blood glucose from baseline to 4h post treatment per patient will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

11.1.5. Exploratory Analyses

11.1.5.1. % wound area surgically excised for eschar removal

The % wound area surgically excised for eschar removal per patient (computed as in section 10.3.5.5) will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

11.1.5.2. Incidence of surgical Escharotomy procedures on circumferential extremities target wounds

Two analyses will be done for this exploratory endpoint. The number and percentage of circumferential extremities target wounds that went through escharotomy will be presented by treatment group (see Table 5). The proportion of circumferential extremities target wounds that

went through escharotomy will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

The number and percentage of patients having at least one circumferential extremities target wound, which went through escharotomy, will be presented by treatment group (see Table 5). Furthermore, bar plots will be displayed. The proportion of patients having at least one circumferential extremities target wound which went through escharotomy will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

11.1.5.3. Incidence of reduction in interstitial/compartment pressure in circumferential extremities target wounds

This is a binary (yes/no) variable indicating whether the patient experienced reduction in interstitial/compartment pressure in circumferential extremities target wounds for each target wound and each procedure separately. Rates of patients with this event will be analyzed by displaying counts and percentages by treatment group (see Table 5). Furthermore, bar plots will be displayed. The proportion of patients with reduction in interstitial/compartment pressure in circumferential extremities target wounds will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

11.1.5.4. Incidence of surgically harvested donor site wounds

This is a counting variable, giving the number of donor site wounds per patient. Rates of patients with different numbers of donor site wounds will be analyzed by displaying counts and percentages by treatment group (see Table 5). Furthermore, bar plots will be displayed (see Figure 2). The number of donor site wounds will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

11.1.5.5. % area of surgically harvested donor site wounds

The % wound area of surgically harvested donor site wounds per patient (computed as in section 10.3.5.11) will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

11.1.5.6. Blood loss using changes in hematocrit following eschar removal procedures

The measure of blood loss defined in Section 10.3.5.12 will be computed for each patient, analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

11.1.5.7. Blood loss using changes in hemoglobin following eschar removal procedures

The measure of blood loss defined in Section 10.3.5.13 will be computed for each patient, analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

11.1.5.8. Blood loss in NexoBrid procedures vs. surgical procedures

The measure of blood loss defined in Section 10.3.3.3.1 will be computed for each procedure, and analyzed descriptively with number of procedures, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between NexoBrid procedures and surgical procedures with a one way analysis of variance. A graphical representation of the data will be done using histograms.

11.1.5.9. Autograft related parameters

Total number of autografting procedures

The total number of autografting procedures (per patient) will be analyzed by displaying counts and percentages by treatment group (see Table 5). Furthermore, bar plots will be displayed. The distribution of the total number of autografting procedures will compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

This analysis will be repeated on a wound level.

Incidence of repeated autografting

This is a binary (yes/no) variable indicating whether the patient had more than one autograft procedure during the study (based on different dates on the same TW). Rates of patients with

more than one autograft procedure will be analyzed by displaying counts and percentages by treatment group (see Table 5). Furthermore, bar plots will be displayed. The proportion of patients with repeated autograft procedures will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

This analysis will be repeated on a wound level.

Area of repeated autografting

The area of repeated autografting per patient will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as shown in Table 4 and compared between groups with a one way analysis of variance. A graphical representation of the data will be done using histograms.

This analysis will be repeated on a wound level.

Early autografted wounds

A subgroup analysis will be performed on the subgroup of target wounds that are early grafted (for a definition see Appendix 13.1.1). The primary endpoint, the secondary endpoints "incidence of surgical excision" and "time to complete eschar removal", as well as the safety endpoint "time to complete wound closure" will be analyzed while only taking wounds that are early grafted into account.

Late autografted wounds

A subgroup analysis will be performed on the subgroup of target wounds that are late grafted (for a definition see Appendix 13.1.1). The primary endpoint, the secondary endpoints "incidence of surgical excision" and "time to complete eschar removal", as well as the safety endpoint "time to complete wound closure" will be analyzed while only taking wounds that are late grafted into account.

11.1.5.10. Duration of hospitalization

The duration of hospitalization will be analyzed descriptively with Kaplan-Meier plots (see Figure 3) and compared between groups with a log rank test.

11.1.6. Pharmacokinetic Variables

Pharmacokinetic variables will be analyzed separately. The analysis is described in a separate document.

11.1.7. Multicenter Data

Randomization will be stratified by center group as detailed in the Section 13.7.2 of the protocol.

The analyses of the primary and secondary endpoints of this trial will adjust for possible effects of the variable center by including it as covariate in the analyses.

11.1.8. Handling of Multiple Comparisons

Unless otherwise specified, all statistical tests will be conducted against a two-sided alternative hypothesis, employing a significance level of 0.05. The primary and secondary endpoints of efficacy will be tested in a hierarchy, so as to preserve the nominal significance levels:

1. Primary
2. Incidence of surgery
3. Time to complete eschar removal
4. Blood loss.

11.1.9. Interim analysis

No interim analyses will be performed.

11.1.10. Stratification

The following factors are used to stratify the study design:

A. Total TBSA % per patient

A1: Total TBSA <=15%

A2: Total TBSA >15%

B. Overall depth per patient

B1: All TWs are full thickness;

B2: Mixed TWs – full thickness and deep partial thickness

B3: All TWs are deep partial thickness.

C. Center Group, to address possible differences in SOC procedures between sites (if any), to allow comparison of the results within group of centers and to reduce the likelihood of a group of centers including patients in only one arm of the study (5 groups of centers were formed based on similarity of SOC practice).

These factors are accounted for in the analyses of the primary and secondary endpoints by including them as adjusting variables.

11.1.11. Analysis of Subgroups

The following subgroup analyses were pre planned and will be conducted to explore efficacy and safety within certain pre-defined subgroups of the population. All analyses presented here are of exploratory nature.

11.1.11.1. Target wounds in the anatomical area of the hand

A subgroup analysis will be performed on the subgroup of target wounds that are found, at least partly, in the anatomical area of the hand. The primary and secondary endpoints will be analyzed while only taking wounds that are found, at least partly, in the anatomical area of the hand into account.

11.1.11.2. Patients with < 25% SPT area

Patients will be classified as having < 25 % or ≥ 25% SPT area as a percentage of the area of all TWs.

For the subgroup of patients with < 25 % SPT area descriptive statistics (counts and percentages) of patients achieving complete eschar removal will be displayed for patients treated with NexoBrid or Gel Vehicle as shown in Table 5. The two groups will be compared using Fisher's exact test. The results will further be described by a bar plot (see Figure 2).

For the secondary endpoint "surgical excision performed" counts and percentages of patients needing surgical excision will be presented (see Table 5). The treatment groups will be compared using a chi square test (of Fisher's exact test in case of small expected cell counts).

The secondary endpoint "time to complete eschar removal" Kaplan-Meier estimates will be presented graphically and in a table (see Figure 3 and Table 8). Time to complete eschar removal will be compared between treatment groups by a log rank test.

For the secondary endpoint "blood loss" the number of subjects, number of missing values, means, standard deviations, medians, quartiles, minimums and maximums will be calculated

and presented as in Table 4. Furthermore, the blood loss will be described graphically by histograms (see Figure 1). Treatment groups will be compared using a t-test.

The safety endpoint “time to reach complete wound closure” will be analyzed by presenting Kaplan-Meier estimates graphically and in a table (see Figure 3 and Table 8). Time to complete wound closure will be compared between treatment groups by a log rank test.

11.1.11.3. Patients with ≥ 25% SPT area

The same analysis as in Section 11.1.11.2 will be done but on the subgroup of patients with ≥ 25% SPT area.

11.1.11.4. Total TBSA ≤ 15%

The same analysis as in Section 11.1.11.2 will be done but on the subgroup of patients with total TBSA <=15%.

