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

Protocol No.: MW2010-03-02

IND No: 65,448

EudraCT No: 2014-001672-55

Clinical Phase: III

Sponsor:

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This clinical study will be conducted in accordance with Declaration of Helsinki and its updates, current Good Clinical Practice (GCP), the provisions of ICH (International Conference on Harmonisation); US Code of Federal Regulations (Title 21, CFR Part 11, 50, 54, 56 and 312) and/or EU Directives; local country regulations and the sponsor's Standard Operating Procedures (SOPs)

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The undersigned are the persons authorized to sign the protocol on behalf of the sponsor

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1. LIST OF DEFINITIONS AND ABBREVIATIONS

1.1 DEFINITIONS[1]

Hereafter is a list of terms and definitions that are acceptable in the burn care area.

TBSA: Total Body Surface Area; in burn care, referring to the (%) total body surface area affected by second and third degree burns [1].

Burn eschar: A slough (area of necrotic tissue), crust or dry scab resulting from a burn. Eschar is structurally intact but dead and denatured dermis [1].

Burn Depth Definitions [1]:

Second Degree (Partial Thickness) Burns involve the epidermis and portions of the dermis and can be clinically categorized as superficial partial-thickness, deep partial-thickness burns or as most common: combination of different depths:

Superficial partial-thickness burns heal spontaneously (most of them) in less than 3 weeks, and do so typically without functional impairment or hypertrophic scarring.

Deep partial-thickness burns extend into the deeper layers of the dermis. They possess characteristics that are distinctly different from superficial partial-thickness burns. If infection is prevented and spontaneous healing is allowed to progress, these burns may heal in three to nine weeks.

Indeterminate burns are usually second degree burns of changing depths difficult to diagnose on arrival so only after few days when the burn conversion (progression) and demarcation took place they can be diagnosed as deep in need of surgery or superficial. Distinguishing between deeper burns and shallow burns is not always straightforward, and many burn wounds have a mixture of superficial mid and deep burns, making precise classification of the entire wound difficult.

Third Degree (Full-Thickness) Burns involve all layers of the dermis and often injure underlying subcutaneous adipose tissue as well. Burn eschar is structurally intact but dead and denatured dermis. Over days and weeks if left in situ, eschar separates ("sloughs") from the underlying viable tissue, through infectious, inflammatory and maceration processes leaving an open, unhealed bed of granulation tissue. Without surgery, they can heal only by wound contracture and scarring with epithelialization exclusively from the wound margins.

Combination wounds, mixed of second and third degrees: the depth of the wound is not homogeneous and many burn wounds have a mixture of all burn depths from superficial second degree through deep, including full thickness burns, making precise classification of the entire wound difficult.

Zones of coagulation, stasis and hyperemia: Zone of coagulation is the tissue completely destroyed by heat and need to be removed. Zone of stasis, usually peripheral and deeper to the Zone of Coagulation, is characterized by decreased tissue perfusion due to congestion that is caused by the increased hyperemia in the surrounding tissue evoked by the inflammatory

[1] Where possible, definitions were based on the ABA White Paper

reaction (Zone of Hyperemia). The tissue in this zone is potentially salvageable if congestion will be resolved, preventing thrombosis and occlusion of blood vessels that will cause death of smaller or larger areas. The aim of burns resuscitation is to increase tissue perfusion in general as well as in the burned area and prevent any damage becoming irreversible. The dead, coagulated eschar in the zone of coagulation, adjacent to the Zones of Stasis is the main cause of local and systemic eschar-related infectious and inflammatory mediators that may cause burn viable zone of Stasis to die [2-9].

Burn conversion (burn propagation, progression, transformation): Superficial second degree burns may convert to deeper burns due to permanent vessel occlusion of the zones of stasis and hyperemia, desiccation of the wound, or the use of vasoactive agents during resuscitation from shock and infection of the eschar [1].

Debridement of Burn Wounds[2]: The removal of loose, devitalized, necrotic, and/or contaminated tissue, foreign bodies, and other debris on the wound using mechanical or sharp techniques (such as curetting, scraping, rongeuring, or cutting) [1].

Clarification: In the USA and so in this study the removal of the necrotic tissue that ends in clean wound bed ready for wound closure treatment is termed "Eschar Removal" with the term "debridement" reserved to mechanical cleansing of the wound that ends with a wound bed still covered with eschar. In the rest of the world the term "debridement" refers to removal of the **entire** burn eschar ending with a clean, raw wound bed. Therefore, the term "Debridement" has been used in all the previous studies and in all the references as a synonym to "Eschar Removal" in its US terminology [1].

Escharotomy is a surgical incision through burn eschar (necrotic skin) down to intact fat, from healthy skin edge to healthy skin, in order to reduce burn induced increased interstitial and compartment pressure and restore blood perfusion. It releases the compressed and stretched, constricted tissue allowing adequate perfusion to tissues and organs, maintaining their normal perfusion and viability [1].

Circumferential extremity wounds: Limb DPT and/or FT burns which encircle ≥ 80% of the limb circumference.

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:

1. Any of the 5 "P"s; Pain (Pain on passive extension of the fingers, stretching of the affected muscle), Paralysis, Pulselessness, Pallor, Paraesthesia,
2. Direct pressure measurement >25 mmHg, measured in 2 consecutive measurements within 30 minutes under the constricting eschar,
3. SpO_2 <95% and a persisting difference of 6% between a healthy symmetrical extremity and the injured one [10-11],
4. Pulse not detected by US Doppler.

Burn-induced compartment syndrome (BICS):

- Increasing interstitial/compartment pressure >30 mmHg,

[2] Enzymes were specifically excluded as debridement means in the White Paper.

- 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"s: Pain, Paralysis, Pulselessness, Pallor, Paraesthesia.

Target Wound (TW): A TW is the burn area to be treated (Eschar Removal) by NexoBrid, SOC or Gel Vehicle as per the randomization and in accordance with the protocol. Following the enrollment of a patient to the study and prior to randomization, physicians identify one or more TWs per patient according to the following criteria and definitions:

- TW is a continuous burn area composed of DPT and/or FT in depth (might include several anatomical areas) that can be treated in one session,
- Minimal burned area of a single TW should be ≥ 0.5% TBSA (DPT and/or FT in depth)
- Maximal burned area of a single TW should be ≤ 15% TBSA,
- Superficial partial thickness areas may be included in the wound area only if they cannot be separated from deeper areas (e.g. surrounded by or mixed with DPT areas) and trying to separate such more superficial areas, may interfere with the treatment of the deeper areas of that wound,
- Note: *TW can be a portion of a larger wound composed of SPT, DPT, and FT, if the superficial partial thickness areas can be separated. The site should treat only the TW area per the randomization*.
- Note: *TW can be a portion of a larger wound that includes face, genital or perineal areas. Face, genital or perineal areas will be separated and not included in the TW area*.

Substance use disorder: describes a problematic pattern of using alcohol or another substance that results in impairment in daily life 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 following 11 symptoms within 12-months:

- Consuming more alcohol or other substance than originally planned
- Worrying about stopping or consistently failed efforts to control one's use
- Spending a large amount of time using drugs/alcohol, or doing whatever is needed to obtain them
- Use of the substance results in failure to "fulfill major role obligations" such as at home, work, or school
- "Craving" the substance (alcohol or drug)
- Continuing the use of a substance despite health problems caused or worsened by it. This can be in the domain of mental health (psychological problems may include depressed mood, sleep disturbance, anxiety, or "blackouts") or physical health
- Continuing the use of a substance despite its having negative effects in relationships with others (for example, using even though it leads to fights or despite people's objecting to it).
- Repeated use of the substance in a dangerous situation (for example, when having to operate heavy machinery, when driving a car)
- Giving up or reducing activities in a person's life because of the drug/alcohol use

- Building up a tolerance to the alcohol or drug. Tolerance is defined by the DSM-5 as "either needing to use noticeably larger amounts over time to get the desired effect or noticing less of an effect over time after repeated use of the same amount"
- Experiencing withdrawal symptoms after stopping use. Withdrawal symptoms typically include, according to the DSM-5: "anxiety, irritability, fatigue, nausea/vomiting, hand tremor or seizure in the case of alcohol".

Complete wound closure- defined as skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits, 2 weeks apart [12], determined by a blinded assessor.

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.

1.2 ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Transaminase (ALT or SGPT, which stands for serum glutamic-pyruvic transaminase)
AST	Aspartate Transaminase (AST or SGOT which stands for serum glutamic oxaloacetic transaminase)
BICS	Burn-induced compartment syndrome
CRA	Clinical Research Associate
CRF	Case Report Form
DG	NexoBrid Gel
DM	Data Management
DPT	Deep Partial Thickness
DSMB	Data Safety Monitoring Board
EAR	Extremities At Risk
EC	Ethics Committee
ECG	Electrocardiogram
EMA	European Medicines Agency
FDA	Food and Drug Administration
FT	Full Thickness
HR	Heart Rate
HD	Hospital Discharge
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IND	Investigational New Drug
IM	Intramuscular
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITT	Intent to Treat
IV	Intravenous
Kg	Kilogram
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MVSS	Modified Vancouver Scar Scale
mg	Milligram

Ml	Milliliter
PO	Per Os
POSAS	Patient and Observer Scar Assessment Scale
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell (Count)
RDC	Remote Data Capture
SAE	Serious Adverse Event
SC	Subcutaneous
SD	Standard Deviation
SI	Smoke Inhalation
SOP	Standard Operating Procedure
SSD	Silver Sulphadiazine
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBSA	Total Body Surface Area
TW	Target Wound
WBC	White Blood Cell (Count)
WP p/l	ABA White Paper no. page/line

2. SYNOPSIS

Protocol Number:	**MW 2010-03-02**
IND Number(s):	65,448
Name of Investigational Medicinal Product (IMP):	NexoBrid
Name of Active Ingredient:	Partially purified Bromelain
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
Clinical Phase:	3
Study Duration:	The total duration of the study treatment and follow up period of each participating subject is approximately 25 months.
Study Population:	175 Hospitalized adults with Deep Partial Thickness (DPT) and Full Thickness (FT) thermal burns will be enrolled from about 30 sites.
Study Objective(s):	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.
Study Design Overview:	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. 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 definition. All subjects' DPT and FT burns that comply with the specified entrance criteria 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 minimise 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. Following the stratification, patients will be randomized as per their stratification group 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 and undergo wound cleansing and dressing of all wounds with antibacterial solutions. Following wound cleansing and antibacterial treatments, subjects will undergo the eschar removal process as per treatment assignment (NexoBrid, SOC or Gel Vehicle, following randomization). Subsequent to complete eschar removal, all wounds will be assessed and treated in the same manner, in accordance with post-eschar removal wound care strategy. Post start of eschar removal, subjects will undergo daily vital signs and pain assessments, until hospital discharge (HD) and weekly assessments of wound progress until wound closure. Following wound closure confirmation visit, subjects will be followed up at 1, 3, 6, 12, 18 and 24 months post wound closure for long term outcomes evaluation.
Study arms, Dosage and route of administration:	Three arms will be included in the study; NexoBrid, SOC and Gel vehicle arm (3:3:1 ratio) and each patient will be treated in accordance with randomization. **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). 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 2g 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 (±3% 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. **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 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. Gel Vehicle should not be applied to more than 15% TBSA (±3% TBSA) in one session. **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.
Inclusion/ Exclusion Criteria:	**Inclusion Criteria- Patient level** **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.

	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)[3], All planned Target Wounds (TWs) should meet the following criteria: 1. SPT areas that cannot be demarcated from DPT and FT areas should be less than 50% of the % TBSA of the TW, 2. Wound's blisters can be removed/ unroofed, as judged by the investigator. **Exclusion Criteria- Patient level** 1. Patients who are unable to follow study procedures and follow up period, 2. Modified Baux index[4] ≥ 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, 7. The following pre-enrollment dressings: a. Flammacerium, b. Silver Nitrate ($AgNO_3$), 8. 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 hrs SSD treatment), 9. Patients with pre-enrolment 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,

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

[4] 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).

	15. ASA greater than 2 (see Appendix 15 in 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, 23. Enrollment in any investigational drug trial within 4 weeks prior to screening, 24. Current (within 12 months prior to screening) severe alcohol or drug use disorder (see definition in section 1.1), 25. Prisoners and incarcerated, 26. Patients who might depend on the clinical study site or investigator.
Outcome Measures:	**Primary Endpoint** The below primary endpoint will be investigated at a 'per patient' level. 1. Incidence of complete eschar removal- Demonstrate superiority of NexoBrid over Gel Vehicle for eschar removal as measured by incidence of complete eschar removal at the end of the topical agent soaking period by a blinded assessor. **Secondary Endpoints** The following secondary endpoints will be evaluated in this study and compared between NexoBrid and SOC. 1. Reduction in surgical need- Demonstrate superiority of NexoBrid over SOC in reduction of surgical need for excisional eschar removal as measured by an analysis of incidence of surgical eschar removal (tangential/ minor/ avulsion/ Versajet and/or dermabrasion excision). 2. Earlier eschar removal- Demonstrate superiority of NexoBrid over SOC in

	reduction of the time to achieve complete eschar removal. 3. Blood loss- Demonstrate superiority of NexoBrid over SOC with regard to the blood loss incurred during the eschar removal procedures **Safety Endpoints** The following group of safety endpoints will be evaluated in this study and compared between NexoBrid and SOC. These endpoints are used to confirm that NexoBrid does have an inferior deleterious effect. 1. Time to reach complete wound closure assessed in days, starting from Randomization. 2. Cosmesis and Function - will be measured using MVSS, to demonstrate that treatment with NexoBrid does not have any clinically meaningful deleterious effect on burns scars quality as compared to the quality of burns scars treated with SOC, measured at 12 months from wound closure date, by a blinded assessor. 3. Cosmesis and Function - will be measured using MVSS, to demonstrate that treatment with NexoBrid does not have any clinically meaningful deleterious effect on burns scars quality as compared to the quality of burns scars treated with SOC, measured at 24 months from wound closure date, by a blinded assessor. *Additional Safety Outcome Measures:* 1. General parameters: Systemic adverse events, vital signs, pain assessment (using VAS and as reported as AEs), laboratory tests, units (and volume) of blood transfusion given during hospitalization, Immunogenicity evaluation for NexoBrid patients, Pyrexia and Hyperthermia, Systemic infections, Incidence of increased interstitial/compartment syndrome[5] and QT prolongation, 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). 2. Additional long term functionality evaluation of the extremities using the 'Lower Extremity Functional Scale', 'QuickDASH' questionnaires and 'Range Of Motion' measurements, 3. Long term Quality of Life using EQ5D and Burn Specific Health Scale- Brief (BSHS-B).

[5] Increased interstitial pressure/compartment syndrome will be defined as following: Increasing pressure >25 mmHg, Difference of >20 mmHg of diastolic pressure between the burned extremity to the opposite uninjured extremity, Decreased SpO2 reading during treatment with difference of >6% compared to an uninjured extremity, or deterioration of clinical signs and/or any of the 5 "P" signs (Pain (Pain on passive stretch of the affected muscle), Paralysis, Pulselessness, Pallor, Paraesthesia).

	4. Local parameters: Local adverse events defined by treating physician or designee; graft loss, wound related infections, etc. **Exploratory Endpoint** 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. POSAS will be used to further assess cosmesis and function, 3. Incidence of surgical Escharotomy procedures on circumferential extremities target wounds, 4. Incidence of reduction in interstitial/compartment pressure in circumferential extremity wounds (measured immediately following eschar removal), 5. Reduction in surgical need as measured by analysis of incidence of surgically harvested donor sites wounds, 6. Reduction in surgical need as measured by analysis of % area of surgically harvested donor site wounds, 7. Blood loss following eschar removal procedures using changes in Hematocrit following eschar removal procedures, Cosmesis MVSS and POSAS will be used to assess the quality of the donor sites scars, 8. PK evaluation for a subset of NexoBrid patients, 9. Autograft related parameters: I. efficacy and safety analyses for early and late grafted wounds II. total number of target wound grafting procedures III. incidence of repeat/additional grafting procedures IV. area of repeat grafting 10. Duration of hospitalization.
Sample size:	The following sample size calculations are based on the consideration of the primary and two secondary endpoints for this study. **Primary Endpoint: Incidence of complete eschar removal (NexoBrid vs. Gel)** Estimated proportion achieving complete eschar removal in all TWs from study MW2004-11-02: 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.

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.

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:

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

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.

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).**
Stages of Analysis	The analysis is planned to be carried out in three stages, as described below. 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 above, 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 nearly 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 Section 13.8.4 and the SAP. The complete data set documented so far will be locked and analyzed as described above. Although the treatment code will be revealed at the end of this analysis, the independent assessor of the cosmesis and function and QoL of an individual patient will remain blinded to the treatment given to that patient until after the third and final analysis (see below), so as to preserve the un-biasedness of the assessments. The second analysis will be performed for the 12 month 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 above. 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 24m 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 above (hard lock).

3. INTRODUCTION

3.1 MEDICAL OVERVIEW

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 [2, 9, 13].

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 [2-3, 8-9, 14]. 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 [2-6, 8-9, 14-15]. 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 [4, 6, 8-9, 16].

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 [3, 17].

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 [2, 6, 8-9, 14, 18]. 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 [3, 17, 19-20]. 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) [8-9, 14, 21].

In current standard of care, removal of the eschar may be accomplished by surgery, usually 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 [2, 9, 17, 21-22] and heat loss [23]. 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-dependant 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.

The optimal 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.

These characteristics were well-demonstrated throughout the clinical development of NexoBrid. A recent confirmatory phase 3 study (MW 2004-11-02) demonstrated the clinical benefit of NexoBrid for effective and selective removal of eschar from burn wounds as was shown in both primary and secondary endpoints, e.g. for % treated burn wound excised, % treated burn wound autografted and timely eschar removal. These findings corroborated those from a previous Phase 2 study (MW 2002-04-01) and retrospective data collection study (35-98-910) (Please refer to Section 3.2).

3.2 CLINICAL DEVELOPMENT OF NEXOBRID

3.2.1 Efficacy

The clinical development of NexoBrid[6] was based on clinical data retrospectively collected from files of hospitalized burn patients with burn wounds treated by NexoBrid and on discussions and guidance from regulatory authorities.

Study 35-98-910 evaluated the safety and efficacy of NexoBrid in subjects between 0.5 and 82 years of age with deep partial and/or full thickness burns of ≤67% TBSA, hospitalized in Soroka University Medical Center (Israel) Plastic Surgery and Burn Unit, from 1985-2000. Data from 154 subjects having complete file documentation (including signed informed consent form (ICF) and pre- and post-eschar removal photographs) were analyzed.

The clinical development program of NexoBrid includes six prospective clinical studies that evaluated the safety and efficacy of NexoBrid in partial and full thickness burns.

Study MW2001-10-03 evaluated the efficacy and safety of three doses of NexoBrid. Twenty hospitalized adult, male and female subjects, with Deep Partial Thickness (DPT) and/or Full Thickness (FT) burns of 1-15% TSBA were randomized to a 1 g (6/20 subjects), 2 g (7/20 subjects) or 4 g (7/20 subjects) dose of NexoBrid powder per 20 g of Gel Vehicle. The study

[6] NexoBrid was formerly named Debridase and Debrase.

further confirmed the use of 2 g NexoBrid mixed with 20g Gel per 1% TBSA as safe and effective dose.

Study MW2002-04-01 evaluated the safety and enzymatic eschar removal efficacy of NexoBrid as compared to SOC and Vehicle[7]. A total of 140 adult hospitalized male and female subjects, with DPT and/or FT burns ranging from 2-15% TBSA, but not more than 30% TBSA in total, were randomized in a 2:1:1 ratio to NexoBrid, Vehicle alone (only Gel), or SOC treatment.

Study MW2005-10-05 evaluated the safety and exploratory efficacy of NexoBrid in comparison to Vehicle, and SOC in hospitalized male and female adult subjects, with DPT and/or FT burns ranging from 1-5% TBSA. Thirty hospitalized subjects were randomized to receive NexoBrid (10 subjects), Vehicle alone (9 subjects), or SOC treatment (11 subjects).

Study MW2008-09-03 evaluating the safety and exploratory efficacy of NexoBrid, as well as the systemic absorption of NexoBrid when applied topically on thermal burns using a recently validated, sensitive ELISA method, through pharmacokinetic (PK) assessments in hospitalized adults and children, male and female subjects, aged 4 to 70 years with partial thickness and full thickness burns of 4-30% TBSA.

Study MW2004-11-02 A phase III, multicenter, controlled study, that confirmed the safety and efficacy of NexoBrid in comparison to SOC in hospitalized adults and children, male and female subjects between 4 and 55 years of age with DPT and/or FT burns of 5-30% TBSA with total burn wounds of no more than 30%. Of the subsequently enrolled subjects, 75 were randomized to NexoBrid and 81 subjects were randomized to SOC treatment [24].

Study MW2012-01-02 evaluated the long-term scar formation and Quality of Life in adults and children who had participated in study MW2004-11-02, 2-4 years post-wound closure and included blinded assessment of scars. A total of 89 patients were enrolled into this study including 72 adults and 17 pediatric patients [24].

Summary of the efficacy results from the phase 2 and phase 3 studies is presented here below.

Eschar Removal

Eschar removal is the direct intended activity of NexoBrid and as such was evaluated in all clinical studies.

Data from the two comparative studies MW 2002-04-01 and MW 2005-10-05 shows that eschar was effectively removed from 92.5% of the treated wound area for NexoBrid subjects as compared to 94.7% for the SOC subjects. The Vehicle showed negligible, if any, eschar removal activity (2.2% eschar removed in one case). Vehicle-subjects' eschars were removed by SOC methods following end of the Vehicle gel treatment and diagnosis of eschar removal failure. Data from the two comparative studies MW2002-04-01 and MW2005-10-05 shows that the time to start of eschar removal from injury was 1.5 days in both the NexoBrid and Vehicle groups compared to 2.9 days in the SOC group. Time to completion of the initial eschar removal procedure (as designated by the protocol per treatment group) from injury was 1.5 days in the NexoBrid group as opposed to 11.2 days in the SOC group.

[7] Vehicle treated patients were treated by eschar removal SOC methods following end of the Vehicle Gel treatment and diagnosis of eschar removal failure.

Data from the Phase 3 study MW2004-11-02 showed that 90.5% (67/74) of NexoBrid subjects had successful eschar removal as compared to 90.1% (73/81) of the SOC-treated subjects (p=0.9301). Time to achieve successful eschar removal from the ICF date in the NexoBrid group was 0.8 days as opposed to 6.7 days in the SOC group (p< 0.0001). Thus, NexoBrid was able to successfully remove the eschar from the burn wounds much earlier than SOC.

Following the EMA CHMP/COMP recommendation, study MW 2004-11-02 evaluated two co-primary endpoints consisting of (1) % treated wound excised, and (2) % treated wound autografted (both on DPT wounds). These endpoints complement each other since less area surgically excised demonstrates the eschar removal efficacy of NexoBrid and less area grafted demonstrates the clinical benefit in the reduction of the extent of non-selective eschar removal by surgical excision of deep partial thickness wounds where viable tissue can be spared[8]. Both endpoints contribute to the overall reduction of surgery [24].

Area of Wound Excised

Data from the Phase 3 study (MW2004-11-02) shows that the incidence of DPT wounds that were excised was only 15.1% (16/106 wounds) as compared to 62.5% (55/88 wounds) in the SOC. Moreover, the DPT wounds treated with NexoBrid had significantly less wound area excised (5.5%) compared to the SOC treated (DPT) wounds (52.0%, p<0.0001). In children (<18 years of age), the incidence of DPT wounds that were excised was only 21.7% (5/23 wounds) as compared to 68.2% (15/22 wounds) in the SOC. Moreover, less wound area was excised (7.3%) in NexoBrid treated wounds compared to the SOC treated wounds (64.9%). In addition to the analyses described above, the percent treated wound excised was analyzed for all treated wounds, including those with third degree components, to investigate NexoBrid ability to remove the eschar from all burn wound depths (ITT population). In the NexoBrid group the incidence of wounds that were excised was 24.5% (40/163 wounds) as compared to 70.0% (119/170 wounds) in the SOC. Significantly less treated wound area was excised in all the NexoBrid treated wounds (13.1%) as compared to the SOC group (56.7%); p<0.0001. This pattern is comparable to that seen in the DPT wounds discussed above and confirms the results in a greater number of wounds of all depths.

Area of Wound Autografted

In the Phase 3 study (MW2004-11-02) wound area autografted was evaluated in DPT wounds. Wounds that were entirely FT or had FT areas were excluded from the analysis of % wound area autografted (in accordance with EMA CHMP/COMP protocol assistance recommendations).

In this study, the incidence of DPT wounds that were autografted was only 17.9% (19/106 wounds) as compared to 34.1% (30/88 wounds) in the SOC (p=0.0099). Moreover, DPT wounds of all subjects had significantly less wound area autografted in the NexoBrid group (8.4%) compared to the SOC group (21.5%; p=0.0054). In the children subset, the incidence of DPT wounds that were autografted was 21.7% (5/23 wounds) as compared to 31.8% (7/22 wounds) in the SOC. Moreover less DPT wound area was autografted in the NexoBrid group (6.1%) compared to the SOC group (24.5%). The potential tissue-sparing effect of NexoBrid

[8] Full thickness wounds require autografting regardless of the eschar removal technique since they have no remnants of viable dermis. Therefore, the clinical benefit of DGD versus the non-selective surgical approach is more apparent in deep partial thickness wounds where the potential tissue-sparing effect, leading to less autografting, is demonstrated.

and its applicability to the issue of reduction in autografted wound area suggest an additional clinical benefit to children, due to their smaller size and limited donor site area.

Long term scar assessment results- study MW2012-01-02

Cosmesis was assessed in study MW2012-01-02 by a blinded assessor using the Modified Vancouver Scar Scale (MVSS). The Vancouver Scar Scale (VSS) [25] is the first validated and widely used scar assessment scale developed by Sullivan et al 1990 and it includes assessments of 4 scar variables; vascularity, height, pliability, and pigmentation. MVSS is an improved version of the VSS, which includes two additional scar variables (pruritus and pain) [23]. The Vancouver Scar Scale is the most commonly used scale that includes multiple subjective and objective parameters [25-27].

The study included data from 89 subjects (72 adults and 17 pediatric) with whom contact was made and who represent the MW2004-11-02 study population based on their demographic and baseline wound characteristics.

Scar assessment (per wound) using MVSS was conducted for 113 wounds in NexoBrid group and 78 wounds in SOC group. The total scar outcome was slightly lower (better results) in the NexoBrid arm compared to SOC (3.12 vs. 3.38, respectively), without statistically significant difference (p=0.88) (MVSS range 0-18).

3.2.2 Safety

The burn trauma is a multi-factor disease involving most if not all body systems and associated with many adverse events (AEs). Overall, AEs associated with NexoBrid were minimal, generally transient, manageable and mostly attributable to the burn process and its care and not to the introduction of the eschar removal agent.

Taken together, there appear to be several common but manageable AEs, many attributable to the burn process and not the introduction of the debriding agent, NexoBrid. The most frequently reported AEs (≥3.0%) whether or not treatment–related, are summarized in the following Table 1 (based on the safety data from the prospective phase II and phase III studies).

Table 1- Summary of Adverse Events in Descending Order of Frequency Whether or Not Treatment-Related and Occurring in ≥3.0% of Subjects* (Safety Population)

Adverse Event*	NexoBrid N=208(%)	SOC N=127 (%)
Pruritus	32 (15.4%)	24 (18.9%)
Anemia	13 (6.3%)	8 (6.3%)
Nausea	13 (6.3%)	6 (4.7%)
Insomnia	6 (2.9%)	5 (3.9%)
Headache	6 (2.9%)	5 (3.9%)
Skin graft failure	6 (2.9%)	2 (1.6%)
Diarrhoea	6 (2.9%)	1 (0.8%)
Vomiting	5 (2.4%)	7 (5.5%)

* Subjects from the five prospective studies; MW2001-03-02, MW2002-04-01, MW2005-10-05, MW2004-11-02 and MW2008-09-03

Across all studies, pruritus was the most frequently reported AE occurring at an incidence >10% in both treatment groups. More subjects in the SOC group reported pruritus compared to the NexoBrid group (18.9% vs. 15.4%). Pruritus, that torments the patient for months, is very common. As reported in the literature, up to 87% of all patients will have a longer or shorter spell of pruritus. The scratching may be so intense that the patient might scratch-open their healed wounds, destroying healed grafts [1, 28-29].

Anemia in response to burns has been acknowledged for over 60 years, and is considered an acute and sub-acute complication. Close evaluation has suggested the response to thermal injury to include initial rapid hemolysis of thermally exposed blood proportional to the burn % TBSA (<18% in 24h in 15-40% TBSA burns [30]. Excisional surgery for eschar removal and skin graft harvesting leads to acute blood loss. Catabolic processes, inflammatory and septic processes and depressed bone marrow hematopoietic activity may all contribute to anemia though a clear etiology has yet to be established [31-33]. Anemia in NexoBrid clinical studies was reported at similar incidences in both treatment groups (6.3% in the NexoBrid and SOC group).

Though skin grafts have been used for more than a century and considered to be a standard wound closure tool, the details of their use vary between centers, surgeons, patients and body areas. The reported incidence in the literature of graft loss ranges may reach up to 71%. Traditionally it was thought that wound infection is the main reason for skin graft loss but with use of effective and simple control means (local antibacterial soaking and systemic antibiotic) infection ceased to play a crucial role in graft loss. At present, the causes for skin graft loss (besides infection) are (by the order of importance): 1. Sub-graft collection (i.e. seroma, hematoma); 2. Lack of graft adherence to the wound bed (ineffective fixation, movement); 3. Hypoalbuminemia; 4. Granulation tissue; 5. Nature of graft (by trend: very thin, sheet, thick and meshed). Other mechanical factors may cause loss of smaller or greater parts of the graft, earlier or later after grafting. Such factors are: shearing, trauma and abrasion (scratching) of the graft, minimally vascularized body areas (lower extremities), areas prone to trauma (buttocks, legs), areas difficult to fixate and dress and mobiles joints (knees, elbows, axilla neck and trunk) [34-37]. Use of negative pressure wound therapy for 2-3 days to stabilize the graft improves its take. Late (2-3 weeks post grafting) graft loss may be caused by autografting over dermis that epithelializes under the graft, detaching it from the healing bed. During the clinical development, graft failure was reported in low percentages, 2.9% in the NexoBrid arm and 1.6% in the SOC arm. Autografting may cause long term sequelae such as scarring at the edges, mesh pattern when mesh grafts are used, overgrafting when used over dermis, color differences and at the donor sites: weeks of painful healing process and scars on unburned skin areas.