11.1.11.5. Total TBSA > 15%

The same analysis as in Section 11.1.11.2 will be done but on the subgroup of patients with total TBSA > 15%.

11.1.11.6. All target wounds full thickness

The same analysis as in Section 11.1.11.2 will be done but on the subgroup of patients with all target wounds classified as full thickness wounds.

11.1.11.7. Mixed target wounds – full thickness and deep partial thickness

The same analysis as in Section 11.1.11.2 will be done but on the subgroup of patients with mixed thickness target wounds (full thickness and deep partial thickness).

11.1.11.8. All target wounds are deep partial thickness

The same analysis as in Section 11.1.11.2 will be done but on the subgroup of patients with all target wounds classified as deep partial thickness wounds.

11.1.11.9. Wounds that are entirely full thickness

The same analysis as in Section 11.1.11.1 will be performed but on the subgroup of wounds that are entirely full thickness wounds.

11.2. Second stage analysis – 12 month short-term follow-up (STFU12)

11.2.1. Demographics and Other Baseline Characteristics

Not applicable for this stage of analysis since all information on demographics and other baseline characteristics was already analyzed in the first stage analysis (see 11.1.1).

11.2.2. Primary Efficacy variable

Not applicable for this stage of analysis since all information on the primary efficacy variable was already analyzed in the first stage analysis (see 11.1.2).

11.2.3. Secondary Efficacy variables

Not applicable for this stage of analysis since all information on the secondary efficacy variables was already analyzed in the first stage analysis (see 11.1.3).

11.2.4. Safety variables

11.2.4.1. Safety Endpoint: Time to wound closure

Not applicable for this stage of analysis since all information on this endpoint was already analyzed in the first stage analysis (see 11.1.4.1).

11.2.4.2. Safety Endpoint: Cosmesis and function at 12 months from wound closure

The MVSS score for a patient will be calculated as described in Section 10.3.4.2. The mean and standard error of this MVSS score (for the target wounds) at 12 months will be estimated for each treatment group and presented as shown in Table 4. The treatment groups will be compared using a linear model with MVSS score as the dependent variable. The explanatory variables in the model will include treatment, and the following variables: overall TW depth (all TWs full thickness, mixed TWs, all TWs deep partial thickness), “Total %TBSA per patient” and number of TWs (1, 2, ≥3). The coefficient corresponding to the treatment group will be estimated and will represent the estimated mean difference in MVSS score between NexoBrid and SOC adjusted for any imbalance in the stratification factors. The results of the regression analysis will be presented as in Table 7.

A clinically meaningful difference will be incorporated into the analysis that will represent a 1.9 or more units advantage to the SOC group, so that the analysis will show whether the NexoBrid group is estimated to have on average a MVSS score that is not worse by more than 1.9 units than the score for the SOC group. That is, the following hypothesis are about to be tested:

$$H_0: \Delta \geq 1.9 \quad vs. \quad H_1: \Delta < 1.9$$

Where Δ is the mean difference in MVSS score between NexoBrid and SOC adjusted for any imbalance in the stratification factors. These hypotheses will be tested by comparing the 95% confidence interval for the coefficient of the treatment group from the linear model outlined above to the clinically meaningful difference 1.9. The null hypothesis will be rejected if the upper bound of this interval is smaller than 1.9.

For handling of missing data see Section 10.1.2.2.

11.2.4.3. Safety Endpoint: Cosmesis and function at 24 months from wound closure

Not applicable for this stage of analysis since the relevant information for all patients is not available yet. This endpoint will be analyzed in the third stage analysis (see 11.3.4.3).

11.2.4.4. Adverse Events

In this analysis, only the adverse events occurring after the 3 months follow up and until the 12 months follow up (including AEs from the first stage that are still ongoing) will be analyzed.

All adverse events will be listed (see Table 14).

Adverse events are classified as being general, local – TW related or local – not TW related in the eCRF. Per each of these categories, the AEs will be listed as given in Table 9 and Table 10. The listing will be presented separately by:

- System organ class
- Preferred term
- Severity (mild / moderate / severe AEs)
- Relatedness (not related / remotely related / possibly related / probably related / related AEs)
- Time of onset (until 3 months follow up [i.e. AEs from first stage that are still ongoing] / between 3 months follow up and 12 months follow up)

SAEs will be analyzed in the same way as AEs.

11.2.4.5. Concomitant medication

In this analysis, only the concomitant medications given after the 3 months follow up and until the 12 months follow up (including CM with a start date before the 3 months follow up but an end date after that or still ongoing at 12 months) will be analyzed.

The number of concomitant medication and patients involved will be tabulated with reference to ATC coding (see Table 12).

11.2.4.6. Lower Extremity Function Scale (LEFS)

The LEFS scores of the 12 months follow up assessment will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean LEFS scores between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of LEFS values.

11.2.4.7. Disabilities of the Arm, Shoulder and Hand (QuickDASH)

The QuickDASH scores of the 12 months follow up assessment will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean QuickDASH scores between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of QuickDASH values.

11.2.4.8. Range of Motion (ROM)

The 12 months range of motion will be evaluated using 3 binary (yes/no) variables. The variables indicate whether all ROM were normal, whether there was at least one abnormal (NCS) finding and whether there was at least one abnormal (CS) finding. This information is present per patient and per visit. Rates of patients with this event will be analyzed by displaying counts and percentages by treatment group (see Table 5) per variable (all normal / at least one abnormal (NCS) / at least one normal (CS)) and per visit. Furthermore, bar plots will be displayed. The proportion of patients with all normal / at least one abnormal (NCS) / at least one normal (CS) range of motion will be compared between treatment groups using a chi square test (or Fisher's exact test in case of small estimated cell counts).

11.2.4.9. EQ-5D (Quality of Life)

The 12 months follow up assessment of the EQ-5D will be analyzed as follows.

The EQ-5D consists of 5 items with 3 possible answers each and an EQ visual analogue scale. For each item (Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) the

count and percentages of each possible answer will be presented per age group and per treatment group as in Tables 3 and 4 of the EQ-5D-3L user guide [32]. Furthermore, bar plots will be provided. The difference between treatment groups will be tested using a chi square test (or Fishers' exact test in case of small expected cell counts).

The values of the EQ visual analogue scale (EQ VAS) will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 3 of the EQ-5D-3L user guide [32]. The mean EQ VAS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of EQ VAS values.

11.2.4.10. Burn Specific Health Scale – Brief (BSHS-B)

The 12 months follow up assessment of the BSHS-B will be analyzed as follows.

Each domain specific sub score and the overall BSHS-B score will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4.The mean (sub-) score values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of (sub-) score values.

11.2.5. Exploratory Analyses

11.2.5.1. POSAS

The POSAS for target wounds will be calculated as outlined in Section 10.3.5.7 and will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean POSAS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of POSAS in the treatment groups.

The analysis will be repeated for the patient scale and for the observer scale separately.

The analysis will be repeated for donor site scars.

The analyses will be conducted for the POSAS assessments of 1, 3, 6 and 12 months.

11.2.5.2. MVSS (target wounds)

The MVSS for target wounds at 1, 3, and 6 months will be calculated as outlined in Section 10.3.4.2 and will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean

MVSS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of MVSS in the treatment groups.

11.2.5.3. MVSS (donor site scars)

The MVSS for donor site scars at 1, 3, 6 and 12 months will be calculated as outlined in Section 10.3.4.2 and will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean MVSS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of MVSS in the treatment groups.

11.2.6. Pharmacokinetic Variables

Not applicable. All pharmacokinetic data was already analyzed in the first stage analysis (see Section 11.1.6).

11.2.7. Multicenter Data

See Section 11.1.7.

11.2.8. Handling of Multiple Comparisons

Not applicable. All testing done during this second stage analysis is exploratory in nature. So no adjustment for multiplicity is needed.

11.2.9. Interim analysis

No interim analyses will be performed.