The incidence of the AEs pain, pyrexia/hyperthermia, and wound infection were analyzed separately for the earlier clinical studies (Group 1: MW 2001-10-03, MW 2002-04-01) and for the later studies (Group 2: MW 2005-10-05, MW 2004-11-02, and MW 2008-09-03) as prior

to the Group 2 studies, corrective measures[9] were implemented for these AEs observed and investigated in the Group 1 studies. Data are presented in Table 2.

Table 2- Comparison of the Incidence of Adverse Events Pain, pyrexia/ Hyperthermia and Wound Infection

Adverse Event	Group 1*		Group 2**	
	NexoBrid N=90	SOC/Vehicle N=70	NexoBrid N=118	SOC/Vehicle N=101
	N (%)	**N (%)**	**N (%)**	**N (%)**
Pain	21 (23.3)	8 (11.4)	4 (3.4)	4 (4.0)
Pyrexia/ hyperthermia	32 (35.6)	13(18.6)	20 (17.0)	16 (15.8)
Wound infection	6 (6.7)	4 (5.7)	7 (5.9)	7 (6.9)

* Group 1: MW 2001-10-03, MW 2002-04-01
** Group 2: MW 2005-10-05, MW 2004-11-02, and MW 2008-09-03

Pain Events

Burn injury is associated with prolonged and significant pain. The pain can be sub-divided into background pain (at rest), breakthrough pain (during activities such as changing positions), and procedural pain during wound care such as Sulfamayalon, bathing, dressing changes, etc [38-39]. Patient's scores vary widely from patient to patient and in the same patient: from day to day. Pain score are higher in the more superficial burns with procedural pain related to the extent of the second degree burns [38].

As shown in Table 2, the incidence of pain in the NexoBrid group was reduced from 23.3% to 3.4% after corrective measures had been taken for adequate pain management, as commonly practiced during routine dressing change to burn patients, and occurred at a comparable incidence in both treatment groups in the later clinical studies (3.4% vs. 4.0%). Use of NexoBrid requires somewhat less analgesia and anesthesia medication compared with SOC and so it is not medication-dependent more than any routinely used SOC treatment.

A few prospective descriptive studies were conducted to investigate the extent of pain in burn patients, reporting more intense, at rest background pain in the first week, two-thirds of the patients had mild or less-than-mild pain, and the others reported moderate-to-severe pain. During therapeutic procedures, 51% of patients suffered intense pain though analgesic medications were administered. The burn induced pain and its long term sequelae are all underestimated and seem to be in many cases greatly undertreated by routine analgesia [1, 28, 39-42].

Pyrexia/ Hyperthermia

The burn patient is catabolic, with a very high basal metabolic rate (BMR), and often has elevated body temperature which may continue for months post healing [43-45]. The hypermetabolic process with the systemic and local inflammatory reactions involved in the

[9] The following instructions and protocols were implemented: Provision of a pre-treatment pain management protocol as commonly practiced during routine dressing change in standard of care of burns in order to prevent NexoBrid related pain and the use of approved topical antibacterial solutions during the soaking stages prior to and post-treatment in order to minimise the potential for infection and associated fever.

wound healing process as well as wound contamination and/or infection contribute to pyrexia. However, elevated body temperature is not considered clinically reliable in predicting local or bloodstream infection, in some cases it starts a few hours post burn, much before any infection can develop. Later it may be due to exposure to catabolic and bacteria products [46-47]. Occlusive dressings may cause fever by reducing heat dissipation [48-49]. Therefore, temperature elevation is not considered clinically significant pyrexia until >39°C (102°F) and even then it is not always related to a clear pathology [50] or correlates consistently with septicaemia [46-47]. Other common sources for pyrexia may be IV catheter infection and/or thrombophlebitis with reported incidence of 8-57%. Urinary Tract Infection (UTI) is another source of pyrexia (as report suggests that most catheterized patients will have bacteruria after 72 hours) and pneumonia with an incidence of 10-65% [51].

The incidence of pyrexia/hyperthermia in the NexoBrid group was reduced from approximately 35.6% in the earlier studies to 17.0% in the later studies and was also decreased from 18.6% to 15.8% in the SOC group. In the later studies pyrexia/hyperthermia occurred at a comparable incidence in both treatment groups (17.0% vs. 15.8%).

Infection

The definition of "infection" in the case of burns is a very general one and can relay to several different pathological entities. Some of the more common burn-related infections are burn wound infections with an incidence of >30% of infection and >9% of bacteraemia [51-52]. The general criteria of pyrexia, leucocytosis, thrombocytopenia may indicate that an infection is developing but will rarely point to the exact source (organ or system) of the infection. Even local signs of burn wound infection will not preclude differential diagnosis of other very common sources (i.e. IV catheter infection, thrombophlebitis, urinary tract infection so common in catheterized patients or pneumonia) [51].

Wound Infection

All burn wounds are contaminated and clinical diagnosis between wound contaminated to various degrees of wound infection and invasive wound infection depends on objective criteria of quantitative culture and microscopic histology. Other definitions of wound infections are not clear, not uniformly accepted by all and greatly depended on aetiology and subjective impressions [51].

Most infections in patients with burn injuries are burn wound infections. The burn wound is a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin [51-53]. Incidence of infections in children was identified in 33.9% of the patients, with burn wound infections being the most common: 10-24.6% [54-55]. Other studies found similar incidence of infection >30 % with 30-70 % of such infections reported as burn wound infections [56-59].

Death in patients with infections ranged from 6 to 25% [56, 60-64].

In the clinical development, incidence of wound infection was similar across studies, 6.7% to 5.9% in the NexoBrid group and 5.7% to 6.9% in the SOC group. In all studies wound infection occurred at a comparable incidence in both treatment groups and with comparable use of antibiotics between NexoBrid and SOC.

There were 31 SAEs reported by 25 subjects. Only one SAE (skin graft failure) was considered possibly related to NexoBrid treatment. Fewer AEs, SAEs, and severe AEs were observed in children compared to adults. Nearly all cases of wound and blood infections reported as AEs were determined to have been not related to study treatment.

There were six deaths in the NexoBrid clinical development program, 5/362 (1.4%) in the NexoBrid group and 1/127 (0.8%) in the SOC group. None of these deaths was considered to have been related to study treatment by the Principle Investigators and Data Safety Monitoring Board (DSMB).

3.2.3 Pharmacokinetic profile

The pharmacokinetic evaluation of NexoBrid following single or double applications of NexoBrid Gel Dressing in 36 subjects with partial thickness thermal burns was performed as part of study MW2008-09-03. In all subjects dosed, NexoBrid exposure was observed with quantifiable serum concentrations through 48 hours post dose administration. Concentrations increased relatively rapidly after each application, with median T_{max} values between 2.0 to 4.0 hours and mean terminal half-life of 12 ± 4.4 hours.

Systemic exposure appears to generally increase with dose and %TBSA. Statistical differences between the PK parameters were not observed as a function of depth of burn.

The plasma levels calculated after the first and second administration shows only slight accumulation of NexoBrid in the blood after the second application indicate that on average the safety margins are maintained after the second application, a conclusion that is also supported by a similar safety profile of patients treated with 2 applications (up to 30% TBSA) that was consistent with the safety profile after a single application (up to 15% TBSA).

3.3 RISK ASSESSMENT

3.3.1 General

Any decisive treatment strategy (such as a decision to execute post eschar removal surgical wound closure) can only be planned and executed if the true extent of the tissue damage is known at each stage of the healing process. Only once the eschar is removed and the viable wound bed is revealed, can the true damage be observed and assessed and the appropriate wound closure modality can be prescribed [2, 13, 65].

The two major characteristics defining the burn wound severity are area, which is referred to in terms of % TBSA, and depth, which is referred to in terms of ‘degree’ of burn or described in words (please refer to Burn Wound definitions, Section 1.1*)*.

Depth assessment will be based on the investigators' visual assessments as used in routine burn care. Burn specialist visual assessment and clinical examination is the technique generally used for the diagnosis, monitoring and documentation of burn depth [1]. All the participating investigators shall be experienced, national or international burn specialists. At present, the burn experts' visual assessments are considered the standard diagnostic tool since efforts to develop and validate objective burn depth diagnostic measures have not been successful. None of the other tools which have been developed (e.g. tissue biopsy, ultrasound, laser Doppler flowmetry, Thermography, nuclear magnetic resonance imaging (MRI), reflectance under different illuminations, vital dyes, fluorescein and fluorometry) have been validated in large

scale clinical trials or accepted as standard diagnostic means by national or international professional burn organizations. All these methods have been compared to the visual assessments of experts but similar results have been attained only occasionally and usually only to several-day old burns where the process of progression has ended and infection not yet started [65-66].

Assessment of very superficial and very deep burns may be in some cases more straight-forward, however, the most frequent burns in hospitalized patients, those of mixed or indeterminate depth, create a challenge in terms of both diagnosis and choice of treatment modalities. Indeterminate burns are usually second degree burns of changing depths difficult to diagnose on arrival so only after several days when the burn conversion (progression) and demarcation have taken place can they be diagnosed as deep in need for surgery or superficial [1]. These burns are the most common in hospitalized patients (>40%). In some cases they may eventually evolve into deep full thickness or slow to heal wounds. In other cases they may heal fast, and spontaneously epithelialize following maceration and separation ("sloughing") of the eschar. The depth of these burns is not constant, their appearance continuously changing during the first days due to the burn progression and to the inflammatory/infectious processes. Due to their varying appearances and behavior they pose a real diagnostic challenge and thus it is common practice to follow/monitor these wounds for a few days until the diagnosis becomes clearer and with this, realization of the preferred wound closure strategy, i.e. surgical or non-surgical or a combination of both. When a non-surgical strategy is chosen, topical wound care designed to prevent infection of the necrotic eschar and promote its separation (sloughing) starts. This treatment includes topical medications and physical procedures (bathing and showering, scraping etc), follow up and monitoring until the eschar sloughs off and the clean bed epithelializes, or until granulation tissue appears and it becomes obvious, usually within a 3 week period, that the wound will not heal without heavy scarring. For the later scenario, a surgical process including excision and autografting will usually be performed [1, 18, 67].

In spite of the general agreement that the eschar should be removed in a timely manner, as soon as possible, very few centers dare to excise the entire burn eschar as early as possible from admission [68]. In immediate eschar removal, defined within 24hrs from injury ("primary eschar removal", as coined in 1995 by Voinchet et al), one does not wait until better diagnosis of the burned skin depth is feasible but rather excises all the suspected tissues and grafts the raw wound bed.

Since in partial, mixed thickness, and intermediate depth wounds, the extent of the burn is difficult to diagnose, early surgical eschar removal will usually be avoided and a more conservative eschar removal approach using topical medications will be pursued. The conservative eschar removal lasts for days until the eschar decomposes or until decision is taken to excise the offending burn eschar. The non-surgical eschar removal process involves the combined activity of topical medicaments, contaminant microorganisms, tissue autolytic processes and the inflammatory process. The infectious-inflammatory processes are slow (lasting between 10-14 days) and may involve significant systemic and local complications.

Burn Induced Compartment Syndrome (BICS) is an acute progressive condition that starts a few hours post injury and develops during the acute, edematous stage of the first 2-3 days (after which edema and increased pressure subside) in deep circumferential burns. The edema and pressure reach their maximum within several days post injury and start to slowly decrease

thereafter. Soft tissue can withstand temporary pressure increase for 6-8 hours without permanent damage, allowing 4-6 hours for decision-making regarding escharotomy.

The optimal solution would be the use of a non-surgical, least demanding eschar removal means, immediately after patients' admittance and stabilization. Such debriding means that will be specific to the eschar, removing it selectively without harm to the non injured bed will enable physician visual diagnosis of the burn wound trauma and the most appropriate choice of wound closure treatment for each wound area.

There are several main characteristics of an ideal eschar removal means:

1. Early (as early as possible), effective and thorough eschar removal that allows early direct visualization and assessment of the primary burn damage, prevention of eschar-related deterioration and complications and initiation of non-surgical or surgical wound closure treatment. The eschar removal should be fast enough to timely release BICS.
2. Selective, avoiding unnecessary damage of viable, non-burned tissues, which may be essential for the wounds' spontaneous epithelialization potential.
3. Safe, both for the patients' general and local (non- or partially-injured tissues) conditions.

Timely (as soon as possible after patients stabilization: early initiation and early completion) and thorough eschar removal, is the primary goal of treatment initiation. Eschar removal will not only stop the vicious eschar progression, eschar-induced effects and resolve (like escharotomy) increased burn induced interstitial/compartment syndrome, but will allow accurate assessment of the primary burn damage and initiation of the closure, healing process. In this regard, burn eschar removal does not differ from the care of all other necrotic wounds, where timely removal of the offending necrotic eschar is the gold standard.

3.3.2 Extremities Wounds

Burns to the extremities (hands and feet) have a special place in the field of burn care, demanding special attention and treatment.

In more than 80% of severely burned patients, the hand is involved [69].The functional and aesthetic importance of the hand, coupled with its complex and crowded anatomy, with risk of burn-induced increases in interstitial and compartment pressure, make hand burn care extremely important and with current SOC, as further elaborated below, challenging.

Due to the special anatomy of the hand in which numerous delicate structures are crowded in a small space and covered by scarce sub-dermal soft tissue, surgical debridement of the burned tissue, or escharotomy in these areas, are technically demanding and may cause considerable morbidity. The maintenance of perfusion is the first and foremost aim in the acute treatment of hand burns. During the acute phase, deep dermal, circumferential or near circumferential burns should be cared for most attentively because they can cause circulatory impairment at the level of the nerves and muscles as well as stretching the partially burned skin and collapsing the marginal blood supply, transforming a partial thickness burn into a full thickness one. Even for experienced surgeons it is sometimes extremely difficult to determine whether an escharotomy of the hand will be necessary or not [69]. Technical difficulties in estimation of the burn depth may lead to late or even unnecessary surgical debridement, especially in light of a previous study which has demonstrated that the excised burn eschar often contains viable tissue:

microscopically unaltered normal deep dermis, unaltered dermal collagen fibers and transected normal capillaries [70].

Contact burns are often palmar burns. Surgical intervention is very rarely indicated because the skin of the palm is thick and provides good protection. Surgical debridement is difficult to carry out due to the palm's distinct anatomy and the tight fibrous septi attached coherence to the palmar aponeurosis. Substitution of the specialized palmar skin is only applicable to a limited degree. These factors justify conservative treatment for 3–4 weeks, whenever possible [69].

Within the clinical development program, NexoBrid was used to treat 130 deep partial and full thickness burn wounds of the hands (in 101 patients; 68 adults, 33 children and adolescents), mainly in 2 clinical studies: retrospective data collection study 35-98-910 and the confirmatory, controlled phase 3 study MW2004-11-02 [71].

In a post-hoc analysis of the subgroup of hand wounds treated with NexoBrid and SOC in study MW2004-11-02, the following results were generated:

Timely Eschar Removal

Similar number of wounds reached successful eschar removal in both groups; 90.3% (28/31) in the NexoBrid group and 97.6% (40/41 wounds) in the SOC group ($p = 0.1843$).

Time to achieve successful eschar removal was significantly shorter in the NexoBrid group (mean 2.57± 1.95 days from Injury compared to the SOC group (mean 7.75± 5.41 days from Injury) ($p<0.0001$).

Excisional Eschar Removal

Four (4) out of 31 hand wounds (12.9%) required excisional debridement in the NexoBrid group as compared to 29 out of 41 (70.7%) in the SOC group ($p<0.0001$).

The mean percentage of the burn wound area excised in the NexoBrid group was 4.4 ± 13.1% as compared to 52.0 ± 41.4% % in the SOC ($p<0.0001$).

Autograft

In the DPT sub-population, only one of the 24 (4.2%) hand wounds was autografted in the NexoBrid group compared to 10 of 20 (50%) in the SOC group ($p=0.0005$).

The percentage of the burn wound area autografted in the NexoBrid was 2.1 ± 10.2% as compared to 30.5 ± 38.4% in the SOC ($p=0.0017$).

Wound Closure

The Mean Time to wound closure measured from injury date was similar in the two treatment groups: NexoBrid 25.0 ± 12.5 days, SOC 28.0 ± 15.8 days ($p=0.3814$)

Hand wounds were further assessed as part of the Long term study (MW200-12-01-02) which included a blinded assessment of scar quality. The mean total overall MVSS score was lower (better results) in the NexoBrid arm compared to SOC: 3.14 ± 2.59 vs. 4.05 ± 2.82, respectively, without yet reaching a statistically significant difference due to sample size ($p=0.2756$).

Burn Induced Compartment Syndrome (BICS) is an acute progressive condition that starts a few hours post injury and develops during the acute, edematous stage of the first 2-3 days (after which edema and increased pressure subside) in deep circumferential burns. The edema and pressure reach their maximum within few days post injury and start to slowly decrease thereafter. Soft tissue can withstand temporary pressure increase for 6-8 hours without permanent damage, allowing 4-6 hours for decision-making regarding escharotomy (see below).

Release of increased pressure can be achieved by formal surgical escharotomy, excision of the constricting eschar and as was observed by Krieger et al [71-72] as well as during the confirmatory phase 3 study MW2004-11-02, by early application of an effective, fast acting eschar removal agent that underwent a development program and studied for this specific condition such as NexoBrid [65, 72-73].

Due to the very high incidence of hand burns and the risk of BICS (Extremity At Risk: EAR) all burn physicians are trained and skilled in the routine diagnosis and treatment of this clinical entity. Due to the severe complications potentially associated with the current SOC of the interventional measure of BICS release: surgical escharotomy (scars, bleeding & damage to deeper structures), physicians often choose to monitor the patient with circumferential extremity burns for a few hours (by clinical signs and direct pressure measurement), continuously assessing the hand's condition, before committing to escharotomy [1, 64, 73, 92-96]. In the clinical development program, none of the hand burns treated with NexoBrid required escharotomy. Moreover, none of the NexoBrid subjects' hands had to be surgically escharotomized to resolve burn induced high interstitial/compartment pressure- 0% (0/130 hand wounds) in the NexoBrid arm, vs. 9.7% (4/41) of the hands treated by SOC.

To investigate release of pathologically elevated interstitial pressure, a sub-analysis was performed for the circumferential wounds that did have a measurement of elevated interstitial pressure prior to treatment in the confirmatory phase 3 study, MW2004-11-02. There were six (6) such wounds with measured high pressures that were treated with NexoBrid and two (2) that were treated by SOC. NexoBrid application reduced the elevated pressure below the threshold without need for surgical intervention (escharotomy or excision), whereas in the SOC-treated wounds the pressure had to be reduced by surgical intervention (escharotomy or excision). Both methods effectively reduced and released burn-induced elevated pressures, but SOC is associated with the sequelae of surgical escharotomy.

These results are further supported by the retrospective study (35-98-910) which demonstrated that enzymatic debridement decreased both the number of deep hand burn injuries that ended-up in an operative procedure, and the wound area grafted in those who did undergo skin grafting [73].

The effective pressure release achieved with NexoBrid is also consistent with the results that were obtained in a controlled in vivo study. In a burn pig model, NexoBrid resolved burn induced compartment syndrome within 30-45 minutes after application, even before the complete dissolution of the eschar [72].

NexoBrid appears to address the unmet needs of an eschar removal means by being as fast and effective as surgery yet selective, non-invasive, not diagnosis-dependent, and least demanding. Its use resolves the first stage of wound bed preparation in the comprehensive wound care

process and in addition, facilitates the second step of non-surgical or surgical wound closure by enabling earlier, direct visual assessment of the wound bed and by reducing the incidence and/or extent of excision, which is a substantial, demanding part of the surgical wound closure process. The potential tissue-sparing effect of NexoBrid that offers the option of reducing autografting of the healing wound suggests an additional clinical benefit to the burn victim. Autografting, besides being a demanding surgical procedure that needs general anaesthesia, OR facilities and surgical personnel, also sacrifices healthy donor site skin that needs 2-3 weeks of painful healing and often ends with permanent scars.

Early non surgical eschar removal and prevention/resolution of increased interstitial/ compartment pressure in circumferential burns of extremities may decrease the time to resolution of such increased interstitial/compartment pressures reducing or preventing the need for surgery. It is especially important and beneficial to the patient and treating physician as it not only reduces or eliminates the need of traumatic and risky surgery but it also alleviates the weight of the difficult decision of performing a potentially mutilating surgical escharotomy, greatly extending the safety margins available for the treating physician. Such hands are routinely monitored before and after treatment and so were and will be the NexoBrid treated hands allowing intervention if needed as has been shown in its routine use.

All these benefits may also have special importance in preparedness for a burn mass casualty disaster scenario where surgery is the main treatment bottleneck (as has been seen in the 9/11 crisis: Dr. Bessey from Cornell, NY September 2012 & February 2013 FDA Burn Mass Casualty Incident Medical Countermeasures Public Workshops [74]. Study Rationale

Enzymatic eschar removal with NexoBrid allows initiating and completing the phase of removal of the offending eschar earlier upon admission, enabling earlier visualization of the wound bed for assessment of burn wound depth as well as preservation of viable tissues, as further elaborated and supported by previous clinical studies. The depth determination is important for the planning and execution of the post eschar removal stage of wound closure phase (grafting or spontaneous epithelialization).

Additional clinically meaningful attributes of NexoBrid enzymatic eschar removal is the ability to lower surgical burden as it allows to remove eschar in wounds that otherwise would have to undergo surgical excision.

This phase 3 study MW2010-03-02 will be a multi-center, multi-national, assessor blinded[10], randomized, controlled, three-arm study intending to demonstrate superiority of NexoBrid treatment over Gel vehicle placebo control treatment and over Standard of Care (SOC) treatment, in thermal burn subjects.

4. ETHICS STATEMENT

The study will be carried out in accordance with accepted international standards, which meet regulations relating to Good Clinical Practice (GCP). These standards are drawn from the following guidelines: ICH Guideline for Good Clinical Practice, 1 May 1996 amended September 1997 and Declaration of Helsinki (as amended in Seoul, October 2008), concerning medical research in humans.

10 Complete Eschar Removal assessment will be performed by a blinded assessor in the NexoBrid and Gel vehicle treatment arms.

The investigator(s) will ensure that this study is conducted in full conformity with the principles of the "Declaration of Helsinki" and with the laws and regulations of the participating countries, whichever affords the greater protection to the individual. It is the responsibility of the investigator to obtain informed consent in written form (according to local legal requirements) from each subject participating in this study. All patients will be informed of the aims, methods, anticipated benefits, potential hazards and confidentiality of data. Candidates will also be told that they are free to refuse participation at any time.

5. STUDY OBJECTIVES

The study objectives are:

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.

6. INVESTIGATIONAL PLAN

6.1 PRIMARY ENDPOINTS, SECONDARY ENDPOINTS AND OTHER ENDPOINTS

6.1.1 Primary Endpoint

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 Section 1.1 for Target Wound definition):

Incidence of complete eschar removal: Demonstrate superiority of NexoBrid over Gel Vehicle for eschar removal as measured by incidence of complete eschar removal at the end of the topical agent soaking period by a blinded assessor.

6.1.2 Secondary Endpoints

The following secondary endpoints will be evaluated in this study and compared between NexoBrid and SOC to further support the clinical benefit of NexoBrid.

1. ***Reduction in surgical need***- Demonstrate superiority of NexoBrid over SOC in reduction of surgical need for excisional eschar removal as measured by an analysis of incidence of surgical eschar removal (tangential/ minor/ avulsion/ Versajet and/or dermabrasion excision).
2. ***Earlier eschar removal***- Demonstrate superiority of NexoBrid over SOC with regard to the time when complete eschar removal has been achieved. For definition of complete eschar removal see primary endpoint.

3. ***Blood loss related to eschar removal***- Demonstrate superiority of NexoBrid over SOC with regard to the blood loss occurred during the eschar removal procedures.

6.1.3 Safety Endpoints

The following group of safety endpoints will be evaluated in this study and compared between NexoBrid and SOC. These endpoints are used to confirm that NexoBrid does not cause an unacceptable level of harm

1. ***Time to reach complete wound closure*** assessed in days, starting from randomization date
2. ***Cosmesis and Function***- will be measured using MVSS, to demonstrate that treatment with NexoBrid does not have any clinically meaningful deleterious effect on burns scars quality as compared to the quality of burns scars treated with SOC, measured at 12 months from wound closure date, by a blinded assessor.
3. ***Cosmesis and function-*** will be measured using MVSS, to demonstrate that treatment with NexoBrid does not have any clinically meaningful deleterious effect on burns scars quality as compared to the quality of burns scars treated with SOC, measured at 24 months from wound closure date, by a blinded assessor.
4. Additional Safety Outcome Measures:
   a. General parameters: Systemic adverse events, vital signs, pain assessment (using VAS and as reported as AEs), laboratory tests, units (and volume) of blood transfusion given during hospitalization, Immunogenicity evaluation for NexoBrid patients, Pyrexia and Hyperthermia, Systemic infections, Incidence of increased interstitial/compartment syndrome (as defined in Section 1.1) and QT prolongation, 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).
   b. Long term functionality evaluation of the extremities using the 'Lower Extremity Functional Scale', 'QuickDASH' questionnaires and 'Range Of Motion' measurements,
   c. Long term Quality of Life using EQ5D and Burn Specific Health Scale- Brief (BSHS-B).
   d. Local parameters: Local adverse events defined by treating physician or designee; graft loss, wound related infections, etc.

6.1.4 Exploratory Endpoint

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. POSAS will be used to further assess cosmesis and function at the long term follow up visits,
3. Incidence of surgical Escharotomy procedures on circumferential extremities target wounds,
4. Incidence of reduction in interstitial/compartment pressure in circumferential extremity wounds (measured immediately following eschar removal),
5. Reduction in surgical need as measured by analysis of incidence of surgically harvested donor sites,
6. Reduction in surgical need as measured by analysis of % area of surgically harvested donor site,
7. Blood loss related to eschar removal assessed by changes in Hematocrit incurred during the eschar removal procedures
8. Cosmesis evaluation (MVSS and POSAS) will be used to assess the quality of the donor sites scars,
9. PK evaluation for a subset of NexoBrid patients[11],
10. Autograft related parameters:
    I. Efficacy and safety analyses for early and late grafted wounds
    II. Total number of target wound grafting procedures
    III. Incidence of repeated/additional grafting procedures
    IV. Area of repeat grafting
11. Duration of hospitalization.

6.2 STUDY DESIGN

This is a multi-center, multi-national, randomized, controlled, assessor blinded (see Section 6.3), three-arm study aiming to demonstrate superiority of NexoBrid treatment over Gel vehicle control and over SOC treatment in thermal burn subjects with burns.

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.

All subjects' DPT and FT burns that comply with the specified entrance criteria under Section 7.1 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 minimise 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 for full description of the randomization procedure.

[11] Please see *Appendix 10- Procedures for specific blood tests* for details.

Following the stratification, patients will be randomized as per their stratification group 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*) and undergo wound cleansing and dressing of all wounds with antibacterial solutions (as specified in Section 8.1). 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.

Subsequent to complete eschar removal, all wounds will be assessed and treated in the same manner, 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 wound closure. Following wound closure confirmation visit, subjects will be followed up at 1, 3, 6, 12, 18 and 24 months post wound closure for long term outcomes evaluation.

6.3 MEASURES TAKEN TO MINIMIZE/AVOID BIAS

This study includes 3 arms: NexoBrid, SOC and Gel Vehicle.

In this study, wound characterization will be performed prior to randomization into treatment arms. The randomization procedure will be initiated only after all TWs of a patient have been defined and recorded in the eCRFs. All subjects' DPT and FT burns that comply with the specified entrance criteria under Section 7.1 and TWs definition (Section 1.1) are intended to receive study treatment per the randomized study arm. Randomization will be performed by stratifying eligible subjects into different groups and by center in order to standardize baseline characteristics and minimise potential bias between study groups in terms of efficacy and safety outcomes that are expected to be correlated to the depths of the patient's TWs. The planned stratification by center further enables the standardization of the treatment of all study arms further reducing possible site effect.

Wound size (%TBSA) will be clinically assessed by the 'rule of palms[12]'.

Using this stratification, patients will be randomized in a 3:3:1 ratio (NexoBrid: SOC: Gel Vehicle). Please refer to Section 13.7 for further information.

To further minimize bias, the Investigator will define the TWs of a patient based on the criteria for defining target wounds, as described in Section 1.1. All Patient's TWs will be treated in accordance with the randomized treated arm; NexoBrid, Gel vehicle or SOC (as described in Section 6.4), thus allowing appropriate collection of safety information, which is being reported per patient, presenting the patient as a whole.

A subject who was randomized, but did not receive treatment will be analyzed in the ITT population (see description of population in Section 13.1).

6.3.1 Assessments of Clinical Measures

The following measures are predefined and will be taken in specific time points per the study protocol to ensure minimization of potential biases:

6.3.1.1 *Extent of eschar removal assessment using topical agent; NexoBrid or Gel Vehicle*

The topical arms are 2 different topical preparations (NexoBrid- a gold color gel and Gel Vehicle- a transparent gel) that are impossible to disguise in terms of preparation procedures and appearance, even if supplied in masked containers. Therefore, the topical product application (NexoBrid or Gel Vehicle) will be done by a health care professional and the wound assessment will be performed by a different health care professional who is blinded to the randomized study arm. This will allow a blinded assessment of the primary endpoint of the study (incidence of complete eschar removal in NexoBrid vs. Gel Vehicle arm).

Eschar removal procedure will be initiated at the end of the cleansing and blister removal session (debridement), after wound soaking. Product will be applied by a health care professional to the wound within 15 minutes of mixing, and left on for a 4-hour period. After 4

[12] Using the rule of palms, the surface of the patient's palm represents approximately 1% of body surface area and is helpful in estimating the area of small burns.

hours, the wound bed with the remains of the dissolved eschar and topical agent will be wiped away and the wound bed will be soaked for an additional 2 hours to remove any remains of the mixture. Following removal of the soaking dressing (6 hours of treatment in total), the wound bed will be photographed in a standardized manner, and the extent of eschar removal will be clinically assessed by a different health care professional- assessor blinded to treatment assignment. The un-blinding tests that are done only for the NexoBrid patients (i.e. PK and Immunogenicity) will be done by non blinded site staff and will be recorded in a separate worksheet for the purpose of keeping the eschar removal assessor blinded to study arm. 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. The blinded assessor will sign the "blinded assessor memo" (see Appendix 15- Blinded Assessor Memo).