11.2.10. Stratification

See Section 11.1.10.

11.2.11. Analysis of Subgroups

11.2.11.1. Total TBSA ≤ 15%

A subgroup analysis will be done for the patients with Total TBSA ≤ 15%.

The 12 months MVSS will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4.

The mean MVSS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of MVSS in the treatment groups.

11.2.11.2. Total TBSA > 15%

The same analysis as in Section 11.2.11.1 will be done but on the subgroup of patients with total TBSA > 15%.

11.2.11.3. All target wounds full thickness

The same analysis as in Section 11.2.11.1 will be done but on the subgroup of patients with all target wounds classified as full thickness wounds.

11.2.11.4. Mixed target wounds – full thickness and deep partial thickness

The same analysis as in Section 11.2.11.1 will be done but on the subgroup of patients with mixed thickness target wounds (full thickness and deep partial thickness).

11.2.11.5. All target wounds are deep partial thickness

The same analysis as in Section 11.2.11.1 will be done but on the subgroup of patients with all target wounds classified as deep partial thickness wounds.

11.3. Third stage analysis – 24 month long-term safety follow-up (LTSFU24)

11.3.1. Demographics and Other Baseline Characteristics

Not applicable for this stage of analysis since all information on demographics and other baseline characteristics was already analyzed in the first stage analysis (see 11.1.1).

11.3.2. Primary Efficacy variable

Not applicable for this stage of analysis since all information on the primary efficacy variable was already analyzed in the first stage analysis (see 11.1.2).

11.3.3. Secondary Efficacy variables

Not applicable for this stage of analysis since all information on the secondary efficacy variables was already analyzed in the first stage analysis (see 11.1.3).

11.3.4. Safety variables

11.3.4.1. Safety Endpoint: Time to wound closure

Not applicable for this stage of analysis since all information on this endpoint was already analyzed in the first stage analysis (see 11.1.4.1).

11.3.4.2. Safety Endpoint: Cosmesis and function at 12 months from wound closure

Not applicable for this stage of analysis since all information on this endpoint was already analyzed in the second stage analysis (see 11.2.4.2).

11.3.4.3. Safety Endpoint: Cosmesis and function at 24 months from wound closure

The MVSS score for a patient will be calculated as described in Section 10.3.4.3. The mean and standard error of this MVSS score (for the target wounds) at 24 months will be estimated for each treatment group and presented as shown in Table 4. The treatment groups will be compared using a linear model with MVSS score as the dependent variable. The explanatory variables in the model will include treatment, and the following variables: overall TW depth (all TWs full thickness, mixed TWs, all TWs deep partial thickness), “Total

%TBSA per patient" and number of TWs (1, 2, ≥3). The coefficient corresponding to the treatment group will be estimated and will represent the estimated mean difference in MVSS score between NexoBrid and SOC adjusted for any imbalance in the stratification factors. The results of the regression analysis will be presented as in Table 7.

A clinically meaningful difference will be incorporated into the analysis that will represent a 1.9 or more units advantage to the SOC group, so that the analysis will show whether the NexoBrid group is estimated to have on average a MVSS score that is not worse by more than 1.9 units than the score for the SOC group. That is, the following hypothesis are about to be tested:

$$H_0: \Delta \geq 1.9 \quad vs. \quad H_1: \Delta < 1.9$$

Where Δ is the mean difference in MVSS score between NexoBrid and SOC adjusted for any imbalance in the stratification factors. These hypotheses will be tested by comparing the 95% confidence interval for the coefficient of the treatment group from the linear model outlined above to the clinically meaningful difference 1.9. The null hypothesis will be rejected if the upper bound of this interval is smaller than 1.9.

For handling of missing data, see Section 10.1.2.2.

11.3.4.4. Adverse Events

In this analysis, only the adverse events occurring after the 12 months follow up will be analyzed.

All adverse events will be listed (see Table 14).

Adverse events are classified as being general, local – TW related or local – not TW related in the eCRF. Per each of these categories, the AEs will be listed as given in Table 9 and Table 10. The listing will be presented separately by:

- System organ class
- Preferred term
- Severity (mild / moderate / severe AEs)
- Relatedness (not related / remotely related / possibly related / probably related / related AEs)
- Time of onset (until 3 months follow up [i.e. AEs from first stage that are still ongoing] / between 3 months follow up and 12 months follow up [i.e. AEs from second stage that are still ongoing] / after 12 months follow up)

SAEs will be analyzed in the same way as AEs.

11.3.4.5. Concomitant medication

In this analysis, only the concomitant medications (CM) given after the 12 months follow up (including CM with a start date before the 12 months follow up but an end date after that or still ongoing) will be analyzed.

The number of concomitant medication and patients involved will be tabulated with reference to ATC coding (see Table 13). The tabulation will be done by treatment group.

11.3.4.6. Immunogenicity evaluation

The immunogenicity evaluation will be done separately and is described in a separate document.

11.3.4.7. Lower Extremity Function Scale (LEFS)

The LEFS scores of the 24 months follow up assessment will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean LEFS scores between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of LEFS values.

11.3.4.8. Disabilities of the Arm, Shoulder and Hand (QuickDASH)

The QuickDASH scores of the 24 months follow up assessment will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean QuickDASH scores between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of QuickDASH values.

11.3.4.9. Range of Motion (ROM)

The 24 months range of motion will be evaluated using 3 binary (yes/no) variables. The variables indicate whether all ROM were normal, whether there was at least one abnormal (NCS) finding and whether there was at least one abnormal (CS) finding. This information is present per patient and per visit. Rates of patients with this event will be analyzed by displaying counts and percentages by treatment group (see Table 5) per variable (all normal / at least one abnormal (NCS) / at least one normal (CS)) and per visit. Furthermore, bar plots will be displayed. The proportion of patients with all normal / at least one abnormal (NCS) / at least one normal (CS) range of motion will be compared between treatment groups using a chi square test (or Fisher’s exact test in case of small estimated cell counts).

11.3.4.10. EQ-5D (Quality of Life)

The 24 months follow up assessment of the EQ-5D will be analyzed as follows.

The EQ-5D consists of 5 items with 3 possible answers each and an EQ visual analogue scale. For each item (Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) the count and percentages of each possible answer will be presented per age group and per treatment group as in Tables 3 and 4 of the EQ-5D-3L user guide [32]. Furthermore, bar plots will be provided. The difference between treatment groups will be tested using a chi square test (or Fishers' exact test in case of small expected cell counts).

The values of the EQ visual analogue scale (EQ VAS) will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4 of the EQ-5D-3L user guide [32]. The mean EQ VAS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of EQ VAS values.

11.3.4.11. Burn Specific Health Scale – Brief (BSHS-B)

The 24 months follow up assessment of the BSHS-B will be analyzed as follows.

Each domain specific sub score and the overall BSHS-B score will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean (sub-) score values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of (sub-) score values.

11.3.5. Exploratory Analyses

11.3.5.1. POSAS

The POSAS for target wounds will be calculated as outlined in Section 10.3.5.7 and will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean POSAS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of POSAS in the treatment groups.

The analysis will be repeated for the patient scale and for the observer scale separately.

The analysis will be repeated for donor site scars.

The analyses will be conducted for the POSAS assessments of 18 and 24 months.

11.3.5.1. MVSS (target wounds)

The MVSS for target wounds at 18 and 24 months will be calculated as outlined in Section 10.3.4.2 and will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean MVSS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of MVSS in the treatment groups

11.3.5.2. MVSS (donor site scars)

The MVSS for donor site scars at 18 and 24 months will be calculated as outlined in Section 10.3.4.3 and will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean MVSS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of MVSS in the treatment groups.

11.3.6. Pharmacokinetic Variables

Not applicable. All pharmacokinetic data was already analyzed in the first stage analysis (see Section 11.1.6).

11.3.7. Multicenter Data

See Section 11.1.7.

11.3.8. Handling of Multiple Comparisons

Not applicable. All testing done during this second stage analysis is exploratory in nature. So no adjustment for multiplicity is needed.

11.3.9. Interim analysis

No interim analyses will be performed.

11.3.10. Stratification

See Section 11.1.10.

11.3.11. Analysis of Subgroups

11.3.11.1. Total TBSA ≤ 15%

A subgroup analysis will be done for the patients with Total TBSA ≤ 15%.

The 24 months MVSS will be analyzed descriptively with number of units, number of missing values, mean, standard deviation, min, max, median and quartiles as presented in Table 4. The mean MVSS values between treatment groups will be compared by a one-way analysis of variance. Histograms will be provided to illustrate the distribution of MVSS in the treatment groups.

11.3.11.2. Total TBSA > 15%

The same analysis as in Section 11.3.11.1 will be done but on the subgroup of patients with total TBSA > 15%.

11.3.11.3. All target wounds full thickness

The same analysis as in Section 11.3.11.1 will be done but on the subgroup of patients with all target wounds classified as full thickness wounds.

11.3.11.4. Mixed target wounds – full thickness and deep partial thickness

The same analysis as in Section 11.3.11.1 will be done but on the subgroup of patients with mixed thickness target wounds (full thickness and deep partial thickness).

11.3.11.5. All target wounds are deep partial thickness

The same analysis as in Section 11.3.11.1 will be done but on the subgroup of patients with all target wounds classified as deep partial thickness wounds.

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13. Appendices

13.1. Definitions

13.1.1. General Definitions

Full analysis Set (FAS): The full analysis set includes all patients who are randomized into the trial. The analysis is focused on the planned treatment

Complete eschar removal: Complete eschar removal is achieved at the end of the eschar removal treatment phase. This stage will be determined by a designated blinded assessor and clearly marked as the end of the eschar removal process and the initiation of specific treatment aimed to close the viable debrided bed by grafting or epithelialization.

Complete wound closure: Defined as skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits, 2 weeks apart, determined by a blinded assessor.

Early grafted wounds: Early autografted wounds were defined as wounds autografted within 7 days or less from injury date.

Late grafted wounds: Late autografted wounds were defined as wounds autografted more than 7 after injury date.

13.1.2. Levels of Sedation

For the analysis of levels of sedation, the definition of general anesthesia and levels of sedation/analgesia of the American Society of Anesthesiologists (see Table 3) is used.

Table 3: Continuum of depth sedation: Definition of general anesthesia and levels of sedation/analgesia*- American Society of Anesthesiologists

	Minimal Sedation Anxiolysis	Moderate Sedation/ Analgesia ("Conscious Sedation")	Deep Sedation/ Analgesia	General Anesthesia
Responsiveness	Normal response to verbal stimulation	Purposeful** response to verbal or tactile stimulation	Purposeful** response following repeated or painful stimulation	Unarousable even with painful stimulus
Airway	Unaffected	No intervention required	Intervention may be required	Intervention often required
Spontaneous Ventilation	Unaffected	Adequate	May be inadequate	Frequently inadequate
Cardiovascular function	Unaffected	Usually maintained	Usually maintained	May be impaired

Minimal Sedation (Anxiolysis) is a drug-induced state during which patients respond normally to verbal commands. Although cognitive function and physical coordination may be impaired, airway reflexes, and ventilation and cardiovascular functions are unaffected.

Moderate Sedation/Analgesia ("Conscious Sedation") is a drug-induced depression of consciousness during which patients respond purposefully** to verbal commands, either alone or accompanied by light tactile stimulation. No interventions are required to maintain a patent airway, and spontaneous ventilation is adequate. Cardiovascular function is usually maintained.

Deep Sedation/Analgesia is a drug-induced depression of consciousness during which patients cannot be easily aroused but respond purposefully** following repeated or painful stimulation. The ability to independently maintain ventilatory function may be impaired. Patients may require assistance in maintaining a patent airway, and spontaneous ventilation may be inadequate. Cardiovascular function is usually maintained.

General Anesthesia is a drug-induced loss of consciousness during which patients are not arousable, even by painful stimulation. The ability to independently maintain ventilation function is often impaired. Patients often require assistance in maintaining a patent airway, and positive pressure ventilation may be required because of depressed spontaneous ventilation or drug-induced depression of neuromuscular function. Cardiovascular function may be impaired.

* Monitored Anesthesia Care ("MAC") does not describe the continuum of depth of sedation, rather it describes "a specific anesthesia service in which an anesthesiologist has been requested to participate in the care of a patient undergoing a diagnostic or therapeutic procedure."

** Reflex withdrawal from a painful stimulus is NOT considered a purposeful response.

13.2. Lay-out and list of tables

For continuous variables, descriptive statistics will be presented as follows:

Table 4: Descriptive statistics of continuous variables per treatment.

Treatment	Variable								
	N	Missing	Mean	Std Dev	Min	Q1	Median	Q3	Max
NexoBrid									
SOC									
Gel Vehicle									
Total									

Table 5: Counts and (column) percentages of categorical variables per treatment.

	Treatment							
Variable	NexoBrid		SOC		Gel Vehicle		Total	
	N	(%)	N	(%)	N	(%)	N	(%)
Category 1								
Category 2								
....								
Total								

Table 6: Confidence interval and test result for odds ratios

Comparison	Odds ratio	Test	p-value	Lower 95 % confidence bound	Upper 95 % confidence bound

Table 7: Presentation of test results and confidence intervals

	Estimate	Std Err	Test	Value of the test statistic	p-value	Lower 95 % confidence bound	Upper 95 % confidence bound
Variable							

Table 8: Table for Kaplan-Meier estimates per treatment

Time in days/hours	NexoBrid				SOC			
	Case	Censoring	At risk	%	Case	Censoring	At risk	%
0								
…								

Table 9: Counts and percentages of Medical History Conditions or Adverse Events by MedDRA System Organ Class.

System Organ Class	SOC		NexoBrid		Gel Vehicle		Total	
	N	(%)	N	(%)	N	(%)	N	(%)
Infections and infestations								
Neoplasmsm benign, malignant and unspecified (incl. cysts and polyps)								
Blood and lymphatic system disorders								
…								
Social Circumstances								
Total								

Table 10: Counts and percentages of Adverse Events by categories of a categorical variable

Variable	SOC		NexoBrid		Gel Vehicle		Total	
	N	(%)	N	(%)	N	(%)	N	(%)
Category 1								
Category 2								
Category 3								
…								
Total								

Table 11: Counts and percentages of concomitant medication with reference to ATC coding (1st stage analysis).

ATC code	Contents	Concomitant medication		Number of Patients		Indication							
						AE		Prophylactic		Pre-existing condition		Other	
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
A	Alimentary tract and metabolism												
B	Blood and blood forming organs												
C	Cardiovascular system												
D	Dermatologicals												
G	Genito-urinary system and sex hormones												
H	System hormonal preparations, excluding sex hormones and insulins												
J	Antiinfectives for systemic use												
L	Antineoplastic and immunomodulating agents												
M	Musculo-skeletal system												
N	Nervous system												
P	Antiparasitic products, insecticides and repellents												
R	Respiratory system												
S	Sensory organs												
V	Various												
Total													

Table 12: Counts and percentages of concomitant medication with reference to ATC coding (2nd stage analysis)

ATC code	Contents	Concomitant medication		Number of Patients		Indication								Start time			
						AE		Prophylactic		Pre-existing condition		Other		Before 3m FU		After 3m FU	
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
A	Alimentary tract and metabolism																
B	Blood and blood forming organs																
C	Cardiovascular system																
D	Dermatologicals																
G	Genito-urinary system and sex hormones																
H	System hormonal preparations, excluding sex hormones and insulins																
J	Antiinfectives for systemic use																
L	Antineoplastic and immunomodulating agents																
M	Musculo-skeletal system																
N	Nervous system																
P	Antiparasitic products, insecticides and repellents																
R	Respiratory system																
S	Sensory organs																
V	Various																
Total																	