If debridement is not complete, but at least 50% of eschar was removed, a second application will be performed, as described in Section 8.2.3.2. Similar procedures will take place following a second application of the topical agent Patients treated with NexoBrid or Gel Vehicle will undergo up to 2 topical applications.

In cases where eschar removal was not complete following the applications of the topical agents, NexoBrid or Gel Vehicle, additional surgical procedures and/or non surgical treatments as described below will be implemented in accordance with physician judgment:

6.3.1.2 *Surgical eschar removal (SOC arm or as additional procedure for the topical arms):*

Extent of wound area excised & incidence of excisions following each surgical excision procedure performed for eschar removal will be recorded in the eCRFs. Eschar removal will be assessed immediately after the eschar removal surgical procedure, before a temporary or permanent dressing will be applied. The extent of eschar removal will be clinically determined by the same assessor who is blinded for the topical arm.

Surgical procedures intended for eschar removal are pre-specified in the protocol for standardization (i.e. tangential/minor/avulsion/Versajet/dermabrasion excision).

6.3.1.3 *Non-surgical eschar removal (SOC arm or as additional procedure for the topical arms):*

Wounds will be assessed by a burn specialist as in common daily practice, following any dressing change. Extent of eschar removal after the last dressing will be clinically determined by the same assessor who is blinded for the topical arm and recorded in the eCRFs.

Non-surgical procedures intended for eschar removal are pre-specified in the protocol for standardization and are detailed in Section 8.2.2.

6.3.2 Main safety measures

To further minimize bias, main safety measures (weekly assessments for wound closure, long term cosmesis as well as some functionality assessment) will be performed by a blinded assessor, different from the first assessor who performed eschar removal assessments. 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 (NexoBrid or Gel 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 and function (as described below) in all patients, and all study arms. The blinded assessor will sign the "blinded assessor memo" (see Appendix 15- Blinded Assessor Memo).

- Wound closure- will be assessed on a weekly basis as described in Section 8.6. **Complete wound closure** defined as skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits, 2 weeks apart [75]. Wounds will be considered as closed when the assessor marks 'Yes' in regards to complete wound closure, which will be further confirmed in the wound closure confirmation visit, 2 weeks later.
- Cosmesis and Function (using MVSS)- will be assessed at the following time-points: at 1 month from reaching complete wound closure confirmation of last wounds and at 3, 6, 12, 18 and 24 months from reaching complete wound closure confirmation.

6.4 STUDY TREATMENTS AND DOSAGE

6.4.1 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).

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 (±3% 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.

6.4.2 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 (±3% 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.

6.4.3 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.

6.5 TRIAL PERIODS

The total duration of the study treatment and follow up period of each participating subject is expected to be 25 months: Following initiation of the Eschar removal procedure, each subject will be followed up weekly (Weeks 1, 2, 3 and 4 etc.) until complete wound closure was 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 of each patient.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 SUBJECT ENTRANCE CRITERIA

7.1.1 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.

7.1.2 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),[13]

   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

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

3. Wound's blisters can be removed/unroofed, as judged by the investigator.

7.1.3 Exclusion Criteria- Patient level

1. Patients who are unable to follow study procedures and follow up period,
2. Modified Baux index[14] ≥ 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,
7. The following pre-enrollment dressings: a. Flammacerium, b. Silver Nitrate ($AgNO_3$),
8. 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),
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),
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,

[14] 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).

21. Current (within 12 months prior to screening) suicide attempt,
22. Mentally incapacitated adults who are incapable of giving legal consent
23. Enrollment in any investigational drug trial within 4 weeks prior to screening,
24. Current (within 12 months prior to screening) severe alcohol or drug use disorder (see definition in section 1.1),
25. Prisoners and incarcerated,
26. Patients who might depend on the clinical study site or investigator.

7.2 SUBJECT WITHDRAWAL 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).

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.

7.2.1 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:

7.2.1.1 *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 ITT 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.

7.2.1.2 *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 [76]. 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 [77], 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.

7.2.1.3 *Post treatment diagnosed infection (until complete wound closure)*

Following the eschar removal treatment, in case 3 subjects develop infections listed in Section 11.9 and are assessed as related to the treatment arm (possibly, probably or related as defined

in Section 12.1), 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.

8. STUDY CONDUCT

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 guidelines [1].

8.1 SCREENING AND BASELINE VISIT

8.1.1 Screening and Baseline Procedures

For subjects who are admitted to the burn unit, and who are willing to participate in the study, the following procedures will be performed:

1. **Signing of consent form**:

   Prior to performing any study related activities/evaluations which are not considered as routine, the subject must be thoroughly informed about all aspects of the study, including scheduled study visits and activities, and must sign the informed consent form. A signed copy of the informed consent form must be given to the subject. Legal representative may be involved in the Inform Consent procedure and sign the form if this is pre approved by the local IRB/EC and only for subjects with temporary medical conditions related to the injury, subjects who might be under the influence of pain medication, sedation, etc. However, legal representative cannot sign the form for mentally incapacitated subjects who are incapable of giving legal consent (entrance criteria, Section 7.1),
2. Review and documentation of complete **demographics, medical history** (e.g. specific cardiac, pulmonary, endocrine and renal diseases), and **concomitant medications**,
3. **Vital signs** measurement including blood pressure, pulse rate, temperature and respiration rate,
4. **Pain assessment** using a routinely accepted pain-scale ruler (Visual Analogue Scale, Please refer to *Appendix 9- Pain Measurement Scale),*
5. **General physical examination** (including diagnosis of smoke inhalation injury, signs for Burn Induced Compartment Syndrome, etc) including a record of weight and height,
6. Obtaining **burn etiology**, mechanism of injury, place of injury, treatments prior to admission and description of the eschar (such as: white, moist, dry, gray, charcoal, etc.),
7. Leukocyte count (**WBC**),
8. Extremities At Risk (**EAR**) burns prone to develop BICS will be identified (See Section 11.8).
9. **Pregnancy test** for subjects of child-bearing potential performed at local laboratory,

10. **HbA_{1c}** in patients with known diabetes as captured in the medical history,
11. **Daily fluid balance** will be monitored for 7 days from injury or until hospital discharge, whichever comes first, for patients with catheter,
12. **Wounds cleansing** as described in Section 8.1.2 below,

    Post wound cleansing, preferably before soaking (to avoid unnecessary dressing removal), **all wounds should be labelled and photographed** (as required in *Appendix 3- Photographic wound documentation*). The TWs numbers assignment per their location will be documented on a schematic drawing of an adult.
13. Post cleansing, preferably before soaking the wounds with antibacterial solution (section 8.1.2, point 3), the **% TBSA (clinically assessed by the rules of palm) and burn depth** (as detailed in *Appendix 1- Wound Depth assessment- Clinical Evaluation*) of each wound and of each TW will be recorded,
14. **Pain management medications** and analgesia/sedation levels should be recorded (see pain management protocol provided in *Appendix 2- Pain Management Procedures*)
15. Completion of the applicable eCRFs,
16. **Randomization** of eligible subjects (as described in Section 13.7.1).

8.1.2 Cleansing and Soaking

The initial care of the burn wound starts with general cleaning that removes all gross contaminants, soot and blisters ("Debridement"). As described in Neligan Plastic Surgery Textbook, volume 4 pg 403 [78], DPT burns contain large blisters. Blister fluid is known to be detrimental to raw exposed tissues and to wound healing as well as being prone to infection that will spread in this occluded space. The blisters tend also to rupture under the dressings and during their changes. Using aseptic techniques the blisters should be removed ("unroofed") and the exposed wound treated as needed [78-82].

Preventive analgesia/sedation medication (pain management) will be mandated as for an extensive dressing change as commonly practiced (see Appendix 2- *Pain Management Procedures*). The following should be performed:

1. Topically applied medication on the wound site must be discontinued,
2. The wound should be cleansed thoroughly with saline, soap or antibacterial solution and the blisters (superficial keratin layer) removed by common procedures (i.e. rubbing with sterile gauze, saline soaking, etc),
3. Soaking of at least 2 hours: A dressing soaked[15] with an antibacterial solution, e.g. 3-5% Sulfamylon, 0.05-0.5% Chlorhexidine, Dakin's solution, hypertonic 5-10 % saline solution or 0.9% Saline must be applied to the wound and left in place. This dressing must remain wet until it is removed prior to application of the eschar removal agent/procedure. In order to prevent desiccation of the wound; the dressing must be changed at least every 12 hours if eschar removal is delayed. After soaking the dressing should be removed using aseptic techniques,

[15] Soaking refers to placing gauze soaked with fluid, e.g. saline or Dakin's Solution on dressing the wound with thick, dry absorbent gauze, inserting inside a feeding tube or the tip of an IV set that offers the option of adding fluids at will.

4. Check that the superficial keratin (blister) has been removed (NOTE: For deep charred, flame burns blister-keratin removal may be difficult. In a deep, charred burn a thorough scraping is needed to remove the charred blister from its dermal eschar). In the event that not all the superficial keratin layer is removed from the burn eschar, repeat steps 2 and 3 as the blister-keratin may be a focus for infection and will prevent the eschar dissolution by isolating it from the topical agent.

Note: The results of the above on-site tests (describe in Section 8.1.1) may be used for study data collection prior to signing of the consent form only in case they are part of routine hospital care regardless of study procedures and within relevant timelines.

8.2 TREATMENT PROCEDURE- ESCHAR REMOVAL: NEXOBRID/SOC/GEL VEHICLE - INITIATED WITHIN 84 HOURS FROM INJURY

Eschar Removal will be performed in accordance with randomization: SOC, Gel Vehicle or NexoBrid.

8.2.1 All arms - Pre first treatment assessments- within 1 h pre treatment

Make sure at least 2 hr soaking were performed and that all blister-keratin has been removed from the TW's eschar. If there are still blisters/keratin remains, clean the wound thoroughly and remove the superficial keratin layer or blisters from the wound area, as the keratin will isolate the eschar from direct contact with the topical agent (as described in Section 8.1.2).

Patients will undergo the following procedures within one hour (+ 30 min) prior to start of first procedure for eschar removal:

1. **Blood culture**,
2. **PTT & INR** (2ml) blood sample,
3. **PK blood sample** (2ml) for NexoBrid patients as described in *Appendix 10- Procedures for specific blood tests*,
4. **Immunogenicity blood sample**[16] (3ml) (NexoBrid patients), see *Appendix 10- Procedures for specific blood tests*,
5. **Central lab blood tests**: Serum chemistry, hematology and urinalysis (as described in Section 11.7)
6. **EAR monitoring:** Clinical assessments, SpO2 and the direct interstitial/compartment pressure measurement will be performed in circumferential extremity wounds. The measurement will be done, as described in Section 11.8,
7. **Pain assessment** using a routinely used, standard pain-scale ruler (Visual Analogue Scale) as described in *Appendix 9- Pain Measurement Scale* should be performed **12-lead ECG** (in triplicate) will be performed, if feasible, at the following time points: 60 ± 5 min, 40 ± 5 min and 20 ± 5 min prior to start of treatment,
8. **Preventive analgesia medication** (pain management) will be mandated as for an extensive dressing change as routinely practiced; it should be initiated at least 15 minutes

prior to dressing change or application in order to ensure pain free care (example of a recommended protocol provided in Appendix 2- *Pain Management Procedures*). Prior to a surgery, preventive sedation and/or general anesthesia will be mandated as routinely practiced. Pain management medications and analgesia/sedation level will be recorded,

9. **Qualitative-swab wound culture** (per Target Wound),
10. All **Target Wounds** should be **labeled and photographed** (please refer to *Appendix 3- Photographic wound documentation*),
11. **% TBSA assessment (using rule of palms) and burn depth** (as detailed in *Appendix 1- Wound Depth assessment- Clinical Evaluation*) of each TW will be recorded.

8.2.2 Standard of Care (SOC) Arm - Eschar removal procedure- (to be initiated within 84 hours from injury)

Subjects in the SOC group may be treated with a combination of surgical and non-surgical eschar removal procedures, for each TW, according to the investigator's judgment. In case of indeterminate burns (second degree burns of changing depths) and/or combination wounds, mixed of second and third degrees, which are difficult to diagnose on arrival, non surgical treatments is usually being initiated for few days when the burn conversion (progression) and demarcation is taking place. Once a diagnosis is feasible, surgical procedures will usually be performed for deep wounds or additional non surgical procedures will be continued for superficial wounds.

Surgical eschar removal procedure

Surgical procedures intended for eschar removal are pre-specified for standardization and will include tangential/ minor/ avulsion/ Versajet/ dermabrasion excision).

Non-surgical eschar removal procedure

Non-surgical procedures intended for eschar removal are pre-specified for standardization and may include the application of Santyl (Collagenase ointment), 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). Non-Surgical procedure is considered as one procedure for whole period of any continuous dressing changes of more than 24h until either complete eschar removal is achieved or until surgical eschar removal is conducted.,

8.2.2.1 *First SOC Procedure (to be initiated within 84 hours from injury)*

8.2.2.1.1 Pre first treatment assessments- within 1 h pre treatment

Pre first treatment assessments are described above in Section 8.2.1. The time-point of start of the SOC eschar removal procedure should be documented. This time point refers to the start time of the first treatment given to the patient, for eschar removal, post randomization (any topical application (non-surgical) or surgical procedure whichever is first).

8.2.2.1.2 During first SOC procedure (surgical or non-surgical), the following assessments will be performed:

1. 12-lead ECG (in triplicate) will be performed, if feasible, at the following time points: 30 ± 15 min, 120 ± 15 min and 4 ± 0.5 hours after start of eschar removal, non surgical or Surgical.

8.2.2.1.3 Procedure (surgical or non-surgical) documentation:

1. Completion time of the eschar removal procedure and type of procedure will be documented,
2. Preventive analgesia medication (pain management) prescribed during treatment and level of analgesia prescribed (as described in Appendix 2- *Pain Management Procedures*), will be recorded,
3. Manpower (number of surgeons, anesthesiologists, registered nurses, practical nurses and unskilled assistances) who participated during the above period of time and disposables used (number of dressings, blades, etc) will be recorded, if applicable,
4. All types of bloods product transfusion (e.g. units of whole blood or packed cells and volume) will be documented,
5. If Quantitative (histological) wound culture biopsy performed, by the discretion of the treating physician, to rule out suspicion of a clinical invasive burn wound infection (*Appendix 8- Biopsy & Histologic Assessment Method*) it will be recorded,

8.2.2.1.4 Post first procedure:

To be performed:

i. For any surgical procedure

And

ii. For non-surgical – only if eschar is completely removed (i.e. the additional procedure is last procedure for eschar removal).

1. Vital signs (blood pressure, pulse rate, temperature and respiration rate) will be taken at the end of the eschar removal procedure,
2. Target Wounds should be labeled and photographed at the end of the eschar removal treatment,
3. Eschar removal will be assessed immediately following the completion of the procedure (by the same assessor who evaluate blindly the eschar removal in the topical arms) and recorded for each TW,
4. TWs depth will be evaluated, and documented in the eCRFs once: post complete eschar removal[17]. For patients treated with only non-surgical treatment, TW depth assessment should be done post first dressing change.

[17] More or equal to 95%

5. Qualitative-swab wound culture (per Target Wound) will be taken at the end the eschar removal procedure,
6. Interstitial/compartment pressure will be measured and recorded (as described in Section 11.8) post-eschar removal, per circumferential extremity wound, at the end of the procedure or escharotomy, whichever is first,
7. Local lab tests: PTT & INR (2ml) blood sample, be performed 4 hours (± 15min) post end of the procedure,
8. Pain assessment using a routinely accepted pain-scale (VAS) as described in *Appendix 9- Pain Measurement Scale* should be performed 4 hours (± 15min) after the end of the procedure,
9. Central lab: Serum chemistry, hematology and urinalysis, should be performed 4 hours (± 15min) after the end of the procedure,
10. Blood culture: 4 h (± 15min) after the end of the procedure,

8.2.2.2 *Additional SOC procedures:*

If eschar removal was not achieved after first eschar removal, additional SOC procedures (surgical or non-surgical), as detailed above, will be performed as needed until complete eschar removal is achieved.

8.2.2.2.1 Pre additional surgical treatment assessments- within 1 h pre treatment

In addition to pre first treatment assessment as described in Section 8.2.1, in case that more than one SOC procedure is performed, the following assessments should be repeated 1 hour (± 15 min) pre **any additional surgical** treatment (do not perform if additional procedure is non-surgical):

1. The time-point of start of the surgical eschar removal procedure should be documented.
2. **Interstitial/compartment pressure** will be measured in circumferential extremity wounds (See Burn wound definitions, Section 1.1). The measurement will be done, as described in Section 11.8, in addition to clinical assessment and SpO_2 monitoring prior to eschar removal or Escharotomy, whichever is first, of each circumferential TW,
3. **Central lab blood tests**: Serum chemistry, hematology and urinalysis (as described in Section 11.7),
4. **Pain assessment** using a routinely used, standard pain-scale ruler (Visual Analogue Scale) as described in Appendix 9- Pain Measurement Scale, **Qualitative-swab wound culture** (per Target Wound),
5. All Target Wounds should **be labeled and photographed** (please refer to *Appendix 3- Photographic wound documentation*),
6. Preventive analgesia medication (pain management): Sedation and general anesthesia as routinely practiced will be administered before and during surgery and recorded.

*** In cases that additional non-surgical procedures are performed, no need to perform the above.**

8.2.2.2.2 Additional treatment documentation **(surgical and non-surgical**):

1. Completion time of the eschar removal procedure and type of procedure will be documented,

2. Preventive analgesia medication (pain management) prescribed during treatment and level of analgesia prescribed (as described in Appendix 2- Pain Management Procedures), will be recorded,

3. Manpower (number of surgeons, anesthesiologists, registered nurses, practical nurses and unskilled assistances) who participated during the above period of time and disposables used (number of dressings, blades, etc) will be recorded,

4. All types of bloods product transfusion (e.g. units of whole blood or packed cells and volume) will be documented,

5. If Quantitative (histological) wound culture biopsy performed, by the discretion of the treating physician, to rule out suspicion of a clinical invasive burn wound infection (Appendix 8- Biopsy & Histologic Assessment Method) it will be recorded,

8.2.2.2.3 Post additional procedure

To be performed:

I. For any surgical procedure

And

II. For non-surgical – only if eschar is completely removed (i.e. the additional procedure is last procedure for eschar removal).

1. All target Wounds should be labeled and photographed at the end of the procedure.
2. Extent of eschar removal will be assessed immediately following the completion of the procedure (by the same assessor who evaluate blindly the eschar removal in the topical arms) and recorded for each TW.
3. TWs depth will be evaluated, and documented in the eCRF once: post complete eschar removal[18]. For patients treated with non-surgical treatment only, TW depth assessment should be done post first dressing change,
4. Qualitative-swab wound culture (per Target Wound) will be taken at the end of the eschar removal procedure,
5. Vital signs (blood pressure, pulse rate, temperature and respiration rate) will be taken at the end of the procedure,

[18] More or equal to 95%

6. Interstitial/compartment pressure will be measured and recorded (as described in Section11.8) post-eschar removal, per circumferential extremity wound, after the completion of the procedure or escharotomy, whichever is first,
7. Pain assessment using a routinely accepted pain-scale (VAS) as described in *Appendix 9- Pain Measurement Scale* should be performed 4 hours (± 15min) after the end of the procedure,
8. Central lab: Serum chemistry, hematology and urinalysis, should be performed 4 hours (± 15min) after the end of the procedure,
9. Blood culture - 4 h (± 15min) after the end of the procedure.

8.2.3 Topical arms; NexoBrid/Gel Vehicle - Eschar removal procedure (to be initiated within 84 hours from injury)

8.2.3.1 *First Application of NexoBrid/Gel Vehicle*

8.2.3.1.1 Pre first treatment assessments- within 1 h pre treatment

Pre first treatment assessments are described above in Section 8.2.1.

The time-point of start of the eschar removal procedure should be documented.

8.2.3.1.2 Preparation

1. Surround the area from which you wish to remove the eschar with Vaseline ointment adhesive barrier by applying it a few centimeters outside of the treatment area (using an adhesive-barrier dispenser). The paraffin layer must not come into contact with the area to be treated to avoid covering the eschar, thus isolating the eschar from direct contact with the topical agent,
2. Sprinkle sterile isotonic (0.9%) sodium chloride solution on the burn wound. The wound must be kept moist during the application procedure.

8.2.3.1.3 Application

Application of the product, as per the randomization, should follow the instruction for use described in Section 6.4. NexoBrid should not be applied to more than 15% TBSA (±3% TBSA) in one session.

1. The wound should then be covered with an occlusive film dressing that adheres to the adhesive barrier material applied as per the instruction above. The topical agent should fill the entire occlusive dressing, and special care should be taken not to leave air in this occlusive dressing. Gentle pressing of the occlusive dressing at the area of contact with the adhesive barrier will ensure adherence between the occlusive film and the adhesive barrier and achieve complete containment of topical agent on the treatment area,
2. The dressed wound should be covered with a loose, thick fluffy dressing, held in place with a bandage,
3. The dressing should remain in place for 4 hours (± 15min),

4. All unused products should be kept for accountability as described in Section 10.1 (all remains and unused bottles),

8.2.3.1.4 During 1st Application

1. 12-lead ECG (in triplicate) will be performed, if feasible, at the following time points: 30 ± 15 min, 120 ± 15 min and 4 hours ± 30 min after initial application of NexoBrid/Gel Vehicle
2. PK blood sample (2ml) will be performed in a subset of NexoBrid patients at the following time points: 30 ± 10 min, 120 ± 10 min and 4 hours ± 15 min from initial application.
3. Circumferential extremity wounds should be monitored to detect EAR. 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. BICS should be treated by the investigator as described in Section 11.8. Circumferential extremity which is assessed as EAR at this stage should not be removed from the study but monitored as described above and in Section 11.8

8.2.3.1.5 Removal of topical agent

1. Administer appropriate preventive analgesia medication,
2. After 4 hours (± 15 min) of treatment at each TW, remove the occlusive dressing using aseptic technique and document the time.
3. The adhesive barrier should be removed using a sterile blunt-edged instrument (e.g. tongue depressor),
4. Remove the dissolved eschar from the wound by wiping it away with a sterile blunt-edged instrument,
5. Wipe the wound thoroughly first with a large sterile dry gauze or napkin, followed by a sterile gauze or napkin that has been soaked with sterile isotonic (0.9%) sodium chloride solution. Rub the treated area until the appearance of a pinkish surface with bleeding points or a whitish tissue. Rubbing will not remove adhering undissolved eschar in areas where the treatment has been ineffective and eschar still remains,
6. Interstitial/compartment pressure will be measured (as described in Section 11.8) post-eschar removal per circumferential extremity wound after removal of dissolved eschar or Escharotomy, whichever is first,
7. Apply a dressing soaked with an antibacterial solution, e.g. 3-5% Sulfamylon, 0.05-0.5% Chlorhexidine, Dakin's solution or hypertonic 5-10 % saline solution for an additional 2 hours. This wet-to-dry dressing will absorb and remove remaining NexoBrid and dissolved eschar that could not be removed by a simple wiping.

8.2.3.1.6 Post first application assessments

1. Document the completion time of the 2 h soaking,
2. Preventive analgesia medication (pain management) prescribed during treatment and level of analgesia prescribed (as described in Appendix 2- *Pain Management Procedures*), will be recorded,
3. Manpower (number of surgeons, anesthesiologists, registered nurses, practical nurses and unskilled assistances) who participated during the above period of time and disposables used (number of dressings, blades, etc) will be recorded, if applicable,
4. All types of bloods product transfusion (e.g. units of whole blood or packed cells and volume) will be documented,
5. If Quantitative (histological) wound culture biopsy performed, by the discretion of the treating physician, to rule out suspicion of a clinical invasive burn wound infection (*Appendix 8- Biopsy & Histologic Assessment Method*) it will be recorded,
6. Target Wounds should be labeled and photographed at the end of the 2hrs soaking,

Eschar removal[19] will be assessed immediately following the completion of 2 hrs soaking by an Investigator or designee, blinded to the treated arm,

[19] Complete eschar removal is defined as more or equal to 95% of the eschar removed

7. TWs depth will be evaluated following the completion of 2 hrs soaking, and documented in the eCRFs once: post complete eschar removal,

8. Qualitative wound culture (swabs), per TW, will be performed at the end of the 2h soaking period,

9. Vital signs (blood pressure, pulse rate, temperature and respiration rate) will be taken at the end of the 2h soaking period,

10. PTT & INR (2ml) will be tested at 4hrs ± 15 min after removal of the topical agent,(2 h after end of soaking)

11. Pain assessment using a routinely accepted pain-scale ruler (Visual Analogue Scale) as described in Appendix 9- Pain Measurement Scale should be performed 4 hours (± 15min) after removal of the topical agent , (2 h after end of soaking)

12. Central lab: Serum chemistry, hematology and urinalysis (as described in Section 11.7), should be performed 4 hours (± 15min) after removal of the topical agent, (2 h after end of soaking)

13. Blood culture - 4 hours (± 15min) after removal of the topical agent, (2 h after end of soaking)

8.2.3.2 *Additional Application of NexoBrid/Gel Vehicle (within 24 hours from start of first application)*

Additional session can be performed in 2 scenarios:

1. In case TWs area is more than 15% TBSA, NexoBrid should be applied in 2 separate sessions of up to 15% TBSA each. The second session can start immediately after the removal of the topical agent applied in first application but no later than 24 hrs after the start time of the previous application.
2. In case eschar removal from the target wound after the first application is not complete, but at least 50% eschar was removed[20], a second eschar removal procedure using the randomized topical agent (NexoBrid/ Gel Vehicle) will be performed. The randomized drug should be re-applied to the entire TW if necessary, but can also be applied only on the areas of the TW left with eschar. Reasons for failure should be recorded in the eCRFs. Repeat eschar removal session on the same area can start immediately after the 2 hrs soaking done post the first application of the randomized arm (In this case pre soaking period is not required before a second application of the randomized arm treatment to the same target wound), but no later than 24 hours after the start time of the first application.

No more than two eschar removal procedures using the randomized topical agent (NexoBrid/Gel Vehicle) will be allowed per TW.

[20] In case that <50% eschar was removed, see section 8.2.3.3

Treatment preparation and application should be conducted as detailed above in sections 8.2.3.1.1 and 8.2.3.1.3.

8.2.3.2.1 During treatment assessments

1. PK samples should be taken as detailed in *Appendix 10- Procedures for specific blood tests*
2. Circumferential extremity wounds should be monitored to detect EAR. 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. BICS should be treated by the investigator as described in Section 11.8. Circumferential extremity which is assessed as EAR at this stage should not be removed from the study but monitored as described above and in Section 11.8.

Topical agent removal should be repeated as described in Section 8.2.3.1.5.

8.2.3.2.2 Post second application assessments

1. Completion time of the eschar removal application and completion time of the 2 h soaking will be documented
2. Preventive analgesia medication (pain management) prescribed during treatment and level of analgesia prescribed (as described in Appendix 2- *Pain Management Procedures*), will be recorded,
3. Manpower (number of surgeons, anesthesiologists, registered nurses, practical nurses and unskilled assistances) who participated during the above period of time and disposables used (number of dressings, blades, etc) will be recorded, if applicable,

4. All types of bloods product transfusion (e.g. units of whole blood or packed cells and volume) will be documented,

5. If Quantitative (histological) wound culture biopsy performed, by the discretion of the treating physician, to rule out suspicion of a clinical invasive burn wound infection (*Appendix 8- Biopsy & Histologic Assessment Method*) it will be recorded,

6. Target Wounds should be labeled and photographed at the end of the 2hrs soaking,

7. Eschar removal will be assessed immediately following the completion of 2 hrs soaking by an Investigator or designee, blinded to the treated arm,

8. TWs depth will be evaluated following the completion of 2 hrs soaking, and documented in the eCRF once: post complete eschar removal,

9. Qualitative wound culture (swabs), per TW, will be performed at the end of the 2h soaking period,

10. Vital signs (blood pressure, pulse rate, temperature and respiration rate) will be taken at the end of the 2h soaking period,

11. Pain assessment using a routinely accepted pain-scale ruler (Visual Analogue Scale) as described in *Appendix 9- Pain Measurement Scale s*hould be performed be performed four hours (± 15min) after removal of the topical agent, (2 h after end of soaking)

12. Central lab: Serum chemistry, hematology and urinalysis (as described in Section 11.7), should be performed 4 hours (± 15min) after removal of the topical agent, (2 h after end of soaking)

13. Blood culture - 4 hours (± 15min) after removal of the topical agent, (2 h after end of soaking).

8.2.3.3 *Additional SOC (Surgical or non surgical) eschar removal procedures*

In case of a recorded failure (< 50% was removed) following the first topical application/s, or not complete eschar removal following 2 topical applications, additional surgical and/or non surgical eschar removal will be performed as needed until complete eschar removal[21] is achieved as described in Section 8.2.2.2.

[21] More or equal to 95%

8.3 GENERAL ASSESSMENTS, POST ESCHAR REMOVAL

The following assessments will be performed for all arms:

1. Vital signs (blood pressure, pulse rate, temperature and respiration rate) will be measured daily, in the morning time (5 AM to 10 AM), starting on the morning after the start of eschar removal, until hospital discharge,
2. Pain assessments will be performed daily, in the morning time (5 AM to 10 AM) using a standard pain-scale ruler (VAS) (please refer to *Appendix 9- Pain Measurement Scale*), starting on the morning after the start of eschar removal, until hospital discharge,
3. Wound coverage type and % of wound covered will be documented upon each dressing change , post complete eschar removal until hospital discharge, if relevant,
4. 12-lead ECG (in triplicate) will be performed, if feasible, at 12 ± 0.5 hours, 24 ± 1 hours and 48 ± 2 hours after start of the eschar removal treatment,
5. PK blood sampling (2ml) will be at 12 ± 0.5 hours, 24 ± 1 hours, 48 ± 2 hours and 72 ± 2 hours from start of the 1st application of NexoBrid (ONLY for NexoBrid patients),
6. Local lab tests: PTT & INR (2ml) blood sample, will be taken 24 ± 1 hours and 48 ± 2 hours after start of Eschar removal procedures,
7. All types of blood product transfusions (e.g. units of whole blood or packed cells and volume) will be documented throughout the patient admission, The physician will be asked to comment on whether the blood transfused is related to any specific procedure performed (e.g. surgical eschar removal, preparation for an autograft, etc),
8. Daily fluid balance will be monitored for 7 days from injury or until hospital discharge, whichever comes first, for patients with catheter,
9. Quantitative (histological) wound culture biopsy – will be recorded, if performed to rule out suspicion of a clinical invasive burn wound infection (*Appendix 8- Biopsy & Histologic Assessment Method*).