Table 13: Counts and percentages of concomitant medication with reference to ATC coding (3rd stage analysis)

ATC code	Contents	Concomitant medication		Number of Patients		Indication								Start time					
						AE		Prophylactic		Pre-existing condition		Other		1st stage		2nd stage		3rd stage	
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
A	Alimentary tract and metabolism																		
B	Blood and blood forming organs																		
C	Cardiovascular system																		
D	Dermatologicals																		
G	Genito-urinary system and sex hormones																		
H	System hormonal preparations, excluding sex hormones and insulins																		
J	Antiinfectives for systemic use																		
L	Antineoplastic and immunomodulating agents																		
M	Musculo-skeletal system																		
N	Nervous system																		
P	Antiparasitic products, insecticides and repellents																		
R	Respiratory system																		
S	Sensory organs																		
V	Various																		
Total																			

13.3. Lay-out and list of figures

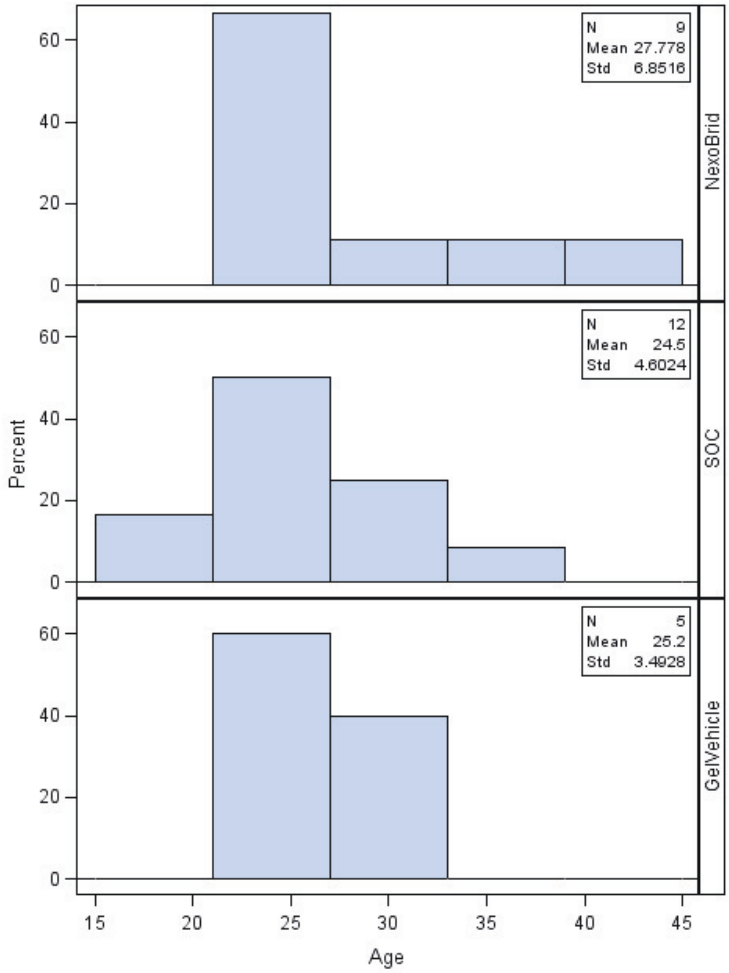

Figure 1: Histogram of continuous variable per treatment

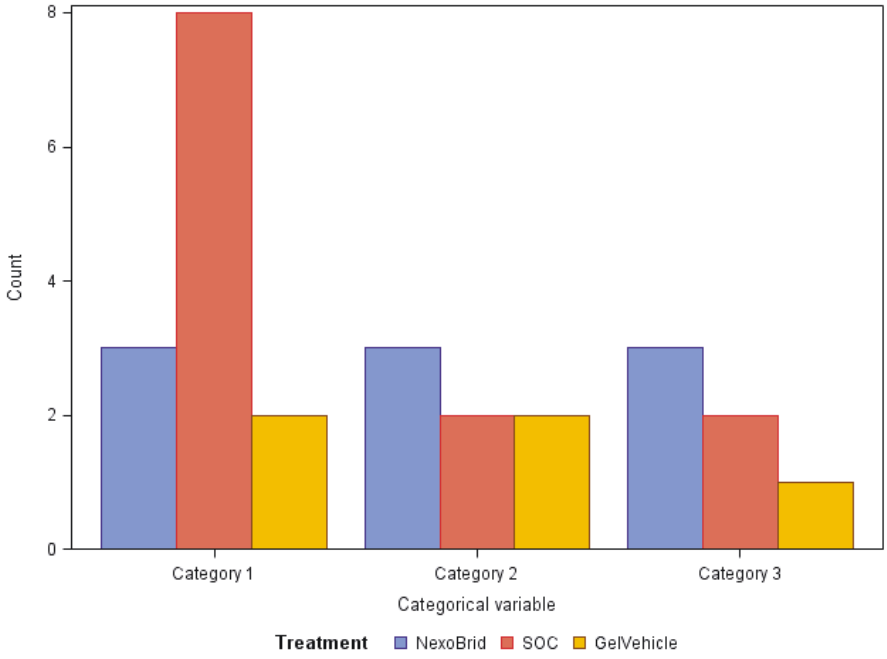

Figure 2: Bar plot for categorical variable by treatment

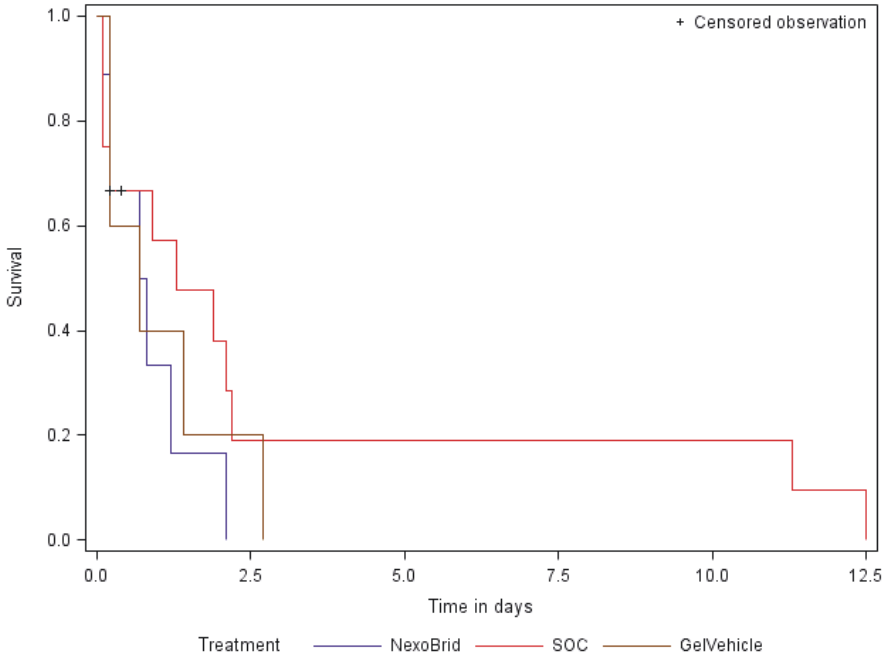

Figure 3: Kaplan Meier plot for time to event data

13.4. Lay-out and content of individual patient data listings (case wise listing)

The case wise listing will not be done by the KKSB and also not form a part of the statistical report. Therefore, in this SAP no lay-out and content is given. The statistical report will refer to the clinical report for the case wise listing.

13.4.1. List of AEs and SAEs

Table 14: Table for listing of all AEs and SAEs.

AE	Subject Id	Start date	Duration in days	Number of AE	Description of AE	Severity grade	Relationship	Outcome	Study treatment	Therapy of AE	SAE
1											
2											
....											