8.4 POST-ESCHAR REMOVAL- WOUND MANAGEMENT

In TWs which were assessed to reach complete eschar removal[22], the consequent wound closure phase can be initiated by grafting or epithelialization. The following section will describe wound management, post eschar removal, and is relevant to all TWs, regardless of the eschar removal method performed in accordance with the randomization.

Following to completion of eschar removal (NexoBrid, Gel Vehicle or SOC), different sites in the same patient may require different treatment strategies [28]. In general, raw bed, regardless of the eschar removal means, should always be protected by a dressing or a topical medicament and never remain open or allowed to desiccate.

[22] More or equal to 95%

Subsequently, wound management for all treatment arms should follow the normal standard care for open, raw surfaces based on the general principles described below. Please refer to *Appendix 5- Wound management; approved covers* for list of approved wound care products:

1. Wounds having **Full thickness** defects should be grafted with autografts or permanent dermal substitutes (i.e. Integra, Matriderm, Hyalomatrix) as soon as possible after eschar removal. If permanent cover such as autografting has to be postponed, a temporary adherent cover (e.g. allograft, Xenograft, etc) should be used until permanent closure is possible. Prior to grafting the clean eschar-free bed should be refreshed by scraping it to open occluded capillaries and remove fibrin deposits and thrombi. In rare cases of exposed tendons, blood vessels or bone, flaps may be used as protection and permanent cover.

   Use of negative pressure wound therapy (e.g. VAC) on top of the autograft for 2-3 days promotes take by ensuring drainage, contact and adherence to the wound bed and is commonly used.

2. Wherever **viable dermal exists**, such as in the **partial thickness burns (mid and deep partial)**, healing by spontaneous epithelialization on these partial thickness areas could be attempted for a period of approximately 3 weeks in order to reduce surgery and autograft use with donor site sacrifice [96], but should be carefully evaluated for a satisfying progress on a weekly basis. In these cases, the following wound management should be performed:

   a. The raw surface should be covered with an adherent biological cover (i.e. allograft, Xenograft), synthetic dressings or skin substitutes (e.g. Mepilex® Ag, Mepilex® transfer Ag, Aquacel Ag, etc) in order to protect the raw surface (mainly from desiccation) and provide the microenvironment necessary for epithelialization (e.g. 5% sulfamylon, Bacitracin, etc). If allografts, xenografts or any film-like products are applied on the debrided wounds, they should be perforated or meshed (and not expanded) for drainage and optimal conforming to the bed. Topical medicaments can also be used as covers as long as they do not interfere with the healing process (as in the case of SSD or Iodine preparations) and moisturize or prevent desiccating the healing bed (the above are suggested coverages to apply on the clean wound bed. For additional approved biological covers, skin substitutes and topical medications please refer to *Appendix 5- Wound management; approved covers*).

   b. Use of negative pressure wound therapy (e.g. VAC) on top of the graft or primary biological cover dressing including autograft for 2-3 days can ensure drainage, contact and adherence to the wound bed. The use of negative pressure wound therapy (NPWT) for a few days in order to stabilize and promote skin graft adherence and take is quite commonly practiced as part of the routine engraftment procedure. It is applied at the end of engraftment over the applied meshed or perforated autograft for 2-4 days and kept until first dressing change [105].

   c. The cover and dressings should be assessed for signs of contamination, infection or sloughing. Contamination or infection may be treated with topical antibacterial soaking or irrigation for a few days as well as systemic antibiotics, according to the investigator's medical judgment. Sloughing tissue should be removed by wiping away the non-adherent parts. The exposed areas should be protected from desiccation and treated either as exposed dermis, aiming at epithelialization, or as a full thickness defect

which should be autografted or closed by permanents skin graft or substitutes. Dry hydrogels, gelfoams, hydrofibres, alginates, Biobrane etc. applied on a viable raw bed can desiccate the wound surface and if used, should be soaked periodically to provide moisture to the healing wound.

d. Re-assessment of wound should be performed at least on a weekly basis (as per Section 8.6)[83]:
    i. If the non healing area is a full thickness wound, the area should be autografted or closed permanently as soon as possible.
    ii. Areas with preserved dermis should be treated with approved topical medications or covered with approved biological covers in order to promote epithelialization and control granulation tissue that may start to develop after 2 weeks. Granulation tissue that appears on the healing surfaces should be treated immediately. Granulation tissue forms-in and results from the proliferative phase of the inflammatory response [78]. Topical corticosteroids' primary activity is anti-inflammatory and/or immunosuppressive effects [84-85] (e.g. Synalar which is indicated for the relief of the inflammatory & pruritic manifestations & Diprolene Ointment which is a super-high potency corticosteroid indicated as well for the relief of the inflammatory and pruritic manifestations). Granulation tissue of wound's bed, being an inflammatory process, is often modulated by topical steroid preparations for short periods (2-3 days), alternating with other topical medications. The topical steroids facilitate control of granulation tissue and the cover that prevents desiccation allows epithelialization[23].
    iii. If the non-epithelialized areas after > 2 weeks of healing are small (< ½ %TBSA or < 50 cm^2) with many interposing epithelializing islands, topical medication can be applied. Medications such as Biafin or antibiotic ointments, creams or hydrocolloid dressings that will prevent desiccation and will provide a good environment for the epithelialization process are recommended. Special attention should be paid upon removal of these medicated dressings so that the epithelializing surface will not be disrupted.

Exposed dermis can be autografted early after eschar removal in order to expedite wound closure but in many cases the dermis may epithelialize under the graft, dislodging it after 2-3 weeks or the graft may take and remain as an over-grafting.

Conservative, non-surgical treatment by the antimicrobial Silversulfadiazine (SSD) dressing followed by daily dressing change and bathing is designed to slowly slough and debride the eschar-covered wound by autolysis without excessive infection. **SSD should not be used on a clean bed as it decreases epithelialization**. The pseudoeschar caused by SSD can sometimes serve as a protective layer to the areas of epithelialization process but it is often misdiagnosed as newly formed eschar. This pseudoeschar **should not be surgically excised (thus sacrificing a viable dermal bed)** and conservative treatment should be continued until the entire

[23] Steroid preparations are indicated and currently used as solutions, creams, ointments and also systemically for shorter and longer periods in most inflammatory process.

pseudoeschar spontaneously sloughs off. After sloughing of the pseudoeschar the resulting surface should be assessed and full thickness non healing areas permanently closed (grafted).

NOTE: **Tangential excision of pseudoeschar will most probably result in a full thickness defect even if it originally covered preserved dermis with epithelialization potential.**

A detailed overview of post eschar removal wound closure is presented in *Appendix 4- Post-Eschar removal Wound Management*.

8.5 WOUND MANAGEMENT PROCEDURES – (REFER TO ANY PROCEDURE POST COMPLETE ESCHAR REMOVAL);

The following relevant details should be recorded:

1. Target wounds and donor sites coverage type (all primary dressing used post-eschar removal and until complete wound closure) and the % area applied with, will be documented
2. Any additional tangential/minor/Versajet/dermabrasion excision(s), which is done as a preparation for cover/autograft should be recorded,
3. The relevant TW should be photographed within 1 hour before and immediately at the end of the excision procedure and % of treated wound excised (out of the original debrided wound) should be clinically assessed,
4. Central lab – hematology, will be taken within 1 hr prior to start of any excision/ autograft and four hours after completion of the procedure,
5. For any autograft performed, the TW should be photographed before (within 1 hour) and after any procedure. The % of treated wound autografted (out of the original wound size) will be clinically assessed,
6. Any autograft performed, if not immediately (within 6 hrs) following the excision- central lab - hematology, should be performed within 1 hr prior to start of the procedure (including any preparations required) and four hours after completion of the procedure,
7. Parameters describing the surgical procedures will be documented in the eCRFs in addition to starting and completion time of the surgical procedure, recovery time, analgesia/anesthesia prescribed during treatment, level of sedation/analgesia prescribed (as described in Appendix 2- Pain Management Procedures), manpower (number of surgeons, anesthesiologists, registered nurses, practical nurses, and unskilled assistances) who participated during the above period of time and disposables used (e.g. number of dressings, blades, etc),
8. All types of blood product transfusions (e.g. units of whole blood or packed cells and volume to be recorded) prescribed to a subject with a record of date and time. The physician will be asked to comment on whether the blood transfused is related to any specific procedure performed (e.g. surgical eschar removal, preparation for an autograft, etc), The site (anatomical area), and size of the donor site will be photographed and clinically assessed (by rule of palm),
9. Background pain should be treated according to the individual needs in order to ensure pain free care,

10. Adverse events and concomitant medication will be recorded.

8.6 FOLLOW-UP ASSESSMENTS: WEEKLY (7 ± 2 DAYS) POST-START OF ESCHAR REMOVAL

The following assessments will be performed and recorded 7 ± 2 days post-start of eschar removal and, thereafter, on a weekly basis until all TWs and donor sites are closed (based on start of eschar removal):

1. The target wound and donor sites should be photographed and documented (as describe in *Appendix 3- Photographic wound documentation*),
2. Clinical assessment of % of target wound area epithelialized and/or closed by graft (see also wound closure assessment described in Section 6.3), and assessment of % donor site epithelialized, will be assessed by a second blinded assessor, blinded to all treatment arms,
3. Assessment of the percent 'take' of any graft should be recorded 2-7 days post-grafting procedure. A photograph should be taken at the time of assessment (as describe in *Appendix 3- Photographic wound documentation*), will be assessed by a second blinded assessor, blinded to all treatment arms,
4. 12-lead ECG (in triplicate) will be performed, if feasible, 7 ± 2 days from initial application,
5. Development of granulation tissue should be monitored. In case of initial appearance, treatment using approved ointment, creams or soaking during the epithelialization stages of wound healing should be applied. Details should be recorded in the eCRFs,
6. Immunogenicity blood samples (3ml) should be taken in NexoBrid patients as per the timelines described in Section 11.11, per *Appendix 10- Procedures for specific blood tests*.
7. Weekly follow-up assessments **will be continued** until complete wound closure[24] for all of a subject's treated target wounds[25] and donor sites (Confirmation of wound closure for each target wound will be performed after 2 weeks, please refer to Section 8.8).

8.7 HOSPITAL DISCHARGE

The following procedures and assessments will be performed and recorded before a patient's discharge from the hospital (within 24 hours prior to discharge):

1. Physical examination and weight,
2. Date of hospital discharge.

Note: Weekly follow-up assessments will be continued post hospital discharge until complete wound closure as per Section 8.6 above.

8.8 FOLLOW-UP ASSESSMENTS: WOUND CLOSURE CONFIRMATION 2 WEEKS POST WOUND CLOSURE

[24] >95% area epithelialized or closed by graft

[25] Complete wound closure- defined as skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits, 2 weeks apart.

A confirmation of wound closure will be performed by a follow-up visit at day 14 (± 2 days) from wound closure, for each subject's target wounds. The following assessments will be performed:

1. The wounds should be labeled and photographed (as describe in *Appendix 3- Photographic wound documentation*),
2. Assessment of % of wound area epithelialized and/or closed by graft,
3. Maintenance of complete wound closure will be confirmed (Yes or No),
4. Patient diary will be provided to the patient to record any change in concomitant medications and or any adverse event, during the following monthly FU period.

8.9 FOLLOW-UP LONG TERM ASSESSMENTS[26]

The following assessments will be performed at 1 month (± 7 days), 3, 6, 12, 18 and 24 months (± 2 weeks for all time points) from the wound closure confirmatory visit:

1. The wounds should be labeled and photographed,
2. Maintenance of complete wound closure (YES or NO) - only on month 1 and 3,
3. Record of scar-modulating procedures and scar reconstructive procedures, if performed. This should include dates of initiation and termination of scar-modulating procedures including any procedure performed (compression therapy, silicone sheeting, steroid injections, etc.),
4. Any Adverse Events and concomitant medications prescribed should be captured in the eCRFs,
5. Quality of Life evaluation will be performed using Burn Specific Health Scale (BSHS-B) and EQ5D as described in *Appendix 12- Cosmesis, Function and Quality of Life Questionnaire,*
6. Functionality evaluation will be performed using self completed questionnaires (as described in *Appendix 12- Cosmesis, Function and Quality of Life Questionnaire*):
    a. The "Lower Extremity Functional Scale" test for burns in the lower extremities,
    b. The "QuickDASH" outcome measure for burns in the upper extremities.
7. Immunogenicity blood samples (3ml) should be taken in NexoBrid patients as per the timelines described in Section 11.11, per Appendix 10- Procedures for specific blood tests,
8. The below evaluations will be performed by a second blinded assessor (who is blinded to all treatment arms):
    a. Cosmesis assessment using POSAS and MVSS for all TWs and donor sites, as described in Appendix 12- Cosmesis, Function and Quality of Life Questionnaire,

[26] Assessments can be done at the patient's home, by the second blinded assessor, if pre approved by the local ethic committee/IRB.

b. Range of Motion measurements of injured joints, if relevant: knee, ankle, shoulder, elbow, wrist and hand. Measurements will be performed on the patient's own non injured joints as well for comparison to burned joints (as described in Appendix 14- Range of Motion).

8.10 FOLLOW-UP ASSESSMENTS: RE-ADMISSION TO HOSPITAL OR DAY CARE

Persistent, small open wounds or reopening of new small areas are expected and can normally continue for months necessitating treatment as in- or outpatients as a part of a normal wound care [1]. A patient may be re-admitted to the hospital for continuation and follow-up of planned treatments or for unexpected events. The following procedures and assessments will be conducted and recorded:

1. Date and reason for the re-admission should be recorded and any relevant TWs planned to be treated,
2. Surgical procedures done during re admission will be documented and assessments will be performed per section 8.5.
3. Adverse events will be recorded throughout the hospital stay,
4. Concomitant medication will be recorded throughout the hospital stay,
5. Date of re hospital discharge will be recorded.

9. TREATMENT FLOW CHART – STUDY SCHEDULE

Table 3- Treatment flow chart- all arms

#- 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[1]- 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[2]	X								
ConMeds & AEs	X	X	X	X	X		X	X	X
Physical examination[3]	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[4]	X	Post S	Pre & Post		Pre & Post	X	X	X
Clinical assessment of the burn[5]	X	X	Post S	Post		Post	X	X	
Local lab - HbA1C, WBC, βhCG	X								

[1] Wound Management will include any, post complete eschar removal additional excisions, Autografts, and donor site data if relevant..
[2] 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
[3] Physical examination will include height and weight. Height will be taken only once, at screening.
[4] Photos should be taken before and after cleansing
[5] Clinical assessments will include % TBSA per depth, % eschar removed, % excised, % autografted, % wound epithelialization (TW and donor site, if relevant) as relevant.

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[1]- surgical procedures assessment	HD or premature discontinuation visit	Weekly FU*	Long Term FU (1, 3, 6,12,18 & 24 months)*
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[1]	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, 48 h			week 1	
PK (for a subset of 20 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[2]	Long term FU: 6, 24
Local lab PTT & INR		X	4 h post R - first application only	4 h post - first treatment only	24,48 h				

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

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

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[1]- surgical procedures assessment	HD or premature discontinuation visit	Weekly FU*	Long Term FU (1, 3, 6,12,18 & 24 months)*
Circumferential extremity wounds monitoring[1]	X	X	, during andpost 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			
Wound closure confirmation[2]								X	Long term FU: 1, 3
Cosmesis, Function and Quality Of Life[3]									X
Scar-modulating procedures and scar reconstructive						#			#
Blood transfusion (units & volume)	#	#	#	#	#	#	#	#	#
Histological biopsy	#	#	#	#	#		#	#	#

[1] 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

[2] Wound closure should be confirmed 2 weeks after the initial wound closure assessment for each TW.

[3] Cosmesis will be evaluated in donor site scars as well

Table 4- NexoBrid/ Gel vehicle Eschar Removal

	Preparation for Treatment	Treatment
At least 2 hours soaking	X	
Preventive analgesia medication (pain management) will be mandated as for an extensive dressing change as routinely practiced; it should be initiated at least 15 minutes prior to dressing change or application in order to ensure pain free care	X	
Removal of any blister-keratin remnants[1]	X	
Surrounding of the TW with a paraffin ointment adhesive barrier[2]	X	
Sprinkle sterile isotonic (0.9%) sodium chloride solution should be sprinkled on the burn wound[3]	X	
Application of the product, as per the randomization, should follow the instruction for use described in Section 6.4 of the protocol		X
Occlusive film dressing should be applied to cover the wound		X
The dressed wound should be covered with a loose, thick fluffy dressing, held in place with a bandage		X
The dressing should remain in place for 4 hours		X
Starting and completion time of the procedure will be recorded		X
Manpower (specify the number of surgeons, anesthesiologists, RN, nurses and unskilled assistances) who participated during the procedure will be recorded in the eCRFs		X
Any disposable used (number of dressings, blades, etc) will be recorded in the eCRFs		X
2 hrs soaking[4]		X

[1] If there are still blisters/keratin remains, clean the wound thoroughly and remove the superficial keratin layer or blisters from the wound area, as the keratin will isolate the eschar from direct contact with the topical agent

[2] Vaseline ointment should be applied a few centimeters outside of the treatment area (using an adhesive-barrier dispenser). The paraffin layer must not come into contact with the area to be treated to avoid covering the eschar, thus isolating the eschar from direct contact with the topical agent

[3] The wound must be kept moist during the application procedure

[4] Antibacterial solution, e.g. 3-5% Sulfamylon, 0.05-0.5% Chlorhexidine, Dakin's solution or hypertonic 5-10 % saline solution

10. DRUG STORAGE AND DRUG ACCOUNTABILITY

The bottles of NexoBrid powder and the Gel Vehicle must be kept refrigerated (2-8ºC).

10.1 ACCOUNTABILITY AND COMPLIANCE

The principal investigator is responsible for maintaining accurate records of the receipt and dispensing of all investigational materials. The investigator may dispense investigational drug only to patients enrolled in the study. All dispensing will be conducted from the site listed in the Statement of Investigator.

MediWound Ltd. will provide drug accountability forms to assist the investigator in maintaining current and accurate inventory records covering receipt, dispensing, and the return of investigational drug supplies. When a shipment is received during site's initiation or during the study, the investigator or pharmacist will verify the quantities received, complete an Inventory log form and return the acknowledgment to the study monitor or designee. The investigational drug accountability record includes the identification of the patient to whom the drug is dispensed, the quantity and the date of dispensing and any returned or unused drug. This record is in addition to any drug accountability information recorded on the Case Report Form. These records will be readily available for inspection by a monitor or MediWound Ltd. audits and are open to any other regulatory authority inspection at any time.

When the supplies are returned or destroyed, the investigator or pharmacist signs the investigational drug return or destruction log to verify that all unused or partially used supplies were accounted for and confirms that no study supplies remain in the investigator's possession. One copy of all inventory records and the return statement are retained by the investigator for the study files.

At study termination, unless otherwise specified, all unused drug supplies including partially used containers and the study drug accountability log should be returned to: MediWound, Ltd., Clinical Research Department, 42 Hayarkon Street, North Industrial Area, Yavne, 8122745, Israel.

10.2 PREVIOUS AND ON-GOING MEDICATIONS/THERAPIES.

10.2.1 Disallowed Previous and On-Going Medications/Therapies

Pre-enrollment dressings with flammacerium or silver nitrate ($AgNO_3$) are disallowed.

10.2.2 Allowed Previous and On-Going Medications/Therapies

All other systemic medications except in the above section are allowed.

11. ASSESSMENT METHODS

Efficacy Assessment Methods will include:

11.1 PRIMARY END POINT

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 Section 1.1 for Target Wound definition):

Incidence of complete eschar removal: Demonstrate superiority over Gel Vehicle for eschar removal as measured by incidence of complete eschar removal assessed by the blinded assessor at the end of the topical agent soaking period (6 hours after start of the 1st topical application). Or, in cases where 2 applications are performed, complete ER is assessed 6 hours after start of the 2nd treatment

Reasons for Incidence of complete Eschar Removal as Primary Endpoint

Following the goal to seek an indication for NexoBrid that focuses on non surgical eschar removal, the evaluation focus is set on this measure as a primary endpoint of the study. This primary endpoint will assess whether non-surgical eschar removal by NexoBrid allows effective removal of the offending eschar compared with its comparator. Complete eschar removal assessment will be captured in the eCRFs for each TW and has to be marked as complete for all TWs to be considered as complete per patient.

11.2 SECONDARY ENDPOINTS

The following secondary endpoints will be evaluated in this study and compared between NexoBrid and SOC. Comparisons between NexoBrid and Gel vehicle will be carried out in the same manner as between NexoBrid and SOC, but will be regarded as supportive analyses.

11.2.1 Reduction in surgical needs

Demonstrate superiority of NexoBrid over SOC in reduction of surgical need for excisional eschar removal as measured by an analysis of incidence of surgical eschar removal (tangential/ minor/ avulsion/ Versajet and/or dermabrasion excision).

11.2.2 Earlier eschar removal

Demonstrate superiority of NexoBrid over SOC with regard to the time when complete eschar removal has been achieved. For definition of complete eschar removal see primary endpoint.

11.2.3 Blood loss

Demonstrate superiority of NexoBrid over SOC with regard to the blood loss incurred during the eschar removal procedures. 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 [86]:

ABL= (EBV * (Hb_{before}- Hb_{after}))/((Hb_{before}+ Hb_{after})/2))+ (500*T_u)

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

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

T_u= sum of autologous whole blood, packed red blood and cell saver units 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 the debridement procedure and 4 hrs later i.e. the before-after periods that are spanned by these HB measurements.

Reasons for Incidence of Surgical Excisions as Secondary Endpoint

A further aspect of the clinically meaningful attributes of NexoBrid's non surgical eschar removal is the ability to reduce surgical needs, as NexoBrid allows removal of eschar in wounds that otherwise would have to undergo surgical excision. Therefore, this secondary endpoint would provide information on treatment failures that required follow-on surgical management and will further provide supportive evidence of efficacy and demonstration of clinical benefit over SOC.

Reasons for Earlier Eschar Removal as Secondary Endpoint

This secondary endpoint will assess whether nonsurgical and effective eschar removal by NexoBrid allows for the removal of the offending eschar earlier upon admission, enabling earlier visualization of the wound bed for assessment of the burn wound's depth as well as preservation of viable tissues [87-88]. The depth determination is important for the planning and execution of the post-eschar removal stage of wound closure (i.e., grafting or spontaneous epithelialization). The dynamic of burn wound progression of the eschar-covered burn wound is the first delaying factor in initiating SOC surgical eschar removal. The non sacrifice of healthy dermis by SOC excisional surgery and the overall surgical implications to the patient are the other delaying factors [1]. Therefore, it is hypothesized that NexoBrid will allow eschar removal earlier (post randomization), effectively and in the first few days from injury without the need to first determine the burn depth (as is done in the initial decision-making phase of surgical and non surgical SOC).

Reasons for Blood Loss as Secondary Endpoint

This secondary endpoint will further demonstrate NexoBrid's clinical benefit by showing a decrease of total blood loss post NexoBrid eschar removal compared with the total blood loss in the SOC eschar removal procedures. Reduction in blood loss is considered as an outcome measure that could further demonstrate NexoBrid clinical benefit. Surgical blood loss is attributed mainly to the excisional phase and is directly proportional to the surface excised measured in ml blood loss/cm^2 and may reach 0.8 ml/cm^2 [89-90]. Local inflammation and anti-aggregation treatments may involve more bleeding and need for transfusion. Many publications endorse different means to reduce debridement related bleeding (tourniquet,

subcutaneous epinephrine infiltration of excised burn and donor site etc.). The calculation for the actual blood loss is being used in other invasive procedures as an accurate calculation that reflects the actual blood loss in a patient as this takes into account both the estimated blood loss, changes in Hb levels during the relevant procedure and total blood units transfused to a patient.

11.3 SAFETY ENDPOINTS

The following group of safety endpoints will be evaluated in this study and compared between NexoBrid and SOC. These endpoints are used to ensure that NexoBrid does not cause an unacceptable level of harm. Comparisons between NexoBrid and SOC will be carried out using pre-specified clinically meaningful margins that will assist in the benefit versus risk assessment of the product. These safety measurements are not powered to yield statistical significant non inferior results. Thus their assessment will be used for supportive analyses and sources of additional information about NexoBrid and SOC.

11.3.1 Time to complete wound closure

Time to reach complete wound closure assessed in days, starting from randomization date. Demonstrate that NexoBrid does not cause any clinically meaningful harm to the patients with respect to the wound healing process by comparing NexoBrid mean time to wound closure with SOC mean time to wound closure, where a difference of not more than 7days difference will be interpreted as clinically not relevant.

Reason for this endpoint and the definition of clinically meaningful harm:

Time to reach complete wound closure is taken as a safety criterion because wound closure is a relevant parameter for patients' quality of life and delay in wound closure may increase the risk of local complications. However, it can be assumed that the selective, non surgical eschar removal will probably increase the potential for spontaneous healing as physicians tend to postpone autografting in an effort to reduce autografting by exploiting the wound's preserved dermal potential for spontaneous epithelialization. Such spontaneous epithelialization is an important benefit for the patient (less autografting and less donor sites). This assumption is based on the results of the confirmatory phase III RCT (MW2004-11-02) in which a slight prolongation in time to wound closure (7 days) compared with the standard of care arm was observed.

A difference of **7 days** to wound closure has no clinical significance as patient mobilization starts long before wound closure, and the return to pre-injury lifestyle, following rehabilitation and convalescence, takes weeks, if not months, long after the wounds are closed. This delay does not affect patients' discharged from the hospital as was further demonstrated in the pivotal clinical study. The concern that the few days' delay will cause complications (mainly infections) proved to be non-based as the few AEs happened long before the delay and was similar to the SOC. The importance of delayed wound closure in burn treatment relates mainly to preventing permanent deforming scars, which affect both cosmesis and function.

Data collected in MediWound's clinical study (MW2012-01-02) show that the few days delay did not cause worse scars, and they support the suggested NI margin of 7 days.

A supportive formal derivation of this margin may be based on the FDA proposal of using two values to derive a NI (non-inferiority) margin [91]:

M1 denotes the effect of the standard against placebo;

M2 denotes the largest clinically acceptable difference (towards non-effectiveness) of the test substance.

M2 is usually chosen as a percentage of M1, usually 50%. If no realistic placebo result exists a conservative choice of keeping 80% of the M1 effect may be preferred (M2=0.2*M1). This is the case planning a trial with large thermal burns as a real placebo result is not available and even not ethical, i.e. a placebo comparison measuring the effect of SOC on the wound closure scale is missing.

The following calculation is based on the data from the pivotal study, MW2004-11-02. For a first derivation of a NI margin we disregard possible correlations between the different wounds and treat the wound closure times as independent.

Given that SOC is an active comparator one expects at least 95% of the wounds treated with SOC to have a smaller time to wound closure than a (virtual) placebo mean time to wound closure. From the empirical distribution of the SOC wound closure times of the data set we derive the 95% quantile as 64 days. Taken this as the worst case scenario for a mean placebo wound closure time we obtain a SOC treatment effect of M1=64-27=37 days (SOC mean was 27 days).

M2 may then be taken as 0.2*M1=7.4 days.

Based on the above supportive clinical information, the MAH finds that a NI margin of 7 days is statistically and clinically justified. Therefore, a slight prolongation in mean time to wound closure might occur (up to 7 days)

Therefore, a slight prolongation in mean time to wound closure might occur (up to 7 days) but will not express a clinically relevant imbalance of the risk benefit assessment as patient mobilization starts far before the wounds are closed and the return to pre-injury lifestyle, following rehabilitation and convalescence, takes weeks, if not months, long after the wounds are closed. Thus any difference in means of up to 7 days may be taken as not representing clinically meaningful harm for this safety endpoint.

11.3.2 Cosmesis and function at 12 months from wound closure

A measure of cosmesis, using MVSS, will be used to demonstrate - that treatment with NexoBrid does not cause any clinically meaningful deleterious effect on scars quality as compared to scars quality of burns treated with SOC, measured at 12 months from wound closure date, by a blinded assessor. Clinically meaningful deleterious effect will be defined as a NexoBrid group mean MVSS score higher by 1.9 or more units than the SOC group mean MVSS score.

11.3.3 Cosmesis and function at 24 months from wound closure

A measure of cosmesis, using MVSS, will be used to demonstrate that treatment with NexoBrid does not cause any clinically meaningful deleterious effect on scars quality as compared to scars quality of burns treated with SOC, measured at 24 months from wound

closure date, by a blinded assessor. Clinically meaningful deleterious effect will be defined as a NexoBrid group mean MVSS score higher by 1.9 or more units than the SOC group mean MVSS score.

Reasons for the cosmesis endpoint and the choice of the clinically meaningful difference:

We propose that a clinical equivalence value of 1.9 in the MVSS scale signify NexoBrid non-inferiority in scar quality to the SOC. This clinically meaningful difference is in agreement with the non-inferiority (NI) margins calculated using the MVSS data in study MW2004-11-02 (Sponsor previous long term follow up of patients treated in the MW2004-11-02).

The NI margin was calculated as follows:

Two values are needed to derive a NI margin [91]:

M1 the effect of the standard against placebo

M2 the largest clinically acceptable difference (towards non-effectiveness) of the test substance

M2 is usually chosen as a percentage of M1, usually 50%. If no realistic placebo result exists a conservative choice of keeping 80% of the M1 effect may be preferred (M2=0.2*M1). This is the case when planning a trial with large thermal burns as a real placebo result is not available and even not ethical to measure the effect of SOC on the MVSS scale.

The following calculation is based on the data observed during the clinical study (study MW2012-01-02). The margin may be calculated taking the theoretical maximum score of 18 instead of the result of a (virtual) placebo arm. Consequently the SOC treatment effect is 18-3.83=14.17, with 3.83 being the MVSS mean observed in the SOC group of the data (per patient analysis). Given the standard deviation of 2.27 from the MVSS data a 95% confidence interval for the SOC treatment effect is given by 14.17±0.75. The lower bound is 14.17-0.75=13.42=M1.

M2 may then be taken as 0.2*M1=2.7.

Calculating the MVSS NI margin based on FDA guideline results in a NI of 2.7.

Survey of burn experts as to the clinically significant difference in MVSS scores was performed and a difference of less than 2 would was advised not be considered as clinically meaningful.

Taken into consideration the statistical calculated margin of 2.7 and the expert opinions, NI margin which is slightly below 2 (1.9) is justified.

Additional Safety Outcome Measures will include:

11.3.4 General parameters

Systemic adverse events, vital signs, pain assessment (using VAS and as reported as AEs), laboratory tests, units (and volume) of blood transfusion given during hospitalization, Immunogenicity evaluation for NexoBrid patients, Pyrexia and Hypothermia, Systemic infections, Incidence of increased interstitial/compartment syndrome (as described in Section 1.1) and QT prolongation. 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).

Additional long term functionality evaluation of the extremities using the 'Lower Extremity Functional Scale', 'QuickDASH' questionnaires and 'Range Of Motion' measurements,

Long term Quality of Life using EQ5D and Burn Specific Health Scale- Brief (BSHS-B)

11.3.5 Local parameters

Local adverse events defined by treated physician or designee; graft loss, wound related infections, etc.

11.4 EXPLORATORY ENDPOINTS

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. POSAS will be used to further assess cosmesis and function,
3. Incidence of surgical Escharotomy procedures on circumferential extremities target wounds,
4. Incidence of reduction in interstitial/compartment pressure in circumferential extremity wounds (measured immediately following eschar removal),
5. Reduction in surgical need as measured by analysis of incidence of surgically harvested donor sites wounds,
6. Reduction in surgical need as measured by analysis of % area of surgically harvested donor site wounds,
7. Blood loss following eschar removal procedures using changes in Hematocrit following eschar removal procedures,
8. Cosmesis MVSS and POSAS will be used to assess the quality of the donor sites scars,
9. PK evaluation for a subset of NexoBrid patients (as described in section11.21)
10. Autograft related parameters.
    I. Efficacy and safety analyses for early and late grafted wounds
    II. Total number of target wound grafting procedures
    III. Incidence of repeated/additional grafting procedures
    IV. Area of repeat grafting
11. Duration of hospitalization

11.5 ADDITIONAL ANALYSIS

The following additional analyses will be addressed as supportive efficacy analyses:

1. Primary & secondary endpoints will be analyzed at a per wound level
2. Secondary endpoints will be analyzed vs. the Gel Vehicle
3. Time to reach 100% wound closure assessed in days, starting from randomization date
4. Clinical assessment of complete eschar removal vs. Centralized complete eschar removal from photos.

11.6 SUBGROUP ANALYSES

Descriptive statistics will be determined for all subgroup analyses.

1. 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,
2. Patients will be classified as having <25% or >=25% SPT area as a percentage of the area of all TWs, and descriptive statistics will be provided for each subgroup, including data on the primary, secondary and safety endpoints,
3. Time to wound closure and 12m and 24m MVSS will be analyzed per TWs' depth and % TBSA of the TWs,
4. An analysis restricted to wounds that are entirely FT will be provided.
5. A subgroup analysis of the primary and secondary endpoints will be performed per % TBSA. The subgroups examined will be ≤15% and >15%.

11.7 SAFETY LABORATORY EVALUATIONS

The following tests described below will be performed. Any abnormal findings must be assessed for clinical significance – Abnormal Non Clinically Significant (NCS) or Abnormal Clinically Significant (CS):

Serum chemistry, hematology and urinalysis testing will be performed at a central laboratory during the topical agent eschar removal phase. Any additional procedures require hematology tests, as described in the protocol, will be performed using Central lab. Pregnancy testing, HbA_{1c}, leukocyte count (at Screening, in addition to central lab hematology), wound cultures, blood cultures and biopsies will be performed locally on-site.

The following tests will be 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.

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 hrs 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*) [93].

Clinical Burn Wound Infection will be defined when an Invasive Burn Wound Infection is diagnosed by quantitative bacterial count of $>10^5$ 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

11.8 EAR AND BURN INDUCED COMPARTMENT SYNDROME MONITORING AND DIAGNOSIS

Burn Induced Compartment Syndrome (BICS) is an acute progressive condition that starts a few hours post injury and develops during the acute, edematous stage of the first 2-3 days (after which edema and increased pressure subside) in deep circumferential burns. The edema and pressure reach their maximum within few days post injury and start to slowly decrease thereafter. Soft tissue can withstand temporary pressure increase for 6-8 hours without permanent damage, allowing 4-6 hours for decision-making regarding escharotomy.

Monitoring:

Monitoring will be performed for all circumferential deep burns using the 5 "P"s (Pain, Paralysis, Pulselessness, Pallor, Paraesthesia and , direct pressure measurement and SpO2 .

Clinical monitoring and diagnosis step include:

- Observation of the extremity for swelling and color
- Asking the patient about ability to move his fingers and hand, pain on passive finger extension and other skin sensations such as paresthesia or numbness (anesthesia)
- Pressing the skin to diagnose blanching and tension
- Sensitivity assessment (from superficial to deep sensation, ending with passive extension of the fingers, which is the specific test for muscle ischemia).

The above sequence of tests should start by:

(i) asking the patient to move his/her fingers

(ii) skin sensation

(iii) skin tension and appearance (pallor)

(iv) assessing pain at passive extension

(v) palpation for pulse.

The direct interstitial/compartment pressure measurement will be performed using one of the following:

1. A 19 gauge IV needle or catheter connected to a pressure transducer (e.g. CVP set, direct blood pressure monitors, Striker system). The needle is inserted:
   - Into the interstitial subcutaneous tissue at the anterior and posterior compartment region of upper or lower extremities, in the case of circular burn extending up to the elbow or knee.

Before reading the pressure, the system should be flushed with 0.2-0.5 cc. (depends of the length of the connecting tube) of normal saline.

2. Intra-Compartmental Pressure monitor (ICP monitor) by Stryker® or similar system.

These measurements, similar in their pain and discomfort to IV treatment, are well tolerated by patients and in DPT/FT

- Ultrasound Doppler may be used for the detection of pulsating arteries but even with distal digital artery pulsation damage may still happen. Laser Doppler blood flow is reported to be the least dependable.

Screening - Prior to randomization:

Patients with Extremities at Risk (EAR) should be identified at screening according to the definition below and will be excluded from the study.

Extremities at Risk are circumferential DPT and/or FT burns with either one of the following:

1. Any of the 5 "P"s (Pain on finger extension, Paralysis, Pulselessness, Pallor, Paraesthesia),

2. Direct pressure measurement >25 mmHg, measured in 2 consecutive measurements within 30 minutes under the constricting eschar,

3. SpO_2 <95% and a persisting difference of 6% between a healthy symmetrical extremity and the injured one [10-11],

4. Pulse not detected by US Doppler.

***In order to include a patient with circumferential wound to the study - Points 1, 2 and 3 above must be tested and ruled out.**

1 h pre eschar removal procedure:

Circumferential wounds should be monitored to detect high interstitial/ compartment pressure and diagnosed BICS. BICS should be treated as per SOC.

Clinical assessments, SpO_2 and the direct interstitial/compartment pressure measurement will be performed.

During the 4 hours eschar removal procedure (apply to topical arms only (1st and 2nd if any):

All circumferential wounds should be monitored 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.

If BICS was diagnosed by one of the following, the treatment should be stopped and escharotomy will be considered:

- Increasing 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
- Deterioration of any of the 5 "P" signs.

Since in such cases eschar removal procedure will not be completed as planned, such patients (if any) will still be part of ITT 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.

Post eschar removal procedure:

Clinical assessments, SpO_2 and the direct interstitial/compartment pressure measurement will be performed in all circumferential wounds.

11.9 INFECTION

All definitions of infection, wound infection, invasive burn wound infection etc. will follow the American Burn Association Consensus Conference to Define Sepsis and Infection in Burns [94] as presented in chapter 12, "Treatment of infection in burns" in the Total Burn Care 4th edition, edited by D. Herndon pages 137-156 that included also input from The Society of Critical Care Medicine (SCCM) and the American College of Chest Physicians (ACCP). Nevertheless, it should be remembered that in spite of the enormous effort invested for so long by so many, often even the simple differential diagnosis between burn wound erithema and burn wound infection or even the etiology of a sepsis is difficult. In order to have a pure as possible study that will assess exclusively the burn related phenomena, the principle is to enroll healthy individuals with fresh thermal burn wounds without any confounding co morbidities or trauma.

- Sepsis [94]

Sepsis is a change in the burn patient that *triggers* the concern for infection. It is a presumptive diagnosis where antibiotics are usually started and a search for a cause of infection should be initiated. While clinical interpretation is required, the diagnosis **needs** to be tied to the discovery of a specific infection source (defined below in section C).

The trigger includes at least three of the following:

I. Temperature >39.0° or <36.5°C
II. Progressive tachycardia
    - Adults >110 bpm
III. Progressive tachypnea
    - Adults >25 bpm not ventilated, or minute ventilation >12 L/min ventilated

IV. Thrombocytopenia (will not apply until 3 days after initial resuscitation)
   - Adults <100,000/mcl

V. Hyperglycemia (in the absence of pre-existing diabetes mellitus)
   - Untreated plasma glucose >200 mg/dl or equivalent mM/L
   - Insulin resistance—examples include
     - >7 units of insulin/hr intravenous drip (adults)
     - Significant resistance to insulin (>25% increase in insulin requirements over 24 hours)

VI. Inability to continue enteral feedings >24 hours
   - Abdominal distension
   - Enteral feeding intolerance (residual >150 ml/hr in children or two times feeding rate in adults)
   - Uncontrollable diarrhea (>2500 ml/d for adults or >400 ml/d in children)

In addition, it is *required* that a documented infection is identified:

I. Culture positive infection, or
II. Pathologic tissue source identified, or
III. Clinical response to antimicrobials

A. Continuous fever > 38.5^{0}C present during the week before sustaining burn injury.

B. The below are assessed as present on physical examination or per history during the week before injury according to the described signs [89]

- Rhinitis including mucopurulent nasal discharge
- Sinusitis including cough, headache, stufinness, purulent nasal discharge
- Stomatitis including localized pain in mouth, difficulty swallowing
- Pharyngitis including sore throat, pharyngeal erythema/exudate, neck lymphadenopathy
- Pharyngeal abscess
- Otitis including ear ache, signs per otoscopy
- **Infections of the Lower Respiratory Tract** [95]

Pneumonia including cough, fever, bronchial breath sounds, crackles, radiographic signs

- **Infections of the Heart [95]**

Endocarditis

- **Infections of the Skin and Soft Tissues** (other than in the burns) [95]

Folliculitis

Impetigo

Varicella-Zoster Virus infection

Erysipelas

Cellulitis

Necrotizing Fasciitis

- **Intra-Abdominal Abscess and Peritonitis [95]**

Infectous Gastroenteritis

- **Bone and Joint Infections [95]**

Septic Arthritis including joint pain, swelling, erythema, limitation of movement

Osteomyelitis including pain, tenderness, radiographic evidence

- **Urinary Tract Infections [95]**

Urethritis including pain and burning sensation of the urethra during urination.

Urinary Tract Infection including pain, burning sensations, abdominal pain, positive urine culture

- **Burn Wound Infection – Heavily Contaminated Burns [94]**
    - Invasive Infection: Presence of pathogens in a burn wound at concentrations sufficient in conjunction with depth, surface area involved and age of patient to cause suppurative, purulant separation of eschar or graft loss, invasion of adjacent unburned tissue or cause the systemic response of sepsis syndrome. Pathogen present in the wound at high concentrations (frequently >105 pathogens/g tissue). Invasion or destruction of unburned skin/tissue. Invasive infection may occur with or without sepsis. Many burn wound invasive infections, however, are life threatening and need urgent treatment (usually wound excision).
    - Cellulitis: Bacteria present in the wound and/or wound eschar at high concentrations. Examination of surrounding tissue reveals advancing erythema, induration, warmth and tenderness. Sepsis must be present.
    - (Cellulitis that appears usually after few days (5-7 days) should be differentiated from Burn erythema: Redness around the wound in the first 3 days post injury and is rather benign and may not require treatment).
    - Necrotizing Infection/Fasciitis: Aggressive, invasive infection with underlying (beneath the skin) tissue necrosis.

Burns diagnosed of Invasive Infection, Cellulites and Necrotizing Infection/Fasciitis are considered heavily contaminated burns.

11.10 VITAL SIGNS

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.

Blood pressure and pulse should be recorded in a supine position after resting for 5 minutes.

All measurements and time of measurements will be recorded in the source documents.

11.11 IMMUNOGENICITY EVALUATION

Antibodies presence will be determined based on a three-tiered approach analysis of plasma samples drawn prior to start of eschar removal and at the following timelines in NexoBrid patients:

- At day 28±7 days (week 4 visit) from start of treatment,
- At day 56 ±14 days (week 8 visit) from start of treatment,
- At months 6 & 24, as part of the long term follow up visits.

Please refer to Appendix 10- Procedures for specific blood tests for further instructions.

11.12 ECG (QT EVALUATION)

12-lead ECG will be performed (if feasible) in triplicates as part of the QT prolongation evaluation at the following time-points: baseline 60 ± 5 min, 40 ± 5 min and 20 ± 5 minutes prior to start of Eschar removal process and at 30 ± 15 min, 120 ± 15 min, 4 ± 0.5 hrs, 12 ± 0.5 hrs, 24 ± 1 hrs, 48 ± 2 hrs and 1 week after start of eschar removal.

11.13 PHYSICAL EXAMINATION

A physical examination will be performed and documented by the investigator or a qualified designee. Any abnormal findings, assessed by the investigator as clinically significant, should be recorded in the relevant eCRFs modules (e.g. adverse event).

11.14 PAIN ASSESSMENT USING VISUAL ANALOGUE SCALE (PAIN SCALE RULER)

Pain experience will be recorded using standard widely used pain scale ruler (Visual Analogue Scale=VAS) on admission, (baseline pain assessment) within 24 hours prior to start of eschar removal, One-hour before and following eschar removal procedure and daily from start of eschar removal until hospital discharge.

. Please refer to *Appendix 9- Pain Measurement Scale* for instructions.

11.15 CONCOMITANT MEDICATIONS

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.

11.16 GRAFT LOSS/GRAFT TAKE

An event of graft loss is defined in study protocol as an event in which an autograft needed to be re-applied on the same area. This event, once occurred, will be recorded in the eCRFs as an AE.

Graft Take: Graft adherence and vascularisation following an eschar removal procedure.

11.17 HYPOTHERMIA AND PYREXIA [94]

Hypothermia will be defined as temperature <36.0°c, measured twice, 30 minutes apart.

Pyrexia will be defined in patients with elevated temperature >39.0°c in the first days (before the beginning of any infectious process) measured twice, 30 minutes apart. These criteria, once met, should be monitored closely. The Investigator may still report hypothermia at higher temperatures and Pyrexia at lower temperatures, if these events are considered by the Investigator to be adverse events.

The Investigator will be reminded to evaluate the need to report an AE in case the temperature is higher than 39.0°c or lower than 36.0°c.

11.18 COSMESIS AND FUNCTION ASSESSMENT

11.18.1 MVSS

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

The MVSS method is composed of the following:

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

11.18.2 POSAS

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 [111-112].

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), and 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.

11.19 QUALITY OF LIFE

Quality of Life evaluation will be performed in adults using Burn Specific Health Scale (BSHS-B) and EQ5D as describe in *Appendix 12- Cosmesis, Function and* Quality of Life Questionnaire, 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.

11.20 FUNCTION ASSESSMENTS

Functionality tests were implemented in the proposed protocol MW2010-03-02 for the assessment of specific anatomical function. This evaluation will be composed of the following:

- Self completed questionnaires:
  - The "Lower Extremity Functional Scale" test for burns in the lower extremities,
  - The "QuickDASH" outcome measure for burns in the upper extremities.
- Range of Motion measurements of injured joints, if relevant: knee, ankle, shoulder, elbow, wrist and hand. Measurements will be performed on the patient's own non injured joints as well for comparison to burned joints.

The above tests will be performed 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. ROM will be evaluated at 1, 3, 6, 12, 18 and 24 months after the wound closure confirmation visit.

11.21 PHARMACOKINETIC EVALUATION

1. Blood samples (2ml) for a pharmacokinetic profile to measure NexoBrid absorption will be taken from a subset of 32 patients treated with NexoBrid in accordance with the randomization arm, including: 16 patients with total wounds area of <=15% TBSA will be tested for PK and 16 patients with total wounds treated area of >15% TBSA will be tested for PK and will be analyzed throughout the study for each patient in an ongoing process. Blood samples will be taken accordingly-

- 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.
  - 2nd treatment: time 0, 0.5 hours ± 10 minutes, 2 and 4 hours ± 10 minutes, 12 hours ± 30 minutes, 24 hours ± 30 minutes, 48 and 72 hours ± 30 minutes after the second NexoBrid application.

Blood samples (2ml) for a pharmacokinetic profile will be taken from all subjects planned for 2 NexoBrid sessions in advance.

11.21.1 Real time data capture and Independent Safety Monitoring

The study data will be collected using an Internet-based, validated, electronic data capture system which allows for "real time" safety reviews via the Internet.

An independent study Medical Monitor, masked to the treatment arms, shall review all Adverse Events (AE's) from the entries on the eCRFs on a quarterly basis. SAEs will be reviewed and evaluated on a per case basis, following the occurrence of an SAE, as detailed in the Sponsor's SOPs. In case any concerns are raised by the medical monitor, additional convention of the DSMB could be performed (see Section 14.4).

12. SAFETY AND PHARMACOVIGILANCE

An adverse event is an undesirable or unintentional event that occurs during use of the study Drug, whether or not considered related to the Drug; this includes clinically significant changes in laboratory values and therapeutic failures. Regardless of the severity or relationship to the investigational Drug, all adverse events occurring during the study period should be recorded in a subject's CRFs. The phrase "associated with the use of the Drug" is defined as a reasonable possibility that the event may have been caused by the investigational Drug.

12.1 RELATIONSHIP TO STUDY DRUG

The following definitions to assess the relationship between an adverse event and the study Drug should be 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.

AEs assessed as possible, probably or related will be addressed as Adverse Drug Reaction (AEs related to study arm).

12.2 ADVERSE EVENT INTENSITY

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.

12.3 ADVERSE EVENT REPORTING

Signing of Inform Consent is considered to be the start date of AEs monitoring. All adverse events must be recorded in the eCRFs. When an adverse event occurs, the following information and assessments should be recorded in the adverse event section of the eCRFs:

- The signs, symptoms or diagnosis or the event,
- The date and time of onset of the event using the 24 hour clock where midnight is 00:00 and noon is 12:00,
- The adverse event severity using the criteria outlined above,
- The relationship of the event to the study Drug as outlined above,
- The description of any action taken regarding study Drug disposition,
- Any required therapy, medication, treatment or diagnostic procedure.

Follow-up of a patient should be conducted until resolution of the adverse event. The investigator is responsible for the appropriate medical management of all adverse events.

12.4 SERIOUS ADVERSE EVENTS

A serious adverse event is an event that is: a) fatal b) life-threatening c) results in persistent or significant disability/incapacity d) requires or prolongs inpatient unexpected hospitalization) is a congenital anomaly or birth defect or f) is a medically important event.

Life Threatening is defined as an event in which a patient is at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it was more severe. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions. Medically important events that may not result in death, be life-threatening or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

When patient's hospitalization is planned by the treated Investigator, no AE should be reported and thus, no SAE should be reported. Note to File will be completed and signed by the Investigator.

An unexpected adverse event is one that has not been previously observed, or one that is of a specificity or severity not consistent with the current investigator brochure.

All adverse drug reactions (a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility) that are both serious and unexpected are subject to expedited reporting as defined in ICH E2A "*Clinical Safety Data Management: Definitions and Standards for Expedited Reporting"*. An Unexpected event is one that is not consistent with the applicable product information as described in the current investigator brochure.

12.5 SERIOUS ADVERSE EVENT REPORTING

The investigator shall report all serious adverse events to the Pharmacovigilance by telephone or fax immediately after the event (within of 24 hours, in accordance with MediWound's SOPs). The initial report should be followed-up with a detailed written report within three days. Full details of the event, any sequelae and an assessment of the relationship to the study Drug must be provided in the report.

Fatal or life-threatening SUSARs should be notified as soon as possible but no later than 7 calendar days after Pharmacovigilance has first knowledge of the minimum criteria for expedited reporting. All other SUSARs must be reported as soon as possible but no later than 15 calendar days after Pharmacovigilance has first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

12.6 PREGNANCIES

In the long term follow up period of the study, no special contraception methods are deemed necessary for the study due to the single short term use of the investigational product and its known pharmacokinetic profile were no remnants of NexoBrid were observed after 48 hours had passed from first application. In the early acute phase of the burn wounds, during their hospital stay, patients are hospitalized and thus are not expected to be subject to sexual intercourse. If sexual abstinence within one month after study treatment is not possible acceptable contraception methods should be used by the patient.

1) Acceptable methods of contraception (a Pearl Index < 1%) include:
   - hormonal methods (oral contraceptives, patches or medroxyprogesterone acetate)
   - An intrauterine device (IUD) with a documented failure rate of less than 1% per year

> **NOTE:** *Females who have been surgically sterilized (e.g., hysterectomy or bilateral tubal ligation) or who are postmenopausal (total cessation of menses for >1 year) will not be considered as "females of childbearing potential."*

Nevertheless, to ensure patient safety, each pregnancy in a female patient of childbearing potential on study drug should be reported to the treated physician during the study course. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Pregnancy should be recorded on the form provided by MediWound and reported by the investigator to MediWound. The telephone and fax number of the contact persons are listed on the form provided. Pregnancy follow-up should be recorded on a form provided by MediWound as well and should include an assessment of the possible relationship to the study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

13. STATISTICAL ANALYSIS

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

13.1 POPULATIONS

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

Intent-to-Treat (ITT) population includes all patients who are randomized into the trial.

Evaluable (per protocol)(PP) population includes all subjects who fulfill all inclusion/exclusion criteria and do not have major protocol violations. Analysis of the PP population is for supportive purposes, whereas the analysis of the ITT population provides the primary and secondary efficacy and the comparative safety analyses.

Safety population (SP) includes all patients receiving a treatment and analysis is focused on the treatment actually performed.

13.1.1 Major Protocol Violations

Major protocol violations will be evaluated by clinical personnel and detailed information will be listed. List of protocol violation will be included in the study monitoring plan.

13.2 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and relevant baseline information (medical history, physical examination, treatments prior to admission, burn description, distribution of wound depth and size, TWs description, primary TW description, etc.) will be presented and summarized with appropriate descriptive statistics. 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 will be used to assess the comparability of the baseline factors between the treatment groups.

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 the supportive analysis models for the primary and secondary efficacy endpoints and the comparative analyses of safety endpoints.

Relevant medical history will be tabulated and presented by treatment group.

These summaries will be based on the ITT population.

13.3 EFFICACY EVALUATION

13.3.1 Primary Endpoint

The primary endpoint will be analyzed as described below. Missing data will be handled by the methods described in Section13.8.4. NexoBrid will be compared with Gel Vehicle for the primary endpoint.

13.3.1.1 *Incidence of complete eschar removal measured at the end of the topical agent soaking period.*

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). For handling of missing data see Section 13.8.4. 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.

If numerically possible, the comparison will supportively be adjusted for the stratification factor wound depth (see Section 13.7) 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 %TBSA and the factor treatment center (see Section 13.8.5) will be included in a logistic regression model with the treatment variable. Statistical inference will be based upon exact distribution methods.

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.

13.3.2 Secondary endpoints

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 13.8.4.

13.3.2.1 *Surgical excision performed*

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 (for definitions, see Section 13.7), %TBSA 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.

If numerically possible, as a supportive analysis, the variable treatment center (see Section 13.8.5) will be added into the logistic regression model and the analysis repeated.

13.3.2.2 *Timely 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 (days). For patients who do not reach complete eschar removal, their time will be censored at the initiation of the wound closure treatment phase. Kaplan-Meier curves will be presented graphically to display the distribution of time to complete eschar removal under the two treatments. Median time to complete eschar removal will be estimated for each treatment group with a 95% confidence interval. The treatment groups will be compared using a Cox regression model. The comparison will be adjusted for the same explanatory variables as mentioned in Section 13.3.2.1, by including each of them in the Cox regression model together with the treatment variable (NexoBrid vs. SOC). 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 we will use a generalized Wilcoxon-Gehan test.

Supportive analyses will include adjustment for other baseline variables that are imbalanced, and investigating interactions between baseline variables and the treatment groups.

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.

13.3.2.3 *Blood loss*

The measure of blood loss defined in Section 11.2 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. 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. Supportive analyses will include adjustments for the same covariates as mentioned in Section 13.3.2.1.

13.3.3 **Safety endpoints**

13.3.3.1 *Time to reach complete wound closure*

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. First the proportional hazards assumption will be checked in the same way as in the analysis of the timely eschar removal endpoint. If the proportional hazards assumption is appropriate we will use a marginal Cox regression analysis with a robust sandwich estimator. If not, then we will use a parametric frailty model. Either method can be implemented in SAS (see Gharibvand and Liu, 2009 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. [102]. The parametric frailty model will be implemented using the following SAS-code:

```
PROC PHREG DATA=burn_data;
     CLASS Subject_id treatment_group depth;
     MODEL time*status(0) = treatment|group depth;
     RANDOM Subject_id;
     HAZARDRATIO "Frailty Model" treatment_group;
RUN;
```

where subject_ID is a unique identification number for each patient, time is the time to wound closure on a wound level, status is an indicator whether the wound has achieved complete wound closure or not (censoring indicator), treatment_group gives the treatment group, depth gives the TW depth (on a wound level) and burn_data is a SAS data set containing the required information. These 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. The comparison will be adjusted for the same stratification variables as mentioned in Section 13.3.2.1. A non-inferiority margin will be incorporated into the analysis that will represent a 7-day advantage to the SOC group, so that the analysis will show whether the NexoBrid group is estimated to have on average a time to wound closure that is not later than 7 days after wound closure for the SOC group.

13.3.3.2 *Cosmesis and Function evaluation using MVSS*

The MVSS score for a patient will be the MVSS score averaged over all TW's of that patient. The mean and standard error of this MVSS score at 12 and 24 months will be estimated for each treatment group. The evaluations at these two time points will represent two separate endpoints. 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 same stratification variables as specified in Section 13.3.2.2. 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. For handling of missing data see Section 13.8.4. 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.

Additional Safety Outcome Measures will include:

These parameters will be analyzed in a descriptive way only. More details will be provided in the statistical analysis plan.

13.3.3.3 *General parameters of safety*

General parameters: Systemic adverse events, vital signs, pain assessment (using VAS and as reported as AEs), laboratory tests, units (and volume) of blood transfusion given during hospitalization, Immunogenicity evaluation for NexoBrid patients, Pyrexia and Hyperthermia, Systemic infections, Incidence of increased interstitial/compartment syndrome (as defined in Section 1.1) and QT prolongation, 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).

13.3.3.4 *Local parameters of safety*

Local adverse events defined by treated physician or designee; graft loss, wound related infections, etc.

13.3.3.5 *Further parameters of safety*

Functionality evaluation

The following scales will be used: QuickDASH questionnaire, LEFS (Lower Extremity Functional Scale) and Range of Motion (ROM) measurements. Each scale will be assessed at the following time points: 1 month after reaching complete wound closure and at 3, 6, 12, 18 and 24 months after wound closure. The ROM measurement will be classified as normal or abnormal status.

Quality of life evaluation

The following scales will be used: EQ5D health questionnaire and BHSH-B (Burn Specific Health Scale). Each scale will be assessed at 1, 3, 6, 12, 18 and 24 months after reaching complete wound closure.

13.3.4 Exploratory endpoints and additional analyses

These parameters will be analyzed in a descriptive way only. Additional details will be provided in the statistical analysis plan.

13.3.4.1 *Exploratory endpoints*

2. 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),
3. POSAS will be used to further assess cosmesis and function,
4. Incidence of surgical Escharotomy procedures in 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[1],
11. Autograft related parameters.
    I. Efficacy and safety analyses for early and late grafted wounds
    II. Total number of target wound grafting procedures
    III. Incidence of repeated/additional grafting procedures
    IV. Area of repeat grafting
12. Duration of hospitalization

13.3.4.2 *Additional Analyses*

The following analyses will be addressed as supportive efficacy analyses:

1. Primary & secondary endpoints will be analyzed at a per wound level,
2. Secondary endpoints will be analyzed vs. the Gel Vehicle,
3. Analysis of time to reach 100% wound closure assessed in days, starting from randomization date

   Clinical assessment of complete eschar removal vs. Centralized complete eschar removal from photos

13.3.5 Subgroup Analyses

Descriptive statistics will be determined for all subgroup analyses.

1. 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.
2. Patients will be classified as having <25% or >=25% SPT area as a percentage of the area of all TWs, and descriptive statistics will be provided for each subgroup, including data on the primary, secondary and safety endpoints,
3. Time to wound closure and 12m and 24m MVSS will be analyzed per TWs' depth and % TBSA of the TWs,
4. An analysis restricted to wounds that are entirely FT will be provided.

[1] For more details see Appendix 10- Procedures for specific blood tests

5. A subgroup analysis of the primary and secondary endpoints will be performed per % TBSA. The subgroups examined will be ≤15% and >15%.

13.4 STATISTICAL COMPUTING

The data will be analyzed using the SAS ® version 9.1 (SAS Institute, Cary North Carolina).

13.5 SAMPLE SIZE CALCULATIONS

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.

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

Estimated proportion achieving complete eschar removal (for further definition and explanation see Section 11.1) in all TWs from study MW2004-11-02: 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.

13.5.2 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.

13.5.3 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:

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

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.

13.5.4 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).

13.5.5 Safety endpoints

Because of the importance of the time to wound closure and MVSS safety endpoints, we examined for these two endpoints, under the chosen sample size of 75 patients per group in the NexoBrid and

SOC groups, the probability of demonstrating that the observed average value in the NexoBrid does not exceed the observed average in the SOC group by more than the clinically meaningful margin.

For time to wound closure, assuming, based on previous data, that in reality NexoBrid has an average time that is longer than SOC by 5.77 days (based on wound closure data from study MW2004-11-02 analyzed per the suggested analysis in Section 13.3.3.1), the probability that the observed average NexoBrid time to wound closure will be longer than the observed average SOC time by more than 7 days is calculated to be 0.26.

For MVSS, assuming, based on previous data, that in reality NexoBrid has a score that is less (better) than SOC by 0.51 units, the probability that the observed average NexoBrid score will not be larger (worse) than the observed average SOC score by more than 1.9 units is calculated to be less than 0.001 for MVSS at 12m and 24m.

13.6 ALPHA CONTROL

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.