13.5. Details of the Amendments

13.5.1. Summary of Amendment of version 8 to version 9

Study title: A multicenter, multinational, randomized, controlled, assessor blinded study, performed in subjects with thermal burns, to evaluate the efficacy and safety of NexoBrid compared to Gel Vehicle and compared to Standard of Care

Study plan code: MW 2010-03-02

IND No.: 65,448

EudraCT No.: 2014-001672-55

Amendment summary- protocol MW2010-03-02, version 09, 12 Aug 2015

The following changes were performed in protocol version 9:

1. Pages 40 and 100-102, Randomization and Stratification
    a. Changes: Stratifications levels were revised; stratification per %TBSA was removed and stratification per depth was revised and SPT proportion was removed from the stratification levels. In addition, randomization will be performed per blocked randomization method rather by minimization
    b. Justifications: Per FDA comments sent to the Sponsor on May 2015, stratification levels were reduced and will be performed in the amended protocol per patient's wounds depth and per clinical center. In addition, FDA suggestion to perform "simple randomization" was implemented.
2. Pages 102-104, Data Handling
    a. Changes: Missing data section was slightly revised for the efficacy & safety endpoints
    b. Justifications: The above changes were performed in accordance with FDA comments sent on May 2015.
3. Pages 78 and 93-05 Analysis of endpoints
    a. Changes: Planned analysis of the primary, secondary & safety endpoints was slightly revised per FDA suggested comments (e.g. limiting the planned analysis to only the terms expected to have the greatest impact, pre- define a plan in the protocol for checking assumptions and determining which analysis will be used, etc)
    b. Justifications: The above revisions were implemented per FDA suggested comments sent on May 2015.

13.5.1. Summary of Amendment of version 9 to version 11

The below table summarizes the changes to Protocol MW2010-03-02, version 9 (submitted to the FDA on August 18, 2015, SN0087) as was implemented in protocol, version 11.

Line	Section in version 11	Suggested Change	Justification
1.	Definition (section 1)	Additions to the definition of '*Circumferential extremity wounds' (see in BOLD): "Circumferential extremity wounds: Limb* ***DPT and/or FT*** *burns which encircle ≥ 80% of the limb circumference" and* "Extremities at Risk (EAR) are circumferential DPT and/or FT burns **that may develop BICS during the first 3 days post injury** with at least one of the following…"	Details were added for clarity and standardization among sites
		Definition for '*Increased interstitial pressure syndrome*' was revised to be in line with the Burn-induced compartment syndrome (BICS)- the common medical term	BICS is a medical event described in the protocol which is being monitored by the treating physicians in anatomical areas of the extremities
		Additions to the definition of Target Wounds	Details were added for clarity and standardization among sites. Minimal size of target wound was reduced to 0.5% TBSA (see further justification in line 12 below)

Line	Section in version 11	Suggested Change	Justification
		Addition of definition for substance use disorder in accordance with the DSM-5	This definition was added to support exclusion # 24 (see line #13 below)- to better describe alcohol abuse
		Addition of assessment of complete wound closure and complete eschar removal- by a blinded assessor	This addition is in line with the protocol and former discussions with the agency. Added in this section for clarity and consistency with the study protocol.
2.	Synopsis (section 2)	The following changes were performed: Changes to entrance criteria (see lines #10-13 below), additional safety outcome measures (line #8), Study Treatments and Dosage (line #9) and additional exploratory endpoints (line #5). All these changes are further detailed below.	In accordance with changes performed in the body of the protocol, similar changes are described in the synopsis section. Please refer to more details in the relevant lines below.
3.	Introduction (Section 3)	Addition of Pharmacokinetic profile section from the recently completed study MW2008-09-03- Section 3.2.3	Information could be added following recent completion of study MW2008-09-03
		Minor additions to section 3.3.2 - under extremities wounds	Additional medical information was added to this section
4.	General safety Endpoints (section 6.1.3)	Addition of the following parameters to the general safety endpoints: "*Number and volume of blood transfusions received throughout the hospital admission, Extent of analgesia, anaesthesia and antibiotic use, Rates of hospital readmission, Change in INR/PTT and incidence of change to > upper limit of normal (after treatment), Change in blood*	The proposed changes were added in an attempt to harmonize the protocol and meet both the FDA and CHMP requirements

Line	Section in version 11	Suggested Change	Justification
		glucose and incidence of change to above upper limit of normal (after treatment)".	
5.	Exploratory Endpoints (section 6.1.4)	1. Description of planned autograft analyses were added: efficacy and safety analyses for early and late grafted wounds, total number of target wound grafting procedures, incidence of repeat/additional grafting procedures & area of repeat grafting 2. Duration of hospitalization- added as additional exploratory EP	The proposed changes were added in an attempt to harmonize the protocol and meet both the FDA and CHMP requirements
6.	Assessment of clinical measures (section 6.3.1.1)	Under Extent of eschar removal assessment using topical agent; NexoBrid or Gel Vehicle, the following was added: "*For the purpose of standardization, if feasible, the same blinded assessor should be assigned to assess eschar removal in all patients, and in all study arms*".	This change was added to further improve the standardization of the eschar removal assessment among the sites
7.	Non surgical eschar removal (section 6.3.1.3)	The following clarification was added (in BOLD): "*Wounds will be assessed by a burn specialist as in common daily practice,* ***following any dressing change*****"**	This change was added to further improve the standardization of the eschar removal assessment among the sites
8.	Main safety measures (6.3.2)	The following was added: *"This assessor* ***should not be involved in any eschar removal procedure and should not be aware whether the patient was assigned to the topical arms or to the SOC arm. For the purpose of standardization, if feasible, the same blinded assessor should be assigned to assess wound closure, cosmesis***	This change was added to further improve the standardization of the wound closure and cosmesis and function assessment among the sites

Line	Section in version 11	Suggested Change	Justification
		and function (as described below) in all patients, and all study arms. *The blinded assessor will sign the "blinded assessor memo".*	
9.	Study Treatments and Dosage (6.4)	The following instruction was added: "NexoBrid/Gel vehicle should not be applied to more than 15% TBSA (±3% TBSA) in one session".	This instruction was added to instruct how to use the study drug in patients with targets wounds of more than 15% TBSA that may be included to the study following the change in Inclusion No 4
10.	Inclusion Criteria- patient level (section 7.1.1)	Inclusion No. 1- upper age of 70 was deleted: "**Males and females; ≥ 18 years of age**"	The change was implemented in accordance with the FDA request (letter from May 14, 2015)
		Inclusion No. 4- %TBSA was revised to be maximum of 30% TBSA: "***Patient total burns area should be ≤ 30% TBSA; SPT, DPT and/or FT in depth***"	The change was implemented in accordance with the agreed changes discussed with the FDA (Type C TC, May 25, 2016)
		Inclusion No. 5 was revised as following: "*Informed consent can be obtained within 84 hours of the burn injury*"	Patients will need to sign on ICF before start of treatment which will be no later than 84 hrs from injury (section 8.2 in the protocol, version 11) instead of within 48 hrs. This will allow larger flexibility for signing consent form based on feedback received from clinical sites participating in the study. Start of treatment limitation was changed as

Line	Section in version 11	Suggested Change	Justification
			well. In protocol version 9, the allowed window to start treatment was 72 hrs from injury. This window was expanded to be 84 hrs from injury.
11.	Inclusion criteria- wound level (section 7.1.2)	Addition of inclusion Criterion: *"SPT areas that cannot be demarcated from DPT and FT areas should be less than 50% of the % TBSA of the TW".* In addition, clarification was added regarding the minimum criteria for patient to be enrolled in a wound level.	To increase standardization of treated wounds and to avoid treating wounds with large superficial areas
12.	Inclusion criteria- wound level (section 7.1.2)	Minimal wound size was reduced from 1% TBSA (FT and/or DPT) to 0.5% TBSA.	To avoid having patients with 3% TBSA DPT and/or FT wounds that most of them small and could not be defined as TWs, the minimum size of TW was reduced.
13.	Exclusion Criteria (section 7.1.3)	Exclusion #5: Blast injury was removed and inserted as part of exclusion #18	As blast injury considered as an additional risk in burn patients, this was added to Exclusion 18 which exclude patients with "*Any conditions that would preclude safe participation in the study or adding further risk to the basic acute burn trauma*"

Line	Section in version 11	Suggested Change	Justification
		Deletion of Exclusion #6: "*Patients with DPT and/or FT facial burn wounds from flame, flash, explosion > 0.5% TBSA (scald and contact burns are allowed)*"	This exclusion was originally written in order to avoid inclusion of subjects with diagnosis of smoke inhalation injury however this concern is being addressed by Exclusion criterion # 12 (see below)
		Clarification added to exclusion #7 (in BOLD): "*Patients with circumferential **(≥ 80% of the limb circumference)** DPT and/or FT burns defined as Extremities at Risk (EAR)"*	A more accurate definition of circumferential injury was inserted into this criterion to avoid confusion
		Exclusion criterion #12: "*Any signs or history that may indicate smoke inhalation"* was revised to be "*Diagnosis of smoke inhalation injury*"	This criterion was modified to avoid exclusion of patients with signs which do not necessarily indicate smoke inhalation injury
		Exclusion criterion #14- lower limit of HbA_{1c} for poorly controlled diabetes mellitus was increased from 9% to 11%	This change does not add any safety risk, as assessed medically
		Exclusion #15 was revised- BMI exclusion will be based on the extent of burn area- patients with up to 15%TBSA will be excluded if BMI above 39 and patients with %TBSA above 15% will be excluded if BMI above 34.	This revision was performed following feedback from the US clinical sites relate to the common US population. This change will not add any additional safety concern

Line	Section in version 11	Suggested Change	Justification
		Exclusion criteria #17-19- Medical conditions in injured subjects that should be excluded from the study were better defined (see in bold): 1. Cardio-pulmonary disease (MI within 6 months prior to injury, **severe** pulmonary hypertension, **severe** COPD or pre-existing oxygen-dependent pulmonary diseases, **severe** broncho-pneumonia **within 1 month prior to injury**, steroid dependent asthma or uncontrolled asthma), 2. Pre-existing diseases which interfere with circulation (**severe** peripheral vascular disease, **severe** edema **and/or** lymphedema, **regional lymph nodes dissection**, **significant** varicose veins). Obesity was deleted from this criterion as being addressed in exclusion # 14 3. Any conditions that would preclude safe participation in the study or adding further risk to the basic acute burn trauma (such as **severe** immuno-compromising diseases, life threatening trauma, severe pre-existing coagulation disorder, **severe** cardiovascular disorder, **significant** pulmonary disorder, **significant** liver disorder including post alcoholic abuse impaired function or neoplastic disease, **blast injury**)	To avoid exclusion of patients with condition that will not preclude safe participation
		Exclusion criterion #24- clarification regarding alcohol and drug abuse- this definition was revised in the protocol, section 1 (see line 1 above): ***Substance use disorder: describes a problematic pattern of using alcohol or another substance that results in impairment in daily life***	To avoid exclusion of patients who used drugs/drink alcohol but are not abused and can sign consent and could comply with study procedures

Line	Section in version 11	Suggested Change	Justification
		or noticeable distress. The DSM-5 states that in order for a person to be diagnosed with a severe disorder due to a substance, they must display 6 or more of the 11 symptoms within 12-months. These 11 symptoms are now defined in the protocol and should be assessed by the sites.	
14.	Subject withdrawal criteria (7.2)	Addition of criteria for subject's removal from the study- "***Withdrawal of subjects with EAR identified post randomization but prior to treatment"***	This addition will allow exclusion of subjects with BICS diagnosed after randomization and before start of treatment
15.	BICS monitoring & diagnosis (section 7.2.1.1)	• Clarification was added- all circumferential wounds should be closely monitored during treatment. • Escharotomy "will" be performed was replaced with "may" • Minor wording changes for clarity	• This was added to avoid deviations and ensure close monitoring of circumferential wounds during treatment • Following feedback from the study PIs- SOC does not necessarily include escharotomy following BICS diagnosis as defined in the study protocol hence, escharotomy is at the discretion of the PI.
16.	Post treatment diagnosed infection (section 7.2.1.3)	Timeframe for diagnosed infection, that may be consider as a stopping rule, was added - until complete wound closure	The timeframe was added for clarity of standardization
Section 8- Study conduct			

Line	Section in version 11	Suggested Change	Justification
17.	Screening and Baseline Procedures (section 8.1.1)	Burn description (size and depth) as well as photographs were deleted before cleansing and will be captured only after cleansing	Screening wound assessments will be performed only once after initial cleansing of wounds. Pre cleansing is usually done pre signing informed consent and therefore this assessment was deleted.
18.	Screening and Baseline Procedures (section 8.1.1)	The use of schematic drawing to mark TWs location was added to the protocol	This drawing is being used by the sites and the relevant documentation was added to the study protocol
19.	Assessment within 24hrs prior to eschar removal (section 8.1.3)	Assessments and procedures previously requested within 24 hrs prior to start of eschar removal were moved and will be performed within 1 hr prior to start of treatment (8.2.1)	All procedures before treatment will be performed at the same time and will prevent repeated blood tests for the subjects
20.	Treatment procedure-Eschar Removal (Section 8.2)	Start of treatment was change from 72 to 84 hrs from injury	To allow a wider time frame for treatment preparations (screening, ICF signing, pre treatment procedures, cleansing)

Line	Section in version 11	Suggested Change	Justification
21.	SOC & Topical arms- eschar removal procedures (sections 8.2.2 & 8.2.3)	Most of the changes in these sections are only editorial ones. Additional changes are described below	Editorial changes were performed in order to have the procedures written in chronological order and with similar structure in both arms.
22.	SOC & Topical arms- eschar removal procedures (sections 8.2.2 & 8.2.3)	Pre procedure assessments will be done only when the additional procedure is surgical	The need to perform pre assessments for additional topical procedure or additional SOC that is non-surgical were deleted, since the assessment are done post the previous procedure (topical or surgical) as described in the protocol
23.	sections 8.2.2.1.4, 8.2.2.2.3, 8.2.3.2 & 8.4	Addition of clarification footnote with regard to complete eschar removal assessment	Assessment of complete eschar removal was further specified to improve standardization across centers.

Line	Section in version 11	Suggested Change	Justification
24.	Post first procedure (SOC and topical arms) 8.2.2.1.4 & 8.2.3.1.6	PTT/INR: • Will not be taken after surgical procedure if it is not the first procedure. • Will be taken 4 hours post treatment instead of 4 hours from start of treatment	• To avoid duplications with 24 and 48 hours assessments • In order to reduce time points of taking blood from the patients, PTT/INR will be taken at the same time point of the safety central lab tests
25.	Post first & post second application assessments – 8.2.3.1.6 & 8.2.3.2.2	Assessments post topical procedure will be collected 4 hours post removal of topical agent instead of 4 hours post soaking (2 hours earlier)	This change will result in similar time point as in the SOC arm
26.	Post first procedure (8.2.2.1.4) & Post additional procedure (8.2.2.2.3)	Post non-surgical assessments - Will be taken only if the non-surgical procedure is the last procedure – i.e. resulted in complete eschar removal	If non surgical procedure is not the last it will be followed by a surgical procedure and assessments will be done pre surgical procedure.