13.7 STRATIFICATION AND RANDOMIZATION

13.7.1 Randomization Procedure:

To enter a patient and obtain the randomized treatment allocation, the following information should be verified and uploaded into the eCRFs:

i. Eligibility of the patient (entrance criteria are met)
ii. ICF date
iii. Patient's demographics (date of birth, gender, ethnicity, race)
iv. Patient's burn history and burn description (identification of all wounds and target wounds (TWs) assigning an identifying number to each wound, and providing its anatomical site, its %TBSA, its depth and where relevant its proportion of superficial partial thickness (SPT) area
v. The overall depth, according to its category A1-A3 (see definition below)
vi. The treatment center

Upon finalization of this process, randomization will be performed as described below and a designated person will receive the allocated treatment. With regard to this designated person, this person must understand that the information regarding the allocated treatment must not be given to the designated person who is assessing the TWs following treatment, as further described in the study protocol.

13.7.2 Stratification:

Eligible subjects will be stratified into different groups in order to balance the treatment groups with respect to factors that may be related to efficacy and safety outcomes. Three factors will be used to stratify the subjects.

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 centres and to reduce the likelihood of a group of centers including patients in only one arm of the study (5 groups of centres will be formed based on similarity of SOC practice).

Using this stratification, patients will be randomized in a 3:3:1 ratio (NexoBrid: SOC: Vehicle).

13.7.3 Randomization algorithm:

The treatment allocation will be performed using the method of block randomization with stratification by the factors A B and C, described in the subsection above: %TBSA, overall depth of TWs, and clinical center group. The method will be implemented by computer generation of permuted sequences of treatments NexoBrid, SOC or Gel Vehicle in the ratio of 3:3:1 in blocks of a certain length. The length of the blocks will not be disclosed so as to prevent prediction of the next patient's allocation.

The algorithm will be prepared and automated by an independent study biostatistician, and will operate through the central site with which the clinics will communicate.

13.8 DATA HANDLING METHODS AND DEFINITIONS

13.8.1 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 the endpoints incidence of complete eschar removal, reduction in surgical needs, summary of blood loss and the cosmesis endpoints of safety, if a subject has more than one visit with data at a time-point, the later non-missing evaluation will be used for analyses. An exception to

this rule is in the analyses of time to eschar removal and time to wound closure; for these parameters a value other than the last one or more than one value per visit time-point may be taken into account. More details will be provided in the SAP.

13.8.2 Rules for Data Derivations

The SAP will identify the necessary data transformations and rules for data derivation.

13.8.3 Multiple target wounds per patient

Patients may have multiple wounds being treated (target wounds). All target wounds will be taken into account in the primary and secondary analyses. The methods of analysis are specified in the efficacy analysis subsection. The following calculations will be used for each patient:

1) Total treated burn surface area (%TBSA): is defined as the sum of areas of all target wounds for that patient.
2) MVSS at 12m and 24m are defined as the score averaged over the TWs.

13.8.4 Missing data

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

Little or no missing data are expected for the primary endpoint, since the outcome is assessed only several hours after the start of treatment. 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 randomized subjects. 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. complete eschar removal) and a second analysis will count all patients with missing data as negative (i.e. no complete eschar removal).

The same procedure will be applied for the secondary endpoint, reduction of surgical need.

The analysis of the secondary endpoint time to eschar removal will compute missing values as censored.

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 this basis a multiple imputation will be calculated.

. The analysis of the safety endpoint time to complete wound closure will compute missing values as censored..

A higher ratio of missing values is expected in the case of later MVSS values, especially for 24 months. In order to avoid unnecessary complex imputation for this descriptive endpoint simple LOCF imputation will be done. It is expected that at least one early MVSS value exists. If this is not the case the missing MVSS values will be replaced by the mean MVSS value of all patients who belong to the same treatment group and the same stratum (see Section 13.7.2) at each time point. A best case – worst case imputation using information about the reasons of drop out documented in the CRF will be used as a supporting sensitivity analysis.

The Statistical Analysis Plan will provide more details on the strategy outlined above for handling of missing data.

13.8.5 Stratifying by center in the analysis

Randomization will be stratified by center as detailed in the Section 13.7.1. However, some centers may enroll very few patients so that there are insufficient subjects to conduct a stratified analysis efficiently. To ameliorate this problem, for stratified analysis at the end of trial, "small centers" will be pooled as follows:

All centers will be classified by region (assuming that those in the same region will have a similar standard of care treatment modality) and number of patients randomized. If a center has less than 14 patients randomized, this center will be pooled with the next smallest center within the same region until the pooled center has 14 or more patients. If a region has less than 14 patients randomized, this region will be pooled with a region that is geographically close to it. The pooling strategy will be finalized prior to database lock by a statistician who is blind to the outcome data.

13.8.6 Stages of Analysis

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 above, 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 nearly 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 Section 13.8.4 and the SAP. The complete data set documented so far will be locked and analyzed as described above. 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. Furthermore, 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 second analysis will be performed for the 12 month 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 above.

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 24m 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 above (hard lock).

14. QUALITY ASSURANCE

14.1 QUALITY ASSURANCE PROGRAM

This clinical trial may be audited according to the MediWound Quality Assurance (QA) program.

The purpose of these audits is to determine whether the study is being conducted and monitored in compliance with the protocol and recognized GCP guidelines and local regulations. These audits will also increase the likelihood that the study data and all other study documentation can withstand a subsequent inspection by any regulatory authority. Such audits, if necessary, will be pre-arranged with the site and conducted within a reasonable time frame.

14.2 MONITORING PROCEDURES

The conduct of the study will be closely monitored by representatives of MediWound to verify adherence to ICH GCP guidelines and applicable SOPs. Reports of these monitoring will be archived with the study report.

The Investigator will allow the designated MediWound representatives and/or any regulatory agency to have direct access to all study records, eCRFs, corresponding subject medical records, study drug dispensing records and the study drug storage area, and any other documents considered source documentation. The Investigator also agrees to assist the representative if required.

The Investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information in eCRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient).

The investigator must give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. MediWound monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of data that will be used for all primary and safety variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan. No information in source documents about the identity of the patients will be disclosed.

14.3 REGULATORY AND ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles originating from the Declaration of Helsinki, and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice in compliance with local regulatory requirements.

14.4 DATA SAFETY MONITORING BOARD (DSMB)

A Data Safety monitoring Board (DSMB) will convene in accordance with enrollment rate i.e. after 25% of patients were enrolled and then again after 50%, 75% and after 100% of the patients were enrolled and treated (last patient discharge from the hospital) as long as not more than six months had passed since the previous DSMB- during the enrollment period. DSMB will assess safety data and narratives[1] as described in the agreed DSMB chart. During the follow up period, a DSMB will convene at least once a year.

In addition, special meetings could take place in the following cases:

1. Any of the stopping rules are met, as described in Section 7.2,
2. Following the randomization of 10 NexoBrid patients suffering from deep burns of more than 15% TBSA (TWs area) that were treated with 2 consecutive sessions,
3. Following the randomization of 25 NexoBrid patients suffering from deep burns of more than 15% TBSA (TWs area) that were treated with 2 consecutive sessions.

All DSMB conventions will be in closed session format.

These activities will be coordinated by MediWound Ltd. and the recommendation shall be directed to MediWound Ltd. The DSMB will consist of three independent individuals including burn specialist(s) and a biostatistician. Following selection and agreement to serve, the committee will meet in person or by teleconference to be briefed fully on the procedures defined in the Protocol and Statistical Plan. MediWound Ltd. shall be formally informed of recommendations by the DSMB prior to implementation of said recommendation(s) and must approve, in writing, any new analyses being recommended by the DSMB.

[1] All SAEs will be presented in narratives as described in MediWound's SOPs. In addition, events of repeated debridement in the topical arm and repeat application of autografts to the same wound area will be summarized & introduced as narratives to the DSMB.

15. INVESTIGATOR'S AGREEMENT

I have carefully read the foregoing protocol including all appendices and agree that it contains all the necessary information for conducting the study safely.

I will conduct this study in strict accordance with this protocol and according to the current GCP guidelines and will attempt to complete the study within the time designated.

I will provide copies of the protocol and all other information submitted by the sponsor relating to pre-clinical and prior clinical experience to all personnel for whom I am responsible that participate in the study. I will discuss this information with them to assure that they are adequately informed regarding the drug and conduct of the study.

I agree to keep records on all subject information (case report forms, shipment and drug return forms and all other information collected during the study) in accordance with the current GCP and local regulations.

Site Principal Investigator's name	MediWound Representative's name
Signature	Title
Date (dd/mmm/yyyy)	Signature
Institution	Date (dd/mmm/yyyy)

16. APPENDICES

APPENDIX 1- WOUND DEPTH ASSESSMENT- CLINICAL EVALUATION

The burn literature [18, 97] suggests that clinical evaluation of the burn wound is the most widely used method of assessing burn wound depth. This was, but also recognized in the FDA Guidance for Industry Chronic Cutaneous Ulcer and Burn Wounds, June 2006[1] and also the burn depth assessment method accepted by the ABA [1]. In accordance with the above, further to assessing wound appearance, the following tests will be included [98]:

1. Capillary blanching
2. Capillary refill
3. Capillary staining
4. Wound sensibility to touch
5. Pin prick

Using the above criteria will allow the Investigator to differentiate between the SPT and DPT areas as is widely acceptance in the US [99], according to the following below criteria in addition to the visual assessment:

- SPT burns extend into the papillary dermis and form blisters. Once the blister is removed from a SPT wound, the wound is pink, wet and hypersensitive to touch. These wounds are painful and blanch with pressure.
- DPT wounds extend into the reticular dermis. They also blister but the wound surface appears mottled pink and white. The patient complains of discomfort and pressure rather than pain and when a pressure is applied to the burn, capillaries refill slowly or not at all. The wound is less sensitive to pinprick.

[1] Section IV D, pharagraph 2.

APPENDIX 2- PAIN MANAGEMENT PROCEDURES

Pain Management Procedures

For topical arms treatment, preventive analgesia medication (pain management) will be mandated as for an extensive burn dressing-change.

During NexoBrid treatment it has been observed that the two painful stages are during the first 30 minutes of the treatment and during the dressing removal which is followed by cleansing and dressing (30-45 minutes). Thus, the principle of the suggested pain management is to prevent pain by starting the treatment with sufficient basic analgesia/sedation as detailed below. Once the first painful stage has ended, background analgesia can be administered and the sedation/analgesia regimen stopped.

Before NexoBrid dressing removal the sedation administration should normally be started ensuring that the patient is pain-free until the first post-eschar removal soaking dressing is in place.

Sedation administration can be repeated two hours later, before the removal of the soaking-dressing**.**

Objective:

The object of the analgesia is to ensure that the entire treatment course is performed in a safe and comfortable manner. During the treatment the patient should be free of significant pain and easily arousable, without interference with vital functions.

In the majority of cases the pain can be managed without the need for deep sedation, by the pre-administration of oral analgesics and being prepared to administer intravenous Morphine as needed to control pain surge.

If sedation is used the patient should not need intubation and it should be easily and quickly reversible by discontinuing administration of the sedation agent. Sedation can normally be discontinued after the first painful wave that lasts for 30-60 minutes from NexoBrid application; it should be reinitiated before and during dressing removal and wiping of the dissolved eschar, a process that may last for another 30 minutes.

Attention should be given to background analgesia during the interval between these procedures.

During procedures, the patient should as a minimum be monitored frequently for:

- Blood Pressure (non-invasive)
- Cardiac activity (ECG)
- Respiratory rate
- Oxygen saturation (pulse oximetry)

Example of a medication regimen:

Bolus intravenous analgesia/sedation using appropriate combinations of fentanyl, and midazolam and ketamine. Suggested initial doses are:

Fentanyl- 2 microgram/Kg
Midazolam- 0.06 mg/Kg (1 mg/15 Kg)
Ketamine HCl- 0.5 mg/Kg

Maintenance doses will typically be one-third to one-half of the initial dose at 10-20 minute intervals; considerable inter-patient variation should be expected.

Intravenous continuous sedation using a syringe driver or target-controlled infusion (TCI) driver is a very acceptable technique, using propofol + alfentanil, propofol + remifentanil, or remifentanil alone.

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.

Because sedation is a continuum, it is not always possible to predict how an individual patient will respond. Hence, practitioners intending to produce a given level of sedation should be able to rescue*** patients whose level of sedation becomes deeper than initially intended. Individuals administering Moderate Sedation/Analgesia ("Conscious Sedation") should be able to rescue*** patients who enter a state of Deep Sedation/Analgesia, while those administering Deep Sedation/Analgesia should be able to rescue*** patients who enter a state of General Anesthesia.

* 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.

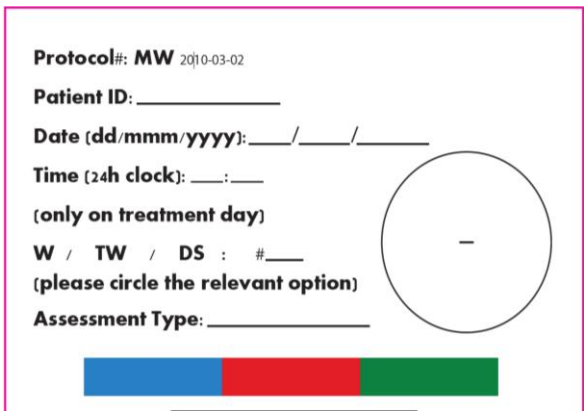
*** Rescue of a patient from a deeper level of sedation than intended is an intervention by a practitioner proficient in airway management and advanced life support. The qualified practitioner corrects adverse physiologic consequences of the deeper-than-intended level of sedation (such as hypoventilation, hypoxia and hypotension) and returns the patient to the originally intended level of sedation. It is not appropriate to continue the procedure at an unintended level of sedation.

APPENDIX 3- PHOTOGRAPHIC WOUND DOCUMENTATION

In accordance with FDA guidance, the photography methods will be standardized in all centers with standard camera type and control of the distance/size (zoom), color, exposure, (lighting) and color bend tag (as a reference for color and size) of the acquired picture. This will be performed using patient ID label (see below) which will be identical in size and content (colors scale) for all patients and should be used as detail below. Photographs will be uploaded into a secure file sharing account.

Photographic Documentation

1. Each wound area is considered an independent entity and should be documented as such.
2. Each wound site should be photographed using a standard digital camera with flash card memory which together with sterile photography patient ID labels, are supplied by MediWound.
3. Patient ID, date and time, TW# and assessment type (e.g. screening, pre-treatment, post-treatment,, weekly visits and monthly visit, etc) should be noted on each ID label, (see below).
4. Each wound site should include the patient ID label.
5. The photograph should be taken using the zoom facility while including the entire treated area and at least two distinctive body parts as reference points.
6. The photograph should be taken 90 Degrees to the desired area. A small angle is acceptable, but any higher angle will make it more difficult to evaluate the wound from photos, at a later time point, if needed. Any pictures taken below 45 Degrees should be avoided.
7. In order to ensure patient confidentiality, facial photographs are not allowed.



Recording Kit

The recording kit consists of:

1. Sterile photography patient ID labels,
2. One digital camera,
3. Flash cards.

APPENDIX 4- POST-ESCHAR REMOVAL WOUND MANAGEMENT

Post Eschar removal Burn Wound Bed(s)

An ideal burn debriding agent should effectively and quickly debride the burn eschar but also preserve viable tissues to source and allow spontaneous healing by epithelialization. It should combine the non-invasiveness of the ineffective and slow non-surgical debriding means and the efficacy and speed of surgery.

Following any eschar removal process, whether surgical or non surgical one, the wound is being assessed for eschar removal efficacy and for presence of residual viable dermis by experienced plastic/burn surgeon. Usually, following an effective eschar removal with visualization of the viable tissue bed, a more accurate burn depth diagnosis can be made. Macroscopically, the components of the human integument are from epidermal, dermal and subdermal origins. Few colors are involved in the final visual appearance of the healthy tissues: the brown-black of the melanin, hemoglobin's shades of reds (depending on the oxygenation level) and the pearly-white of the collagen. Below the skin a yellow fat add another color.

The debrided wounds can be divided into four groups based on the debrided bed condition:

1. Complete eschar removal revealing residual viable dermis.
2. Complete eschar removal revealing a full thickness skin defect requiring permanent cover.
3. Complete eschar removal revealing a wound bed with areas of viable dermis and areas of full thickness defect**.**
4. Incomplete eschar removal to various degrees.

Several means and strategies can be used to remove the eschar, each ending in a typical bed:

<u>Surgically debrided beds:</u>

Sharp dissection (using dermatomes, dermabrasion or hydro dissection such as Versajet) will leave a smooth bed that is composed, in the case of complete eschar removal, of transected structures such as dermis and blood vessels or subcutaneous fat. This bed will readily accept autograft with good take if adherence and drainage are assured.

<u>Autolytically debrided beds:</u>

Slow decomposition and maceration will cause eventually the sloughing of the necrotic tissue but simultaneously, at the end of the second week granulation tissue will start to form and epithelial cell will start to epithelialize clean exposed dermis originating from the edges or the appendages (hair follicles, sweat and sebaceous glands). Granulation tissue will form if wound closure (by epithelialization, grafting or wound edges approximation) is delayed developing into scar. Granulation tissue will, if not infected, readily accept autograft but may contract later pulling with it the wound's edges. In case of significant bacterial infection the process can invade surrounding healthy tissues (Invasive Infection) extending the damage. Infection together with other wound healing intervening factors (e.g. diabetes, hypoproteinaemia, etc) can cause the wound healing process to stop and become chronic. Chronic wounds may be highly contaminated or chronically covered with biofilm with only slowly granulation tissue

formation. Such wounds may behave as chronic, recalcitrant wounds, and will not heal easily or support graft.

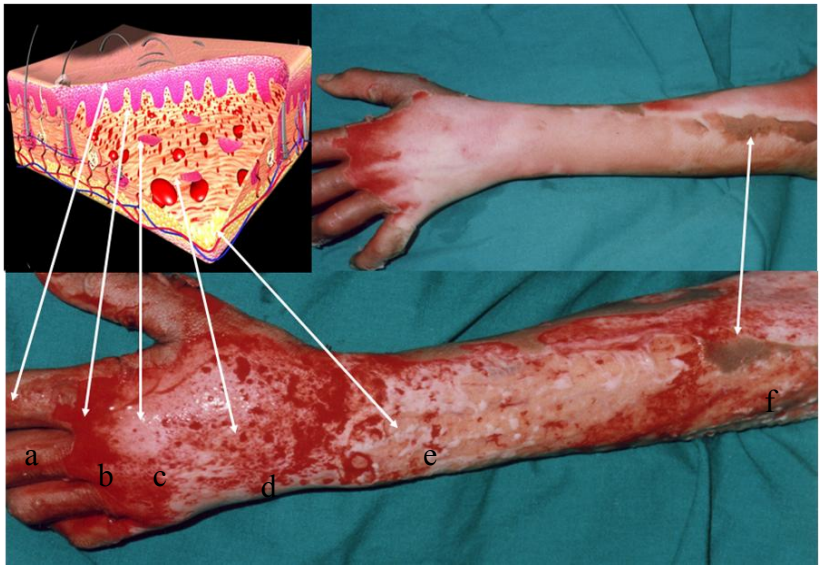
NexoBrid- Enzymatically debrided bed

NexoBrid is an enzymatic debriding agent provided for eschar removal of the eschar. The raw surface that is left after this eschar removal is the Interface Layer (IL), which is comprised of the upper layer of healthy tissue that is made of exposed viable tissues. After wiping away the dissolved eschar the dermal IL has a whitish hue due to the dermal collagen matrix seeded with punctuated bleeding capillaries. The dissolved ends of the collagen fibres are made of unferruled collagen fibrils that may give a "furred" aspect to the surface. This layer preserves all viable components such as deep epithelial elements inside the adnexae and deep dermal remnants (collagen fibres, subdermal fat and blood vessels). In all but the deepest wounds, the presence of the dermal Interface Layer permits spontaneous healing (epithelialisation over dermal remnants); however, the majority of transacted blood vessels are still occluded at their ends. In the event that skin grafting is required (a full thickness burns or after the spontaneous healing potential has been exhausted or when an immediate wound closure is required) a graftable bed is obtained by simple scrubbing the Interface Layer with a dry swab or a sterile nail brush to expose the blood vessels during the graft application session.

Wound bed assessment

Any effective eschar removal will reveal the surface of the exposed viable tissue with its components and structures and the exact extent of the original damage (Figure 1). Being aware of the cutaneous three dimensional macroscopical structures, the form and color of the various components, it is rather easy to assess the depth of the debrided bed. The first step is to assess visually the presence or absence of epidermis which is the criterion of second degree (dermal) burn. Residual, none removed eschar appears opaque, yellowish-grey clearly differing from the pearly-white viable collagen. Bleeding vessels on the background of white collagen indicate that the eschar removal process reached viable tissue. Once the dermis is revealed the bleeding pattern that characterizes the different dermal levels clearly indicates the depth. Very fine, homogeneous bleeding characterizes the superficial papillary dermis (superficial, II° dermal burns). Deeper the burn is, the bleeding vessels are set wider and wider apart. In mid and deep dermal burns, only few bleeders can be seen on the background of white collagen matrix background. The few deep dermal capillary complexes that are situated around the adnexae may give the dermis a pinkish hew. When most of the dermis is removed the yellow subdermal fat gives the debrided bed a yellowish hew. Reaching the deepest part of the dermis, at the transition to the subdermal layer, one can see the yellow fat lobules protrude through the loose net of collagen fibers with few bleeders. This "quasi" full thickness burn behaves as a full thickness burn and only rarely will heal spontaneously.

Figure 1- Wound bed assessment



a. Non burned skin, uninjured by NexoBrid
b. Superficial dermal burn with bleeding
c. Mid depth dermal burn with well preserved dermal collagen matrix
d. Deep dermal burn with larger and wider spaced bleeding capillaries and a very thin dermal matrix
e. Full Thickness burn
f. Non dissolved eschar protected by the blister that was not removed prior to NexoBrid application

Wound healing options post eschar removal

In general, burn wound closure can be achieved by:

Primary Intention: closure of the wound bed by graft that covers and obliterates the raw surface and "heals" the wound completely, similar to using sutures for bringing together the surgical wound's edges. This approach requires a completely clean and viable bed with all cellular and non cellular components exposed for the healing process. The severed end of the blood vessels should be opened to allow endothelial cells to bud and connect the graft's raw underside and eventually supply the graft with the necessary life giving blood. The graft survives the first days by "imbibition phenomenon": graft's nutrition by the serous exudates from the wound bed but its continuous survival depends on blood vessels connection and integration with the graft's capillary bed. The capillary bed in the dermal papillary layer indirectly supplies the epidermis (that does not have any capillary bed of it own) with the necessary nutrients and oxygen so autograft needs a certain dermal layer to provide nutrient to the epidermis for survival. Graft adhesion during first days will provide good contact to the recipient bed, decreased danger of exudates' collection and a better chance of take. Such graft stabilization and adherence is achieved by compressive dressings or using sub atmospheric (vacuum) external dressing over the graft that will stabilize the graft to its bed and remove exudates that can interfere with graft take.

Autograft: grafting the raw bed with autograft that includes epidermis and a thicker or thinner layer of dermis. If the graft take >95% – the engrafted wound is practically closed. Autografting requires surgery under general anesthesia, sacrifice of healthy donor site skin area and surgical facilities (OR, surgical team etc.). The donor site will heal spontaneously during 2-3 weeks by epithelialization but may be scarred and infected. Thicker the harvested dermal layer is better the functional and aesthetic results are but more damage is inflicted on the donor site, longer its healing is and higher is the likelihood to develop scars. Thicker the graft is, higher are the demands of the transplanted tissue from the wound's bed and so are the risks of graft failure.

Permanent Skin Substitutes: products such as Matriderm, Integra and Jalomatrix can be applied on the debrided bed, serving as regenerating matrix for the development of new dermis. Here too the debrided bed should be free of necrotic eschar and contain opened capillaries ends as the source for endothelial cells that will integrate, together with the native fibroblasts, into the artificial matrix forming the neodermis. All these products have to be engrafted sooner or later also with thin autograft (Matriderm immediately and Integra and Jalomatrix, 2-4 weeks later) that contains mainly the epidermal layer. The grafting procedure will necessitate surgical facilities as in a formal autografting but the donor sites heal faster (< 2 weeks) as the harvested graft is thinner with much less sacrificed dermis. The cost and success rate of these products is a severe limiting factor.

Allograft, xenograft and adherent biological covers: will adhere to the bed (assisted by vacuum dressing in the first days), preserve moisture and provide conditions for epithelialization or autograft for later stage, following removal of the temporary dressings.

Epithelialization over preserved dermis: Full thickness defect greater than few cm^2 should be closed by grafting but exposed dermis can heal by epithelialization that will close the wound without granulation and scar formation. When sufficient dermal elements exist, epidermal cells (Keratocytes) originating from the wound's edges, the skin appendages (hair follicles, sweat and sebaceous glands) or Keratocytes cultures/ suspension can proliferate and propagate over the dermal bed creating a new epidermis. This process requires a healthy and clean dermal bed and a dressing that will protect the exposed, raw dermal bed from desiccation and infection and provide the epithelialization process with the necessary conditions (moisture and propagation surfaces for the Keratocytes). There is a plethora of products that can be used for this purpose each with its advantages and disadvantages, each fulfilling a specific need in the healing process.

Healing By Secondary Intention: As real regeneration of full thickness skin defect is impossible, the natural healing process of these wounds is aimed at creating first a temporary protection mechanism followed by a "plug" that fills the defect, replacing the missing, normal skin. Following a generalized inflammation-healing process of three overlapping phases of ***Inflammation, Proliferation*** and ***Modulation*** the granulation tissue forms, covering the wound bed and slowly filling the defect. Granulation tissue originates from connective tissue, containing fibroblasts, macrophages, white blood cells elements and endothelial cells from the severed blood vessels all forming a tortuous mass of new capillaries imbedded in new connective tissue. Keratocytes from the wound's edge slowly strive to cover the granulation tissue but very often their progress is extremely slow and may be completely denied by the fibroblasts. The granulation tissue with all the local and systemic defense mechanisms provides

protection against infection. The fibroblasts deposit collagen and the fibroblasts–originating miofibroblasts pull-in the wound's edges forming a scar with more or less contracture element. The progressive development of the granulation tissue (*proliferative* phase) may be controlled and decreased by the influence of the epithelial cells, the Keratocytes. Once the granulation tissue is entirely covered by the Keratocytes, its growth will decrease and the granulation tissue becomes a scar that will start to mature, often also to contract. Granulation tissue proliferation may be attenuated by topical corticosteroid ointment short (few days) treatment course exploiting the controlling affect of corticosteroids on inflammatory processes. In the granulation tissue, the fibroblasts deposit collagen and the entire tissue undergoes modulation to become fibrous, hard scar filling the original defect. The scar contracts by the miofibroblast, pulling-in the healthy skin margin, reducing the scar's surface but these contractures, especially across joints will interfere with function and cosmesis.

Grafting granulation tissue is routinely practiced: during surgery the granulation tissue is forcefully scraped with a blunt edge surgical instrument (spoon, scalpel handle, metal ruler, etc.), removing the superficial layer with its bacterial load and opening the multitude of capillaries to accept the graft. In burn care healing by secondary intention with granulation tissue scarring and contractions is undesirable and often the scarring is more incapacitating and dangerous than the acute burn itself. The lengthy (sometimes years) of the scar modulation process may be influenced by application of continuous pressure on the scar by made-to-measure pressure "garment" dressings that applies a constant pressure and silicon sheets applied directly on the scar under the pressure garment.

Healing by tertiary (delayed primary) intention: In some cases primary closure by wound edges approximation (suturing) or grafting is not feasible mainly due to local infection, patient's general condition or external restrictions (surgical team, OR, other facilities or graft donor site availability). Another condition is when wounds closed by suturing disrupt (dehisce) or the grafts do not "take" to the wound bed and slough off. In most cases it is either an infection process of the underlining wound bed or edges or collection (blood, serum, pus). Re-suturing or re-grafting the contaminated bed will end only with another closure failure and probably also with spreading the infectious process to adjacent tissues. The accepted practice is to leave the wound open, to treat the infection by topical and sometimes also systemic medications and once the infection is resolved the wound can be closed again. Formation of granulation tissue during the treatment course can favor graft take by providing additional blood supply to the graft but later may promote scar formation.

Summary of wound healing strategy in burns

Ideally burns wounds should be debrided and closed as soon as possible after injury. At present that means: immediate or very early after injury tangential excision and autografting. Due to diagnosis difficulties mainly of the dermal burns, the interest of avoiding the surgical related trauma, morbidity and the surgical needed facilities/costs with the potential (and hope) that the burn may heal spontaneously, the surgical treatment may be delayed. Once the eschar is not surgically debrided a slower, non surgical eschar removal approach is practiced. It is based on the best possible anti-infection care with the promotion of the eschar separation (sloughing) or disintegration (necrosis). This is a slow process that may lead to local and systemic infection and additional tissue damage. The time needed for this non surgical process allows also the formation of granulation tissue. The granulation tissue protects the open wound and offers a good bed for grafting but in many cases will form a heavy, contracted scar in few weeks. During this healing process an educated, cyclic (short spells of 2-3 days) of corticosteroid ointment will reduce the granulation tissue and will offer the bed a better grafting bed.

To graft or not to graft: Full thickness defect will not heal properly without grafting and need always autograft or permanent skin substitute to prevent healing by secondary intention and scaring. Clean dermal bed that can epithelialize spontaneously can be grafted as well in order to close the wound immediately. Several factors should be considered when striving to close the wound "as soon as possible". The true benefit to the burn patient in earlier burn wound closure by few days (the difference between grafted and epithelialized dermal burn) not always is truly advantageous. Autograft involves surgery, surgical and anaesthesia related trauma, donor site morbidity and the healing time of the donor site by epithelialization (≈2 weeks) is very similar to the epithelialization of the burn wound exposed dermis. Donor site morbidity includes infection, pain, additional dressing changes and scars. Grafting will not speed early subject mobilization as the latest normally starts much before wound closure ends and a return to work/normal lifestyle requires at least several weeks of recovery and rehabilitation after the wound is closed. Thus, the decision to graft exposed dermis in order to shorten by few days wound healing should take into consideration the comprehensive well being and benefit to the patient. Issues such as length of treatment course and hospitalization depend on the nature of the dressing and wound care that in many cases allow early hospital discharge and prevent open wound related complications.

Following wound closure the healed wound is dressed with a thin protective primary dressing or silicon sheet followed by pressure “garment” dressing modulating their modulation to a smooth, pale and pliable skin-like scar.

Wound healing requirements

The general prerequisites for good wound healing are based on adequate conditions and microenvironment for this dynamic process.

Tissue factors

- Adequate blood supply: adequate arterial supply, adequate venous return, healthy capillary bed (example to potential problematic conditions: PVD, Varicose Veins, arterial or venous insufficiency, diabetes, peripheral edema of any kind, hypertension, etc.)
- Clean wound bed: no eschar, foreign bodies or materials: all the followings can interfere with the healing process: necrotic eschar such as in burns, pressure sores, exposed bone, tendon or orthopedic implant and suturing material, foreign bodies introduced during injury, intradermal or subdermal hematomas or seromas.
- No severe wound infection: $< 10^5$ count /cm^3, purulent discharge, invasive wound infection, signs of cellulites, fungal infection.
- Balanced healing dynamics: offering adequate conditions for epithelialization propagation and preventing overgranulation and its stagnation.
- Systemic factors such as chronic steroid intake, anticancer drugs, malnutrition, diabetes, etc.
- Post radiation tissue damage
- Avoiding local medication that can interfere with the desired healing process (e.g. SSD, pseudoeschar formation, desiccating dressings).

Local wound bed conditions that promote wound healing

- The healing process is a cell-based process with different cells requiring different conditions besides the very general ones of blood supply and contact with the bed:
- Moist wound surface: desiccation of the healing bed is one of the main dangers. All cells need moist environment for their survival. An occlusive dressing, any adherent- to-the-bed dressing, hydrogels (hydrating dressings) and ointment (oil based medicament) will create a thin moist environment underneath.
- Three-dimensional wound bed topography:
  - Propagation surfaces: Keratocytes "prefer" to propagate and epithelialize on a collagen/HA, dermal surface under guiding, protective, scab like surface.
  - Contact inhibition: Fibroblasts and endothelial cells tend to develop granulation tissue in the direction of the wound's open space.
  - Surface modulation of inflammation process: granular and fibrous surfaces macrophages, fibroblasts, etc.
- Infection free surfaces: early treatment of any apparent infection
- Adherent dressing will keep the undersurface moist, infection free, provide propagation surface and protects from mechanical insults. Usually an adherent dressing is the sign of a safely healing wound though desiccating, hydrophilic dressings may also desiccate a viable bed, deepening the damage.

Wound-bed, Interface Layer (IL)' Contact, Primary Dressings-Covers: Main Demands

The primary dressing, the dressing that is in immediate contact with the IL exposed raw tissue has very well defined role: to protect and promote care and healing.

The followings are the factors that are important in these aspects:

1. Adheres to the Interface Layer (with its special characteristics)
2. Moisture regulation
3. Epithelialization enhancement
4. Fibroblastic activity modulation
5. Infection control
6. Trans-Dressing Diagnosis of changing conditions (TDD)
7. Trans-Dressing Intervention (TDI)

1. **Adherents to the Interface Layer:** Dressing's adherence is important as it provides the protection against microorganism contamination and propagation, serving as a mechanical barrier shielding the healing wound from external insults and maintaining a thin moisture layer over the healing bed. In general, the dressing's adherence may be due to the following situations:

   a. Incorporating, ingrowths of the wound bed tissue into the dressing structure (as may be seen in Allografts, Xenografts, cotton gauze or some of the sponge dressings).

   b. The "dressing" is an integral structural part of the wound (a desiccated eschar, the desiccated, upper layer of the bed or a planned mummification of the eschar by cerium nitrate [*Flamacerium*] or iodine will preserve the original collagen fibers ties with the bed's matrix.

   c. There may be a physical/chemical layer that "bonds" the dressing to the underlying bed (such as scab formation where the fibrin and fibrous clot will adhere and bond dry hydrofiber [*Aquacell*], cellulose [*Velloderm*] etc. dressings). Dressings such as some of the Hyluronic acid [*Jaloskin*], Collagen [*Biobran*] or DL-Lactide (*Vicryl* like poliglycolic acid [*Suprathel*]) dressings may adhere by their undersurface, deeper layer that serves as adhering layer with the bed. In these cases very often, if the bed's viability is maintained (not desiccated) the epithelial or granulation tissue will grow underneath and their advancing fronts will separate the dressing from the healing bed.

      In some of these dressings this bonding surface can bond the dressing also to a not completely debrided beds. If the eschar is very thin and not too contaminated there is a good chance that the dressing will remain adherent and the bed, slowly healing with the eschar remnants, will disintegrate as the healing front progresses. This characteristic is very important as it allows an effective cover to a wide range of wound beds.

      Unfortunately an adherent dressing to the wound bed may also form a barrier to the propagating healing process. The dressings from groups **a.** & **b.** above, though they may effectively prevent infection will not allow propagation of the epithelialization fronts underneath and may also prevent the formation of granulation tissue. The dressings from group **c.** may become also occlusive and increase microorganisme spreading and infection.

2. **Moisture regulation:** The optimal moisture at the wound's surface should be delicately balanced between desiccation that leads to cell death and matrix changes and overhydration that leads to maceration. Adherent dressing may normally keep the optimal

moisture unless they are hydrophilic (desiccating) by nature and in this case may desiccate the wound. Hydrating dressing with high water content may overhydrate the wound acting also as occlusive dressings increasing the danger of infection and infection propagation. The moisture condition in the individual wound is dynamic and may change within hours. A "moist" highly exudating wounds that may need drainage by absorbing dressing may become dry, recalcitrant wound by too active desiccation or even exposure. In the case of burn, "weeping" surface of a dermal scald burn or a fresh, raw donor site may desiccate in few hours of exposure and develop a layer of neo-eschar: a layer of desiccated, newly "killed" dermis. This dry, adhering layer can, if not infected, serve as a biological dressing but at a price: death of tissue that could be instrumental in the healing process and a danger of contamination and infection of this new eschar.

All vital functions of healing and proliferation, healing factors and cellular activity need moist environment for their function. Nevertheless, microorganism survival and propagation need also moisture. The dressing we use should allow us to intervene and correct these changing conditions.

Dressings from the group of "hydrogels", "gelfoams", all occlusive dressings-films, some of the adhering dressings, all topical gels, creams and ointments will maintain the bed moist and offer good healing conditions but a continuous monitoring should be maintained to intervene in time to prevent too much moisture, maceration and/or infection.

3. **Epithelialization enhancement:** The progression of the epithelialization process is a complex one, involving the inbuilt epithelial progression based on the contact inhibition phenomenon with the proliferation of the keratocytes from the wound's exposed edges and the epithelial islands originated in the skin adnexae. The Keratocytes propagate slowly over the dermal matrix in a moist atmosphere. The following are conditions that the propagation and the entire epithelialization process may be disturbed even in presence of sufficient epithelial sources (epidermal wounds edges, skin adnexae or autografts):

- Mechanical disruption: dressing change, forced scraping or wiping of the healing wound will peel off the friable new epithelium from its bed
- Mechanical barrier: such as to adherent dressing
- Infection: may destroy keratocytes
- Eschar presence: beside being a mechanical barrier that will not allow epithlialization over viable bed is also a potent source for infection
- Actively granulating tissue: ("overgranulation") that will stop epithelialization by forming a physical barrier and biological competition by the fibroblastic activity.
- Topical medications: may be noxious to the proliferating keratocytes (i.e. SSD, Iodine compounds)
- Systemic conditions: mainly malnutrition as expressed by hypoproteinaemia and hypoalbuminaemia or Oncological cytotoxic drugs.

4. **Fibroblastic activity modulation:** The Keratocytes and Fibroblasts originate from two different embryonic cellular origins: ectodermis and mesodermis. In spite of a very intimate relation in structuring the skin and forming the Epidermis and Dermis each of

them exerts an inhibitory effect on the other. Fibroblasts are very active cells that proliferate in wound beds forming the three dimensional granulation tissue (together with new blood vessels by endothelial cells, also from Mesodermal origin). The Keratocytes, proliferate and propagate along the dermal superficial plan striving to from the Epidermis. They exert an inhibitory effect on the granulation tissue formation, arresting its development as soon as they cover it with the newly formed epidermis. The Fibroblasts and the granulation process are more robust than the thin epidermal, Keratocytes unicellular epithelialization healing front. Thus, if epithelialization process will not cover the healing bed, modulating and arresting the formation of rich granulation tissue the granulation tissue can become Overgranulation, forming a barrier to the Keratocytes and the epithelialization process and eventually becoming a heavy, contracting and deforming scar.

In view of such a situation of developing granulation tissue in front of epithelialization process we can intervene by providing the Keratocytes with the optimal conditions (moisture, propagation surfaces and protection) and trying to interfere and decrease the overgranulation process. This can be done by mechanically scraping the overgranulation tissue, chemically destroying its surface ($AgNO_3$) or using short (2-4 days) courses of topical corticosteroid treatment. The later treatment will effectively inhibit the granulation process allowing epithelialization to propagate and exert it own inhibitory effect on the fibroblasts. Topical steroids in given in shorts spells of few days each for over a month will not exert systemic effect and may be considered as safe. A wound healing dressing should promote epithelialization (that controls granulation) and allow us to intervene medically or mechanically interfering with overgranulation and promoting epithelialization.

5. **Infection control:** Adherent dressing will prevent infection and occlusive conditions may favor it. Some dressings may be the source or serve as a medium for microorganisme growth (rejected allografts or xenografts, infected collagen or HA based dermal substitutes such as *Integra, Hyalomatrix*,). The dressing's structure may itself harbor, as a foreign body, microrganismes (fibrous dressings such as cotton gauzes, some of the foams, other absorbing materials- *Hydrogels* and even hydrofibres-*Aquacel* to a certain point). Some of the dressings contain antibacterial agents (mainly silver) but they are not innocent. In such case that the dressing itself became contaminated we may wish either to replace it or be able to treat it with anti bacterial agents.
6. **Trans-Dressing Diagnosis of changing conditions (TDD):** Being aware to the rather fast dynamic process, beneficial or not, that may happen in the healing wound the dressing should allow the diagnosis of emerging situations that may need our intervention. Process such as infection, granulation, overgranulation and epithelialization should be diagnosed and monitored in real time. An opaque, adherent, unchanged dressing may make such a diagnosis difficult. Dressings that are changed frequently (every 1-3 days at most, i.e. all topical medications, hydrogels, etc.) make such a diagnosis (on dressing change) easy. Some transparent dressings allow diagnosis through them (i.e. *Jaloskin)* or staining of their external surface when exudates from the wound ooze through (i.e. *Aquacel, Polymem*).
7. **Trans-Dressing Intervention (TDI):** Adherent dressings that can remain for days, preventing infection, offering optimal healing conditions and allowing diagnosis of the

processes that take place underneath are obviously ideal. The last possible criterion for an optimal dressing is the possibility to treat the wound bed through the dressing without disrupting it. Some of the occlusive dressings (*Occludress, VAC+ all vacuum assisted dressings)* could be connected to intake ports others, absorbing dressings such as gauze could be soaked without disruption.

Wound Dressings and Covers

The effect of the dressing's physical properties

As previously mentioned the role of the dressing is to protect and create an optimal healing microenvironment. Moisture and temperature that allow gases exchange and surfaces for epithelial migration, function of cells such as macrophages, neutrophils and fibroblasts. Another alternative are all the topical medications that need replacing or renewing daily or every other day.

Absorbing & Non-absorbing

The absorption function may depend on the physical structure (filament, fibers, hydrofibres, foams, etc.) or specific absorbing (hydrophilic mostly) materials the impregnate the dressing (starch, HA, alginates, collagen to a lesser measure, etc.). The passage of the fluids (exudates, soaking solutions, etc.) to or from the wound surface depend on the concentration gradient between the wound and the dressing and the characteristics of the dressing itself (potential fluid capacity, hydrophilic characteristics). Once fluid completely saturates the dressing the absorption power is cancelled and moisture at the wound surface increases to the point of maceration. Dressings with high absorption power may desiccate the wound to the point of tissue death. In order to maintain the absorption function the dressing should always be under its maximal saturation. This can be done by replacing the dressing before saturation or adding an external dressing that will absorb, through the primary dressing the fluid access.

a. Antibacterial Topical Creams and Ointments

Topical medicaments in the form of creams and ointments are innumerable, based on various antiseptic and/or antibiotic in different excipients preparations *(few examples: Bacitracin, mupirocin, silver sulfadiazine, neomycin, polymyxin, honey inhibine, HA based creams)*

Indications: prophylaxis of infection, ointment maintain moist wound environment and can be used as covers in wound healing process.

Burns care involves often the more effective combination of soaking and creams/ointments (*silver nitrate compresses, chlorhexidine gluconate, mafenide acetate cream 0.5%, povidone iodine, nitrafurazone, silver sulfadiazine*).

Application: once or twice a day after washing and cleansing the wound.

Contraindications: Allergic reactions, decreased cellular activity and viability.

Complications: Drug specific, usually without risk of systemic toxicity. Local adverse effects include contact dermatitis (i.e., neomycin is as high as 34%). Application of silver sulfadiazine has been associated with neutropenia and epithelialization inhibition.

Special points: Bacitracin zinc is extremely popular because of low cost, low toxicity, and low allergic contact dermatitis. Relatively safe; topical antibiotics have low systemic absorption. HA based creams have additional benefit due to the HA base.

b. Solutions for Irrigation & Soakings.

Topical medicaments solutions are routinely used for soaking and irrigation of wound over a primary dressing, usually gauze. They are used for initial cleansing, being effective antimicrobial agents and mechanically washing away contaminants particles. With antibacterial agents they may be used for infection control. Corticosteroid addition will control topical inflammation and over granulation and with hypertonic solutions they can reduce cellulites, edema and swelling and even be used as antiseptics. Leaving the soaked gauze to dry will "suck in" the superficial dissolved materials be it dirt, bacteria or dissolved eschar allowing its removal. If the gauze is allowed to desiccate and bond to the bed surface its removal will tear off the surface ("wet to dry" cleansing). The following are common solutions used in burn and wound care: Saline (0.9%), Hypertonic Saline (<20%), $MgSO_4$ (<10%), Chlorhexidine acetate (0.5%), Chlorhexidine gluconate (0.05%-0.1%), Sulfamylon 3%-5%, povidone-iodine, $AgNO_3$ (0.5%), corticosteroid solutions, antibiotic +/-corticosteroid solutions, PHMB (Polyhexamethylen biguanide) solution, Prontosan® (Betain + PHMB).

Tissue toxicity is possible, caution should be used in the management of open wounds as well as wound desiccation. Corticosteroid long term (weeks), continuous soaking may cause transient, systemic Cushingoid symptoms.

c. Gauze, gauze-like: woven & non-woven, cotton-base, not-cotton-based

Cotton or cotton-like fibrous materials are used since antiquity as dressings. The pliability, adherence, absorption and their capacity to serve as a matrix to many medicaments and materials made them the current choice in wound care. This characteristic make them serve as a"reinforcement" for the creation of the scab that is the natural dressing for the healing wound. The fibrin from the raw bed is absorbed by the dressing that remains embedded in the solidified fibrin, forming a strengthened scab that by its nature (it is nature's raw bed's dressing) adheres to the bed. Due to their fibrous structure they serve also as wicks for soaking, passing the solution and bathing the bed. They can also serve as absorbing dressings passing the exudates to the surface, to be reabsorbed by additional, external, absorbing dressings. Normally, they will not adhere to full thickness wounds unless adhere through fibrous bonds that in every dressing-change (daily) will damage re-epithelialized layer delaying healing. The problem with such dressings is that they may shed particles that some become embedded in the healing bed, forming later foreign body inclusion cysts.

d. Impregnated Dressings including Silver Dressings

Dressings coated or impregnated with any material that is conceived to be beneficial to any of the wound healing phases or factors are produced by all wound care companies. Each of these agents has its advantages and its potential risks and drawbacks and should be assessed individually. The dressings can be made of many kinds of matrixes (cotton woven or non woven, films of various permeabilities and geometries, foams, hydrofibres, alginates, etc.).

The choice of the dressing should be done according to the matrix characteristics (e.g. the difference in the properties of cotton gauze and hydrofibres) and the impregnating medication (e.g. the difference between SSD and Paraffin ointment) considering the specific requirement/s of the wound and its healing phase. Few examples are:

Paraffin with or without antimicrobial medicaments, coating woven, non woven or other primary form of dressings (such as *Jelonet, Neotulle, Aquaphor-Gauze, Paranet, Unitulle,*

Bactigras etc.): It will keep it moist, will not adhere to the bed, may increase infection danger, should be changed every 2 days at most.

Silver compounds of different formulations *(Acticoat, Silvelon, Atrauman Ag, Biatain Ag, Aquacel Ag, Contreet Hydrocolloid, Mepilex, Physiotulle Ag, PolyMem Silver, Silvercel, Suprasorb A+Ag, Tegaderm Alginate Ag, Urgocell Silver, Urgotul Silver):* Silver ions (Ag+) have antibacterial effects, hyper sensitivity and arginosis (staining by silver oxide) of tissues has been reported.

SSD impregnated matrixes *(Allevyn Ag, Urgotul SSD):*widely used as a local antimicrobial, not to be used in sensitive to silver or sulfa drugs, known to delay epithelialization, kerenicterus has been reported.

e. Biological covers (autografts, allografts, xenograft, skin substitutes and equivalents)

The biological covers have a complex three dimensional collagen or collagen-like matrix, saturated by mucopolisaccaride compounds such as Hyluronic Acid and usually covered by semi permeable layer (epidermis, silicone). They are applied on clean, viable surfaces, initially adhere by fibrin bonding and serum imbibition followed by in-growth of new vessels and fibroblasts from the bed. When they adhere ("take") on a clean and viable bed all provide good protection and healing conditions. The ones with antigenic potential (allograft and xenograft) should be replaced before immune rejection occurs.

- **Autografts** are the classical means for permanent wound closure. In full thickness defects they provide a complete closure by primary intention healing. Deep dermal defect will also heal in a similar way by accepting the graft as a permanent cover but dermal remnants that contain epidermal foci (skin appendages) may also heal by epithelialization under the graft forming cysts or unsightly overgrafting patches. Autograft is a costly means, requiring surgery and sacrifice of healthy donor site skin areas and should be reserved for permanent closure for full thickness defects where spontaneous healing is impossible or when a fast closure is so important as to abandon the somewhat slower epithelialization of the dermal surface.
- **Allograft** (fresh or cryopreserved) is used as a temporary solution to close the wound. It will "take" as autograft but will be rejected by the immune reaction 7-14 days later. On a clean IL bed they provide good healing conditions for the dermal IL and a temporary closure for full thickness defect. The epithelialization process will take place under the graft protection with the sloughing graft exposing the healing bed. The allograft keratocyte's growth factors can promote the process but infection may harm the healing process. As the allograft is practically occlusive it is advisable to perforate or mesh it without expanding it to allow for a drainage and potential treatment medication. Use of allograft does not require surgical facilities but the availability of allograft may pose a problem in some countries. Their availability may be limited to the skin bank services and their shelf life is limited to a week when fresh and 3-4 years when frozen. Glycerol preserved allografts have a longer shelf life and do not need skin bank preservation facilities but are not viable, do not "take" but bond to the surface by fibrin, being less effective than fresh or cryopreserved.
- **Xenograft** (fresh or cryopreserved, *Mediskin I*) usually from porcine origin, as allografts they are used as a temporary cover to "close" the wound. Fresh or cryopreserved, it will "take" as allograft but will be rejected by the immune reaction in few days. On a clean IL

bed they provide good healing conditions for the dermal IL and a very temporary closure for full thickness defect. The epithelialization process will take place under the graft protection with the sloughing graft exposing the healing bed. It is more infection-prone and should be applied meshed and medicated with antimicrobial agents. The glycerolized xenograft has a better shelf life but is even less effective and more infection-prone. Use of xenograft does not require surgical facilities but its availability may pose a problem in some countries.

Skin Substitutes, Biodegradable & Non-biodegradable: Collagen, Hyluronic Acid (HA), Synthetic (DL-Lactide)

There are number of materials that the inflammatory and healing process can degrade thus avoiding removing them at the end of the healing process. The problem is always to decide whether we want this degradation process in addition to the normal inflammatory, healing one. Biological materials such as Collagen, Hyluronic acid or DL Lactide- polyglactin (the raw material for Vicryl sutures) are all used as films or three dimensional sponge-like structures. In full thickness wounds, some are designed to serve as templates for new dermis formation (Collagen: *Integra, Matriderm*, HA: *Hyalomatrix*) by providing a growth matrix for capillaries and fibroblasts to be grafted with thin autograft when their survival ("take") is secured. Similar approach is shared by other products: *Dermagraft* (non-immunogenic neonatal fibroblast on polyglactin mesh), *OrCel* (Fibroblasts layered onto bovine collagen matrix), *TransCyte* (Shipped frozen for use on partial- and full-thickness burns, epidermal layer with dead fibroblasts layered onto a nylon mesh), *Apligraf* (dermis-like matrix from cultured heterogenous human foreskins and bovine collagen type 1, has epidermal and dermal components), *Oasis* (porcine, small-intestine submucosal dehydrated dressing with longer shelf-life, rehydrate when needed with a secondary dressing to prevent desiccation), *Alloderm* (Freeze-dried, cell-free).

Another group is design to serve as a cover for clean, dermal wounds providing healing (epithelialization) conditions and disintegrating when epithelialization is completed (Collagen films and sponges, HA *Jaloskin* or DL Lactide *Suprathel)*. All need a clean bed to adhere to, with Suprathel in need of a meticulously dermabraded bed. All are sensitive to infection. Collagen and HA can be used to coat synthetic matrixes or fabric (Nylon-*Biobrane*). *Biobrane* is common in wound care, peeling off at the end of epithelialization but is prone to desiccation of the bed and infection.

f. Inert, synthetic materials

The alternative to biodegradable materials are the ones that are completely inert, most are synthetic providing the required conditions to the wound bed but are not part of the healing process and peel-off, trimmed away as wound heals by the epithelializing front. Another alternative are all the topical medications that need replacing or renewing daily or every other day.

g. Foams

Synthetic (i.e. polyurethane or silicone) foams may by more or less absorbent depending on their structure (large or small, open or partially open cells) and the additive materials (e.g. starch, SSD, silver) (i.e. Allevyn, Flexzan, Lyofoam, Sof-Foam*)*.

They may be considered as a farther development of more simple dressings such as: impregnated multilayer gauze, Polyurethane or gel film coated, hydrophilic or hydrophobic,

semiocclusive, bilaminated dressings. Foam dressings offer most of the advantages of medicated gauze with a better absorption and protection in most cases. Very often they adhere to the bed (incorporation by ingrowths of granulation tissue and/or bonding by fibrin), creating the required adhering, moist environment and absorbing exudates from discharging wounds thus reducing the need for dressing changes. Foams provide also mechanical protection but in many cases their permeability can be obstructed by hardened exudates transforming them to opaque, occlusive dressings that are prone to infection.

h. Films, permeable, non permeable

These dressings, may be completely occlusive or permeable to gas and water vapors, but are a barrier to bacteria and water. Films, Semiocclusive/semipermeable made of polyurethane or copolyester, approximately 0.2 mm thick (*Bioclusive, Polyskin II, OpSite, Tegaderm)*. They are elastic and transparent but difficult to occlude larger that few cm^2 areas. They are commonly used as occlusive dressings, sometimes with additional medicated gauze or topical medication underneath as a primary dressing, creating a microenvironment over the wound that can contain gazes (0_2, 0_3, or vacuum etc.) or medicaments. On clean beds they may adhere through the bonding effect of the wound exudates and fibrin but usually are transparent allowing easy assessment of the wound bed. When adhering to the bed they are generally reserved for the definitive closure of superficial, shallow partial thickness wounds where comfort and ease of management are important. The dressings can be left in place for several days, but their occlusive nature and accumulated exudates make them extremely prone for infection and daily monitoring is important.

i. Hydrocolloids

These dressings are non adhesive, water and gas impermeable membranes (Comfeel, Cutinova, DuoDERM, Intrasite, Replicare, Hyprapad Tegagel®, Intrasite etc.*)*. Composed mainly of water held in a complex network or fibres that keep the polymer gel intact. Water is released to keep the wound moist.

When the inner layer comes into contact with exudates, it forms a gel providing adequate moisture, the liquefied gel and exudates fill the dressing that has to be changed. If the dressing dries it may adhere to the bed interfering with healing and may damage, tear-off new epithelium when removed. Obviously the occluded moist atmosphere is prone to infection propagation and sometime to overhydration and maceration. Hydrocolloids can be applied directly to clean wound but not on eschar or tendons. Perform dressing changes every 3 to 7 days with saline irrigation cleans colloidal layer. Hypersensitivity to materials can develop.

j. Aquacel (with or without Ag.)

Soft non-woven pad made from sodium carboxymethylcellulose fibres. These Hydrofibres absorb exudates and fluids from the wound becoming a gel like material. Germs thus absorbed are eliminated from the wound surface but the dressing itself may dry to become a dry adherent shell. The desiccation may dry viable surface extending the original damage but the dry shell allows discharge before wound is closed with epithelial cells slowly separating the dry dressing from its bed.

k. Alginates

Composed of calcium alginate (a seaweed component). When in contact with wound, calcium in the dressing is exchanged with sodium from wound fluid and this turns dressing into a gel

that maintains a moist wound environment. Do not use on low exudating wounds as this will desiccate the wound. Dressing should be changed daily to avoid desiccation.

E.g. Kaltostat, Sorbsan Alginates are derivatives of seaweed are in the form of dry non-woven mats, absorbing wound exudates to become a hydrophilic gel like hydrocolloids and behave in a similar way (Kaltostat, Sorbsan, Algosteril, AlgiSite, Kaltostat, Sorbsan etc.). Jellified alginates are not adhesive, they are easily washed away but applied on a moist, clean bed they may strongly adhere to become a hard shell that will protect the wound but may slow the healing process and be hard to remove, injuring and stripping the healing bed. It is a strong hemostatic that can be used on bleeding wounds (post tangential excision or graft harvesting) but can interfere with healing later. A secondary wet dressing may prevent desiccation. Soaking and irrigation of the desiccated dressing will jellify it again and ease changing. Hypersensitivity to materials. Thyroid abnormalities can develop.

l. Hydrogels-gelfoams

These gels are usually based on starch polymers providing ample moisture (Carrasyn, Intrasite, Lamin, Nu-gel, Vigilon, etc.). Hydrogels are best suited to autolyse dry necrotic wounds, but they also absorb exudates while maintaining the products of tissue repair and degradation, including growth factors and lysosomes, in contact with the wound. If the wound is clean, the gel only needs to be replaced once or twice a week providing good healing conditions but these conditions are also favorable for infection propagation (especially gram negative bacteria).

m. Negative Pressure Wound Therapy

It is still a constantly evolving technology based on an occlusive dressing connected to a vacuum pump, containing a fibrous or foam matrix that distribute the sub-atmospheric pressure onto the wound bed. There are numerous similar available systems differing mainly in price and nature of matrix. Used initially mainly for chronic ulcers, it is quite effective as a secondary dressing to secure skin graft adherence and take.

APPENDIX 5- WOUND MANAGEMENT; APPROVED COVERS

General remark: the guidelines of this appendix follow the ABA White Paper guidelines with detailed additions of some commonly used topical treatments.

All dressings that are films, sheet-like (including biological dressings such as autografts, allograft and xenografts) and even foams, hydrocolloids and gauzes can become occlusive (sometimes by desiccated exudates) and pose danger associated with occlusion to healing wounds. It is recommended to perforate or mesh (without expansion) such dressings and if possible, apply a negative pressure dressing (e.g. VAC, etc.) on top of them for few days to ensure adherence and "take"). There are many additional dressings that strive to fulfill wound healing goals beside the approved to be used in the study detailed below. Some of them, beneficial as they may, are not standard. If the PI wishes to use then in this study, MediWound clinical department should be approached to verify and approve their use.

Below is a list of the approved wound care products in the study which are all legally marketed in the US as detailed in the ABA guidelines.

In the study protocol, there are 2 main categorizes of wound care products:

Skin substitute [commercial product]: A biomaterial, engineered tissue or combination of materials and cells or tissues that can be substituted for skin autograft or allograft in a clinical procedure.

Skin replacement: A tissue or graft that permanently replaces lost skin with healthy skin.

Temporary Wound Coverage

Temporary skin substitutes are used when the wound is too extensive to be closed in one stage because there is not enough donor skin available, because the patient is too ill to undergo the creation of another wound that results when skin is harvested from a donor site, because there is a question regarding the viability of the recipient bed, or because of a concern regarding potential infectious complications. The gold standard temporary skin substitute is **cadaver allograft**. Other temporary skin substitutes are used to provide transient wound coverage and to create a physiologically homeostatic environment such as- **Skin Xenografts**—also termed heterografts.

Biobrane® is a silicone membrane pressed onto a flexible nylon fabric which has been coated with porcine dermal collagen. Used as a temporary wound cover, it is considered a dressing by the FDA and CPT Editorial Panel.

Transcyte®, a related product, is not a dressing. It is classified as a temporary skin substitute and is composed of Biobrane® and cultured newborn human fibroblasts, which secrete growth factors and matrix proteins onto the wound surface. TransCyte® must be applied to a clean wound bed and monitored closely because it is prone to infection. It can be used in ways similar to Allograft and Xenograft.

Permanent Wound Coverage

A **full-thickness skin graft** contains all components of the skin: epidermis, dermis, hair follicles and nerve endings. The most important advantage of full-thickness grafts is decreased scar formation; however, because there is no dermis to regenerate epithelial coverage, the donor site of a full-thickness skin graft must be closed either with primary direct closure or with a split-thickness skin graft; local advancement of skin flaps is a rarely used option. Full-thickness grafts are thus usually used to cover small, functionally and cosmetically important areas (such as eyelids and digits).

The **split-thickness skin graft** is the most common method used to achieve permanent wound coverage. It includes the entire epidermis but the dermal layer is split by the dermatome blade. With thicker split-thickness grafts, more dermis is transferred with the skin graft (such as 15/1000 of inch or greater). This reduces the risk of scar formation at the recipient site, but it takes longer for the donor site to heal. Furthermore, the thicker the graft, the more likely the donor site will heal with a scar and the less likely the donor site may be reharvested for further grafts.

There are a number of commercially available products to facilitate permanent wound coverage. **Acellular human dermal allograft (Alloderm®)** is devoid of epidermis and must be covered by a thin split-thickness autograft at the time of the initial operation; however, it replaces a portion of the missing dermis on the newly covered wound, thus reducing postoperative scarring. Another permanent wound coverage product is a **dermal regenerative template (Integra®)** constructed with bovine collagen and shark chondroitin sulfate with a silicone surface layer. This is applied to an excised wound bed that is well vascularized and free from infection, and provides wound coverage much like other skin substitutes; however, after the template becomes vascularized, it forms a neodermis (usually within 10 days to three weeks) and when the silicone layer is removed, an epidermal autograft (< 0.008 inch thickness) can be applied. This intermingling of autograft on a biosynthetic neodermis is permanent, unlike the wound coverage provided by temporary skin substitutes. This approach may be lifesaving and provides quality skin coverage.