Line	Section in version 11	Suggested Change	Justification
27.	General assessment & Wound management procedure (sections 8.3 & 8.5)	Coverage used as wound management will be captured until wound closure	This information is important to be captured during the study, until wound closure
28.	General assessment (section 8.3)	Additional time point for PK was added- 72 hours from start of treatment	This point was added based on PK profile information collected to date
29.	Follow-up Assessments (section 8.6)	Addition of clarification footnote with regard to complete wound closure assessment	Assessment of complete wound closure was further specified to improve standardization across centers.
30.	Follow-up Assessments (section 8.6)	Weekly FU visits will be continued until donor sites will be closed	This assessment will provide additional safety data in patient level (i.e. closure of all wounds, including the donor sites, if relevant)

Line	Section in version 11	Suggested Change	Justification
31.	Hospital Discharge (section 8.7)	The following assessments at Hospital Discharge (HD) were deleted: 1. The target wounds and donor sites should be labeled and photographed, 2. Assessment of % of wound area epithelialized and/or closed by graft, 3. Assessment of % of donor site area epithelialized, 4. Type of cover/dressing applied on TWs and donor sites should be recorded	These changes were applied to avoid duplicate assessments of in both weekly FU visits and HD visit which is being performed in parallel to the weekly FU visits
32.	Follow-up assessments: wound closure confirmation (Section 8.8)	Extent of wound closure will be documented in the confirmation visit	This assessment was added In order to collect additional exploratory information
33.	Table 3, section 9	Changes performed in accordance with changes in flow of treatment	
34.	Primary endpoint 11.1	The assessment of the Incidence of complete eschar removal was clarified	The wording was changed to stress that the assessment that is considered for the primary endpoint is the one done by the blinded assessor and at a specific pre defined time point

Line	Section in version 11	Suggested Change	Justification
35.	Safety EPs-Time to complete wound closure (section 11.3.1)	NI margin for time to wound closure reduced to 7 days instead of 7.4 days	The proposed change was added following re assessments and taking into consideration additional clinical data.
36.	Safety EPs-Cosmesis & function at 12 and 24 months (sections 11.3.2 and 11.3.3)	NI margin for MVSS was reduced to 1.9	The proposed change was added following re assessments and further discussion with PIs.
37.	General safety parameters (section 11.3.4)	The following were added: "**Number and volume of blood transfusions received throughout the hospital admission, Extent of analgesia, anaesthesia and antibiotic use (i.e. total dose per kg body weight), Rates of hospital readmission, Change in INR/PTT and incidence of change to > upper limit of normal (after treatment), Change in blood glucose and incidence of change to above upper limit of normal (after treatment)**"	See line #4 above

Line	Section in version 11	Suggested Change	Justification
38.	Exploratory EPs (section 11.4)	1. Description of planned autograft analyses were added: efficacy and safety analyses for early and late grafted wounds, total number of target wound grafting procedures, incidence of repeat/additional grafting procedures & area of repeat grafting 2. Duration of hospitalization- added as additional exploratory EP.	See line #5 above
39.	Additional analyses (section 11.5 & 13.3.4.2)	Additional analyses were added for eschar removal and wound closure	The proposed change was added in attempt to harmonize the protocol and meet both the FDA and CHMP requirements
40.	Subgroup analyses (section 11.6 & 13.3.5)	The following sub groups were added: • Sub group analysis on entirely FT wounds • Sub group analysis on patients classified as having <25% or >=25% SPT area as a percentage of the area of all TWs • Sub group analysis per % TBSA	The first 2 subgroup analyses were added in order to test for possible effects of wounds depth on the study results. The subgroup analysis per %TBSA was implemented in accordance with the agreed changes discussed with the FDA (Type C TC, May 25, 2016). See also appendix #1 for the rational to include sub group analysis per %TBSA as additional analysis and not to include it in the main analysis (primary analysis) as discussed with the FDA during the TC.

Line	Section in version 11	Suggested Change	Justification
41.	EAR and BICS-Monitoring and Diagnosis (section 11.8)	Revision to EAR & BICS section was performed and clarification of monitoring methods on each time point was added to this section	Main reason for change is to reduce complexity in following protocol procedures and reduce sites' potential deviations
42.	Immunogenicity evaluation (section 11.11)	Week 1 test was deleted. Immunogenicity evaluation will now be performed based on weeks 4, 8 and 6 & 24 months visits	The ELISA method is specific for anti NexoBrid IgG. Circulating anti NexoBrid IgG are not expected one week after treatment and hence, this time-point was deleted
43.	Graft loss and Graft take (section 11.16)	Added definition for graft take	Definition was added to further allow standardization
44.	Pharmacokinetic evaluation (section 11.21) & Appendix 10-Procedures for specific blood tests	PK timelines were revised as follows: **In subjects requiring a single application:** Before treatment (time zero), 0.5 hours ± 10 minutes, 2 hours ± 10 minutes, 4 hours ± 10 minutes, 12 hours ± 30 minutes, 24 hours ± 30 minutes , 48 hours ± 30 minutes and 72 hours ± 30 minutes after NexoBrid application. **In subjects requiring two planned applications of NexoBrid:** 1st treatment: before treatment (time 0), 0.5 hours ± 10 minutes, 2 and 4 hours ± 10 minutes after the first NexoBrid application.	Based on the available PK data from study MW2008-09-03, new sampling paradigm is proposed for the Phase 3 MW2010-03-02 study to support PK analysis after the first and second applications

Line	Section in version 11	Suggested Change	Justification
		2nd treatment: time 0, 30 ± 5 minutes, 2 hours ± 10 minutes, 4 hours ± 10 minutes, 12 hours ± 30 minutes , 24 hours ± 30 minutes, 48 and 72 hours ± 30 minutes after the second NexoBrid application	
45.	Pharmacokinetic evaluation (section 11.21) & Appendix 10-Procedures	The subset of patients tested for PK was modified: 1. 16 subjects with total wounds area of <=15% TBSA 2. 16 subjects with total wounds area to be treated of >15% TBSA	This addition of PK samples in patients with more than 15% TBSA will provide PK information in patients with maximal permissible TBSA and data for second application
46.	Safety endpoint-Time to wound closure (section 13.3.3.1)	An addition to the planned Cox regression model	This change was performed in accordance with the FDA request (letters from 14 May 2015 & 03 Nov 15)
47.	Stratification (13.7.2)	Stratification factor of TBSA was added. Stratification levels of the factor Center was reduced by grouping centers into 5 groups.	Stratification by TBSA was added due to the change in inclusion No 4 to include patients with TBSA of more than 15%. Stratification by centre was changed to "group of centers, 5 groups rather 30 centers to allow balance in the randomization.

Line	Section in version 11	Suggested Change	Justification
48.	Missing data (13.8.4)	The analysis of the safety endpoint time to complete wound closure will compute missing values as censored.	This change was performed in accordance with the FDA request (letters from 14 May 2015 & 03 Nov 15)
49.	Section 13	Changes performed in sections 13.3.3.3 (general safety parameters), 13.3.4 (exploratory), 13.3.4.2 (additional analyses) and 13.3.5 (subgroup analyses) as described in lines 24-27 above	Changes performed as described above
Section 14- Quality assurance			
50.	DSMB (section 14.4)	Addition of special cases for DSMB convention- 1.After randomization of 10 NexoBrid patients suffering from deep burns of more than 15% TBSA (TWs area) and were treated with 2 repeated applications of NexoBrid 2. After randomization of 25 NexoBrid patients suffering from deep burns of more than 15% TBSA (TWs area) and were treated with 2 repeated applications of NexoBrid	This change is part of the risk mitigation plan suggested to support the inclusion and treatment of patients up to 30% TBSA as was discussed with the FDA during the Type C meeting held on May 25, 2016
	DSMB (section 14.4)	Timing of the routine meeting was clarified, Review of narratives by the DSMB was added	The proposed changes were added in attempt to better clarify the DSMB role

Line	Section in version 11	Suggested Change	Justification
51.	Appendix 3- Photographic wound documentation	Details were added to the instructions in this appendix, in accordance with the study photograph guideline.	Detailed instructions were added in order to ensure standardized digital images