Cultured epidermal autograft (CEA; Epicel®), also referred to as “test tube” skin, was introduced by Rhinewald & Green in 1975 and is often employed to provide permanent skin coverage for patients with extensive burns.

All of the above surgical or non surgical agents and covers are routinely used in the US, specified in the protocol and are allowed to be used in the proposed study based on the investigator’s medical judgment for the specific wound condition or patient.

APPENDIX 6- REGULATORY AND ETHICAL ISSUES

Compliance with Regulations Applicable to Clinical Trials

The study will be conducted according to the laws, regulations and administrative provisions relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, as applicable by national legislation and EU Directives and US 21 CFR Part 11, 50, 54, 56, 312.

This clinical trial will be registered on the "clinicaltrials.gov" clinical trial registry website as required by 42 USC 282(j) and the State of Maine's PL 205, c. 392, §1.

Informed Consent

The principles of Informed Consent, conducted in accordance with Declaration of Helsinki and its updates, current Good Clinical Practice (GCP), the provisions of ICH (International Conference on Harmonisation); US Code of Federal Regulations (Title 21, CFR Part 50,) and/or EU Directives; local country regulations and the sponsor's Standard Operating Procedures (SOPs) will be followed. A subject is not allowed to enter a clinical study until he/she has been properly informed, has been given time to contemplate participation, and has freely given his/her consent by signing and dating the Ethics Committee/Institutional Review Board (EC/IRB) approved informed consent form. This must be done prior to performing any study related procedures.

The proposed consent form and any other documents relevant to the consent process must be submitted to the EC/IRB, together with the protocol, and must be approved prior to study start.

A copy of the fully signed and dated Informed Consent Form and any other documents relevant to the consent process will be given to the subject and the original will be maintained at the site.

Subject Confidentiality

All subject data will be identified only by a subject identification number, subject initials (may be dummy) and date of birth.

The investigator should keep the initials given in the "Subject Identification Log". After obtaining subject's consent, the investigator will permit the study monitor, independent auditor or regulatory agency personnel to review that portion of the subject's medical record that is directly related to the study. This shall include all study relevant documentation including subject medical history to verify eligibility, laboratory tests results, admission/discharge summaries for hospital admissions occurring while the subject is on study, and autopsy reports for deaths occurring during the study (if applicable).

Good Clinical Practice

The study described in this protocol will be carried out according to the local regulatory requirements (FDA, EU, other) and ICH accepted standards of Good Clinical Practice.

Ethics Committee (EC) / Institutional Review Board (IRB)

The study must have unconditional approval in writing, by an appropriate Ethics Committee/Institutional Review Board (EC/IRB). A copy of the Letter of Approval from the

EC/IRB, which contains specific identification of the documents approved, must be received by local Sponsor prior to site activation.

Any amendments to the protocol or subsequent changes to the informed consent form as a result of changes to the protocol and/or investigator brochure that is approved by MediWound, must also be approved by the EC/IRB and documentation of this approval provided to MediWound. Records of the EC/IRB review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to the sponsor's audit and/or regulatory authority inspection, during or after completion of the study.

Serious adverse events (SAEs) must also be reported to the EC/IRB by the investigator or the sponsor according to local regulations.

The investigator must submit a periodic status reports to their EC/IRB as required, as well as notification of completion of the study and a final report where applicable. A copy of all reports submitted to the EC/IRB must be sent to the sponsor.

Protocol Amendments

Protocol amendments must be prepared by sponsor or designee and approved by the sponsor, Competent Authorities (if applicable), Central EC/IRB and/or each respective site's EC/IRB (if applicable), prior to implementation.

Changes to the protocol can only be made by an approved protocol amendment. In case of urgent safety amendment ICH GCP guideline, should be followed.

For clinical trial sites located in EU Member States, the procedures outlined in Directive 2001/20/EC, Article 10(a), are applicable.

Declaration of the End of the Clinical Trial

For clinical trial sites located in EU, a declaration of the end of the clinical trial will be made according to the procedures outlined in Directive 2001/20/EC, Article 10(c) and for those countries outside EU local regulations will be followed.

Liability And Insurance (If Applicable)

A Certificate of Clinical Trials Insurance will be provided to the study centers by MediWound Ltd.

APPENDIX 7- STUDY ADMINISTRATIVE PROCEDURES

This study will be conducted, recorded and reported according to ICH E6 requirements "*Guideline for Good Clinical Practice*" and its other implementing directives and guidelines.

Study Site Initiation

After the investigator files were updated with the following documents, the site may begin patient recruitment:

1. CV and Medical License of the Principal Investigator,
2. A signed and dated Investigator Protocol Agreement,
3. A Financial Disclosure form,
4. Signed Agreement between MediWound and the trial site,
5. The laboratory certification or proficiency ratings, the normal ranges for laboratory determinations required by the protocol and the curriculum vitae of the laboratory director,
6. A copy of competent authority written notification of protocol approval (EU sites), study-specific informed consent form and any advertisements, notices or bulletins to be used in this study,
7. A copy of the ethics committee/IRB approval,
8. The ethics committee name, address and a list of ethics committee members including occupations and institutional affiliations,
9. Signed Investigator Brochure receipt.

Informed Consent

Protection of clinical trial subjects will be performed as follows:

A clinical trial may be undertaken only if the following elements are fulfilled:

1. The study may be initiated only if the ethics committee and the competent authority come to a conclusion that the anticipated therapeutic benefit justifies the foreseeable risks and inconveniences.
   a. Prior to signing the informed consent, the trial subject had a discussion with the investigator or a member of the investigating team concerning the objectives, risks and inconveniences of the trial and the conditions and has been informed of his right to withdraw from the study at any time.
   b. Neither minor nor patients not being able to consent on their own are not included in the study However, legal representative may be involved in the Inform Consent procedure and sign the form if this is pre approved by the local IRB/EC and only for subjects with temporary medical conditions related to the injury, subjects who might be under the influence of pain medication, sedation, etc (see Section 8.1.1).
   c. The rights of the subject to physical and mental integrity, to privacy and to protection of the data concerning him are safeguarded.

d. If the subject is unable to write, an oral consent in the presence of at least one witness may be given in exceptional cases, as provided in national legislation.

e. Provision has been made for insurance or indemnity to cover the liability of the investigator and the sponsor.

f. The medical care given to, and medical decisions made on behalf of, subjects shall be the responsibility of an appropriately qualified physician. The subjects shall be provided with a contact where he may obtain further information.

2. In addition to the above restrictions the following elements are to be fulfilled in clinical trials on minors and incapacitated adults:

a. The subject should receive information regarding the trial, the risks and benefits, from an experienced staff,

b. The investigator should consider explicit wish of the subject who is capable of forming an opinion, to refuse participation or to be withdrawn from the clinical trial at any time,

c. No incentives or financial inducements will be given except compensation,

d. The study should relate directly to the clinical condition from which the subject concerned suffers,

e. The study is designed to minimize pain, discomfort, fear and any other foreseeable risk, being based on common, routinely practiced thermal burns SOC strategies and techniques

f. The ethics committee, with expertise in the relevant disease and the patient population concerned or after taking advice in clinical, ethical and psychosocial problems in the field in the subject's population involved, has endorsed the protocol,

g. The interests of the patient always prevail over those of science and society.

3. The following issues should be included in the Informed Consent discussion and the written Informed Consent Form:

a. A statement that the study involves research,

b. Discussion with the subject should include: an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed and identification of any procedures that are experimental,

c. The trial treatment(s) and the probability for random assignment to each treatment,

d. Disclosure of any available alternative procedures or courses of treatment that might be advantageous to the subject,

e. A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the regulatory authorities, the institutional ethics committee or MediWound or designee may inspect the records,

f. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is normally entitled,

g. The subject's responsibilities,

h. A statement that the particular treatment or procedure may involve risks to the subject (or the embryo or fetus if the subject is, or may become, pregnant) that are currently unforeseeable,

i. Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent,

j. Any additional costs to the subject that may result from participation in the research,

k. A statement that significant new findings developed during the course of the research, which may relate to the subject's willingness to continue participation, will be provided to the subject,

l. The approximate number of subjects involved in the study,

m. The person(s) to contact for further information regarding the trial and the rights of trial subjects.

The Principal Investigator or designee is responsible for obtaining written informed consent from potential subjects prior to entry into the study. The investigator must keep each subject's signed consent form on file. A copy of the signed document must be given to the subject.

Competent Authority (CA)[1] and the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC)

This study must be approved by the CA (EU sites) and the ethics committee prior to patient enrollment.MediWound will obtain a EudraCT number before submitting an application to the CA. Each member state CA shall be provided with a list of CAs to which application was made, with a copy of their decisions as soon as it is available.

The investigator must maintain an accurate and complete record of all submissions made to the CA including a list of all reports and documents submitted. The investigator agrees to promptly report to the ethics committee all unanticipated problems involving risks to human subjects or others.

MediWound will inform the CA and the ethics committee of any new information relating to the conduct of the trial or the development of the IP where it is likely to affect the safety of subjects. Any safety measures taken should be reported to the authorities. Periodic status reports must be submitted at least yearly to the IRB/IEC.

MediWound Ltd. shall notify the competent authorities of the member states concerned and the ethics committees that the clinical trial has ended within 90 days of the end of trial. If the trial

[1] Relevant for sites located in the EU.

has been terminated earlier than expected, notification should be made within 15 days and reasons clearly explained.

Electronic Case Report Forms (eCRFs)

No data will be directly entered into the e-CRF without source documentation.

The CRF is an integral part of the study and subsequent reports. The Case Report Form provided by MediWound Ltd. must be used to capture all study data recorded in the subject's medical record. The CRF must be kept current to reflect subject status during the course of the study. Only a subject identification number and subject initials will be used to identify the subject. The investigator must keep a separate log of subject names and medical record numbers (or other personal identifiers).

The protocol will use an Internet-Based Remote Data Entry System, primarily to collect clinical trial data at the investigational sites. The system will comply with 21 CFR Part 11 and ICH E6. The system will be used to enter, modify, maintain, archive, retrieve, and transmit data. Paper source documents are to be retained to enable a reconstruction and evaluation of the trial. No original observations will be entered directly into the computerized system. Source documents include the hospital patient files and study worksheets provided by the Sponsor. Data will be recorded in the study worksheets as appropriate in order to complete and/or clarify source data.

The design of a computerized system complies with all applicable regulatory requirements for record keeping and record retention in clinical trials (21 CFR Part 11 and ICH E6 Good Clinical Practice) to the same degree of confidence as is provided with paper systems. Clinical investigators must retain either the original or a certified copy of all source documents sent to a sponsor or contract research organization, including query resolution correspondence. The system is designed so that changes to any record do not obscure the original information. The audit record clearly indicates that a change was made and clearly provides a means to locate and read the prior information. All changes to the data have an electronic audit trail, in accordance with 21 CFR 11.10(e). Electronic signatures will be used in conformance with 21 CFR Part 11.

Corrections are made in source documents by crossing out the error with a single line, making the correct entry in close proximity to the data field, then initialing and dating the strike-through and the new entry.

Maintenance of Study Files

All essential documents listed in ICH Guideline CPMP/ICH/135/95 should be filed in an organized way that will facilitate management of the clinical trial and audit.

Agreements between the involved parties, all Institutional Review Board correspondence including notification of protocol and consent form approval and all annual reports along with the final report to the IRB must be retained.

Study records including patient source documents, Case Report Forms, all test results and signed informed consent forms must be kept on file by the investigator.

Other documents pertaining to the conduct of the study including laboratory normal values, laboratory certification, monitor logs and telephone reports and all written correspondence must also be kept as part of the permanent record.

Storage conditions: Essential documents should be maintained in a legible condition and so that they can be promptly retrieved. Any change in the ownership and location of the documentation should be documented in order to allow tracking of stored records.

The investigator should make the sponsor aware of the storage arrangements for the documents. If the investigator for any reason cannot keep the study records, MediWound Ltd. should be notified in writing so that alternative arrangements can be agreed upon.

Essential documents should be retained until at least 2 years after the last approval of a marketing application and until there are no pending applications or at least 2 years elapsed since the formal discontinuation of clinical development of the investigational product. It is the responsibility of the MediWound Ltd. to inform the investigator/ institution as to when these documents no longer need to be retained.

These regulations are described in: Detailed Guidelines on Trial Master File and Archiving – July, 2006.

Monitoring Procedures

Investigational sites will be monitored periodically to assure satisfactory patient enrollment, data recording and protocol adherence. The frequency of monitoring may vary depending on enrollment rate and the quantity of data collection. The investigator and staff are expected to cooperate with the study monitor and provide to study monitor all relevant study documentation. It is essential that the investigator and study coordinator set aside a sufficient amount of time for these visits to permit an adequate review of the study's progress and of completed Case Report Forms.

Changes in Study Personnel

The 'Authorization Signature Log' which is a list of appropriate qualified persons to whom the Principal Investigator has delegated significant trial-related duties, should be updated if there is a change or addition of any personnel initially listed. CVs and other relevant documents evidencing qualifications should be provided to MediWound Ltd.

On-Site Inspections/Audits

In accordance with section 1.29 in ICH E6 inspections may be requested and coordinated by the European Medicines Agency or the FDA. Inspections may take place at various times: before, during and/or after the conduct of the clinical trial. The investigator and staff are requested to cooperate with all such audits. The investigator must notify MediWound Ltd. of any expected inspection as soon as possible. A representative or designee of MediWound, Ltd. Clinical Research Department, may also conduct similar auditing procedures.

Disclosure and Publication of Information

All proprietary information including: MediWound Ltd. operations, patent applications, formulas, manufacturing processes, basic scientific data and formulation information provided to the investigator or the methodologies used in this study, as well as proprietary information obtained during the course of the study are confidential and will remain the sole property of

MediWound Ltd. The investigator agrees not to disclose any proprietary information supplied by MediWound Ltd. in any way without prior written permission.

Individual patient data obtained during this study are confidential and will not be disclosed to third parties with the following exceptions:

When the data is needed by a patient's personal physician or other medical personnel responsible for the patient's welfare,

For data inspection and verification by MediWound Ltd. or designee, European Medicine Agency inspectors or by the Institutional Review Board (IRB).

Any manuscript or abstract produced by an investigator must be provided to MediWound Ltd. for review 45 days prior to submission. Individual patient identity must not be divulged in any publication.

Protocol Changes or Amendments

The investigator also agrees not to make any changes in the study protocol or its conduct except when necessary to prevent immediate hazards to human life.

Substantial amendments in protocol may be made only by written amendment by the MediWound Ltd. Substantial amendment to the conduct of the clinical trial may arise from changes in the protocol that have impact on safety or physical or mental integrity of the subjects, the scientific value of the trial, conduct or management of the trial or the quality or safety of any IP used in the trial. The Ethics committee and the competent authority must approve protocol amendments prior to implementation. Documentation of IRB/IEC approval for such amendments must be kept on file by the investigator and forwarded to MediWound Ltd.

Notification to the ethics committee/IRBs and the competent authorities (EU sites) on non-substantial amendments should follow the local regulatory policies. Such amendments should be recorded and be available for inspection.

These regulations are described in: Detailed Guidance for the request for authorization of a clinical trial on a medicinal product for human use to the competent authorities, notification and substantial amendments and declaration of the end of the trial – April, 2002.

APPENDIX 8- BIOPSY & HISTOLOGIC ASSESSMENT METHOD

Burn Wound Infection - Biopsy & Histologic Assessment Method

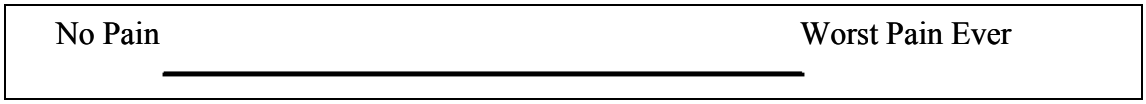
1. Using a scalpel, a 500 mg lenticular sample of eschar and underlying, unburned subcutaneous tissue is harvested from the area of the burn wound showing the most marked changes characteristic of infection.
2. If local anesthetic is required, it should be injected around the planned margin of the biopsy to minimize distortion of the architecture of the biopsied tissue.
3. One-half of the specimen is sent to the quantitative microbiology laboratory for culture and antibiotic sensitivity determinations, and the other half of the specimen is processed for histologic examination by a frozen section technique (requiring 45 minutes) or a rapid section technique (requiring 3.5 to 4.0 hours).
4. If the frozen section technique is utilized, the tissue should be subsequently processed by rapid section technique for definitive examination, since the frozen section method is associated with a 3.6% false-negative rate which can be corrected by careful examination of permanent sections.

APPENDIX 9- PAIN MEASUREMENT SCALE

Pain scores will be measured using a Visual Analogue Scale (VAS) for the adult population at the required timelines. VAS is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured.

As illustrated in Figure 2, the patient marks on the line (100 mm line), the point that they feel represents their perception of their current state. The VAS score is determined by measuring in millimeters from the left end of the line to the point that the patient marks.

Figure 2- Visual Analogue Scale



APPENDIX 10- PROCEDURES FOR SPECIFIC BLOOD TESTS

Immunogenicity blood Samples

Serum samples for antibodies to NexoBrid will be obtained prior to application (time 0), and at the following time points:

- At day 28±7 days (week 4 visit) from start of treatment,
- At day 56±14 days (week 8 visit) from start of treatment.
- At the 6 months FU visit
- At the 24 months FU visit

The blood sampling should be taken after an overnight fast (at least 8 hours if possible) at about the same time of the day (± 2 hours and before noon) due to potential daily fluctuations in the levels of immunological responses. The serum samples will be kept at (-) 20^{o}C until delivery for analysis.

Sample preparation procedures are as follows:

1. For each immunogenicity sample at each time point, draw 3 ml whole blood into a 5 ml pre-labeled tube and check the label for subject number, time point, and type of test.
2. Place the tube in the refrigerator at 2-8^{o}C for 0.5hrs - 2 hours for clotting. Important Note: Do not leave the serum with the clot longer than 2 hours in the refrigerator or at room temperature.
3. Centrifuge the sample at a minimum of 1100 g for 10 minutes, at ambient temperature for red blood cells sedimentation.
4. Divide the serum equally (the upper liquid layer, approximately 1.5 ml) into four pre-labeled 1 ml conical bottom cryotubes resulting in at least 0.3 ml serum each. Check the subject number, time point, and type of test and fill DATE and TIME, 2 cryotubes will be used for analysis and 2 for backup.
5. Store the cryotubes at (-) 20 ± 5 ^{o}C or below until shipped for analysis at Covance.
6. The shipment of samples will be in (-) 20 ± 5 ^{o}C or below.
   If possible, storage and shipment are preferred in (-) 70^{o}±10°C conditions.
7. The set of 2 samples for analysis should be shipped to Covance first. The samples should be sent at the end of the study. Please contact your CRA when shipment is required.
8. The second set of samples (backup) should be dispatched to Covance in a subsequent and separate shipment, after confirmation that the first set has been received, but only if asked by the lab.

Procedures for Pharmacokinetic Samples

PK evaluation will be performed for a subset of 32 NexoBrid patients:

1. 16 patients with total wounds area of <=15% TBSA will be tested for PK
2. 16 patients with total wounds area of >15% TBSA will be tested for PK

PK samples will be obtained prior to application (time 0), and at the following time points:

- 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.
  - 2nd treatment: time 0, 0.5 hours ± 10 minutes, 2 and 4 hours ± 10 minutes, 12 hours ± 30 minutes, 24 hours ± 30 minutes, 48 and 72 hours ± 30 minutes after the second NexoBrid application.

Please carefully follow the procedure below for handling the PK blood samples:

1. For each PK sample at each time point, draw 2 ml whole blood into a 5 ml pre-labeled tube and check the label for subject number, time point, and type of test.
2. Place the tube at room temperature for approximately 30 minutes for clotting.
3. Store the sample at refrigeration (2-8 ºC) for approximately 120 minutes.
4. Following the refrigeration period, Centrifuge the sample for approximately 20 minutes at 2800 to 3000 x g at a temperature of 2-8 ºC.
5. Divide the serum equally (the upper liquid layer) into pre-labeled 1 ml conical bottom cryotubes. Check the subject number, time point, and type of test and fill DATE and TIME.
6. Store the cryotubes in (-) 20 ± 5 ºC or below until shipment for analysis at Quintiles. The shipment of samples will be in (-) 20 ± 5 ºC or below.
7. If possible, storage and shipment are preferred in (-) 80 ± 10°C conditions.
8. The samples should be shipped to Quintiles after 4 patients completed the 48hrs test, but no more than 7 months post first sample was drawn (in that case you may send samples of less than 4 patients). Please contact your CRA when shipment is required.

APPENDIX 11- BURN PATIENTS: POTENTIAL ADVERSE EVENTS

Cardiovascular
Hypotension (decrease of either systolic or diastolic for >/= 2hrs)
Pressor or inotrope requirement for normotension
Sinus tachycardia
Dysrhythmia
Hypertension
Pulmonary
Tachypnea
Pulmonary edema
Pneumonia
Pneumothorax
Pleural Effusions
Atelectasis
Pulmonary Infiltrates
Inhalation injury and/or airway (req. intubation)
Respiratory failure
Neurological
Mental status changes
Sedation
Confusion/delirium
Substance withdrawal
GI
Nausea
Vomiting (>/= 2/shift or >/= 6/day)
Diarrhea (>/= 2/shift or >/= 6/day)
Constipation
Ileus during bum shock
Renal
Acute renal failure (BUN >/= 24; Cr >/= 1.19)
Renal failure
Urinary tract infections
Urinary tract infection, early
Urinary retention
Fluctuation in UOP during burn resuscitation «1mllkg/hr for 2 hrs)
Blood
Anemia (chronic or acute) (based on hemoglobin gm/dL)
Thrombocytopenia (plt </= 145,000)

Thrombocytosis (plt >/= 350)

C-Reactive Protein (>/=30)

Mild coagulopathy as evidenced by ele PT/PTT (PT> 12.3, PTT>36.5)

Asymptomatic electrolyte imbalance (see norm ranges)

Hyperglycemia (glycosuria, insulin drip)

Leukocytosis WBC 2: highest normal range

Leukopenia WBC ~lowest normal range

Other hematologic

Coagulopathy

ODE III/DDDP

Mise

Pain from primary injury

Pruritis

Microbial colonization of burn wounds

Wound infection

Other infection

Excision and grafting of burn injury

Graft loss «/= to 10% ofthe extent grafted)

Hands

Sepsis / septicemia

Line infection

Compartment syndrome (extremity)

Hypothermia «37.2C for 2hrs)

Hyperthermia (>/=39.5C)

Amputation of extremities/digits from deep burn

Cellulitis

Traumatic injury

Graft site contracture requiring release or revision

Death

Multiple organ failure / multi-system organ failure

Permanent neuro/vascular deficit not r/t sev of inj

Unplanned surgical intervention

Autografting to donor site for failure to heal

Repeat autografting to same body site (>10% org grafted)

Auto Graft/regraft

CSS regraft

Intubation due to declining respiratory status not related to edema or inhalation injury

Acute Respiratory Distress Syndrome (ARDS)

Abdominal Compartment Syndrome

APPENDIX 12- COSMESIS, FUNCTION AND QUALITY OF LIFE QUESTIONNAIRES

Modified Vancouver Scar Scale (MVSS)

The six parameters listed below are evaluated on a point system (please tick the correct answer):

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

Patient and Observer Scar Assessment Scale (POSAS)

POSAS Observer scale

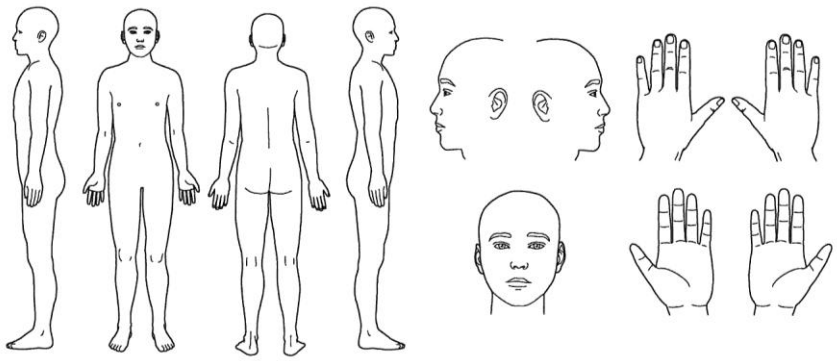
The Patient and Observer Scar Assessment Scale v2.0 / EN

Date of examination: _____ **Name of patient:** _____

Observer: _____

Location: _____ **Date of birth:** _____

Research / study: _____ **Identification number:** _____



1 = normal skin — worst scar imaginable = 10

PARAMETER	1	2	3	4	5	6	7	8	9	10	CATEGORY
VASCULARITY	○	○	○	○	○	○	○	○	○	○	PALE | PINK | RED | PURPLE | MIX
PIGMENTATION	○	○	○	○	○	○	○	○	○	○	HYPO | HYPER | MIX
THICKNESS	○	○	○	○	○	○	○	○	○	○	THICKER | THINNER
RELIEF	○	○	○	○	○	○	○	○	○	○	MORE | LESS | MIX
PLIABILITY	○	○	○	○	○	○	○	○	○	○	SUPPLE | STIFF | MIX
SURFACE AREA	○	○	○	○	○	○	○	○	○	○	EXPANSION | CONTRACTION | MIX
OVERALL OPINION	○	○	○	○	○	○	○	○	○	○	

Explanation

The observer scale of the POSAS consists of six items (vascularity, pigmentation, thickness, relief, pliability and surface area).
All items are scored on a scale ranging from 1 ('like normal skin') to 10 ('worst scar imaginable').
The sum of the six items results in a total score of the POSAS observer scale. Categories boxes are added for each item. Furthermore, an overall opinion is scored on a scale ranging from 1 to 10.
All parameters should preferably be compared to normal skin on a comparable anatomic location.

Explanatory notes on the items:

- **VASCULARITY** Presence of vessels in scar tissue assessed by the amount of redness, tested by the amount of blood return after blanching with a piece of Plexiglas
- **PIGMENTATION** Brownish coloration of the scar by pigment (melanin); apply Plexiglas to the skin with moderate pressure to eliminate the effect of vascularity
- **THICKNESS** Average distance between the subcutical-dermal border and the epidermal surface of the scar
- **RELIEF** The extent to which surface irregularities are present (preferably compared with adjacent normal skin)
- **PLIABILITY** Suppleness of the scar tested by wrinkling the scar between the thumb and index finger
- **SURFACE AREA** Surface area of the scar in relation to the original wound area

POSAS Patient scale

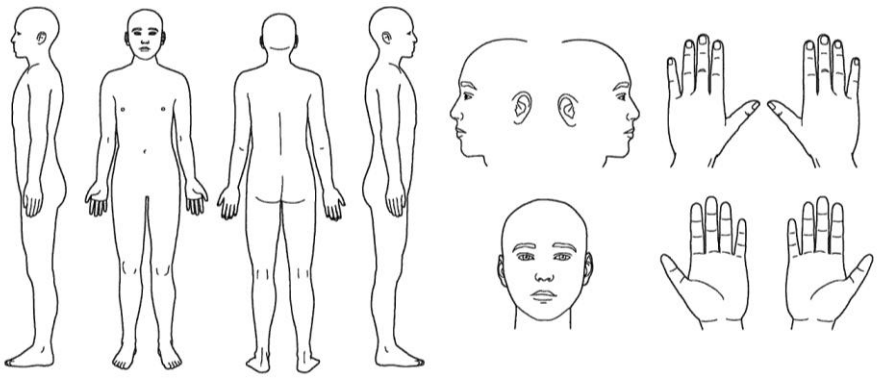
The Patient and Observer Scar Assessment Scale v2.0 / EN

Date of examination: **Name of patient:**

Observer:

Location: **Date of birth:**

Research / study: **Identification number:**



	1 = no, not at all … yes, very much = 10
HAS THE SCAR BEEN PAINFUL THE PAST FEW WEEKS?	1 2 3 4 5 6 7 8 9 10
HAS THE SCAR BEEN ITCHING THE PAST FEW WEEKS?	1 2 3 4 5 6 7 8 9 10

	1 = no, as normal skin … yes, very different = 10
IS THE SCAR COLOR DIFFERENT FROM THE COLOR OF YOUR NORMAL SKIN AT PRESENT?	1 2 3 4 5 6 7 8 9 10
IS THE STIFFNESS OF THE SCAR DIFFERENT FROM YOUR NORMAL SKIN AT PRESENT?	1 2 3 4 5 6 7 8 9 10
IS THE THICKNESS OF THE SCAR DIFFERENT FROM YOUR NORMAL SKIN AT PRESENT?	1 2 3 4 5 6 7 8 9 10
IS THE SCAR MORE IRREGULAR THAN YOUR NORMAL SKIN AT PRESENT?	1 2 3 4 5 6 7 8 9 10

	1 = as normal skin … very different = 10
WHAT IS YOUR OVERALL OPINION OF THE SCAR COMPARED TO NORMAL SKIN?	1 2 3 4 5 6 7 8 9 10

EQ-5D (QoL)

EQ-5D is a standardized measure of health status developed in order to provide a simple, generic measure of health for clinical and economic appraisal. Applicable to a wide range of health conditions and treatments, it provides a simple descriptive profile and a single index value for health status that can be used in the clinical and economic evaluation of health care as well as in population health surveys.

EQ-5D is designed for self-completion by respondents and is ideally suited for use in postal surveys, in clinics, and in face-to-face interviews.

EQ-5D essentially consists of 2 pages - the EQ-5D descriptive system (Figure 3) and the EQ visual analogue scale (EQ VAS) (Figure 4). The EQ-5D descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 3 levels: no problems, some problems, severe problems. The respondent is asked to indicate his/her health

state by ticking (or placing a cross) in the box against the most appropriate statement in each of the 5 dimensions. This decision results in a 1-digit number expressing the level selected for that dimension.

The EQ VAS records the respondent's self-rated health on a vertical, visual analogue scale where the endpoints are labeled 'Best imaginable health state' and 'Worst imaginable health state'.

Figure 3- EQ-5D Descriptive system

EQ-5D (UK English version)

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility
I have no problems in walking about ☐
I have some problems in walking about ☐
I am confined to bed ☐

Self-Care
I have no problems with self-care ☐
I have some problems washing or dressing myself ☐
I am unable to wash or dress myself ☐

Usual Activities (e.g. work, study, housework, family or leisure activities)
I have no problems with performing my usual activities ☐
I have some problems with performing my usual activities ☐
I am unable to perform my usual activities ☐

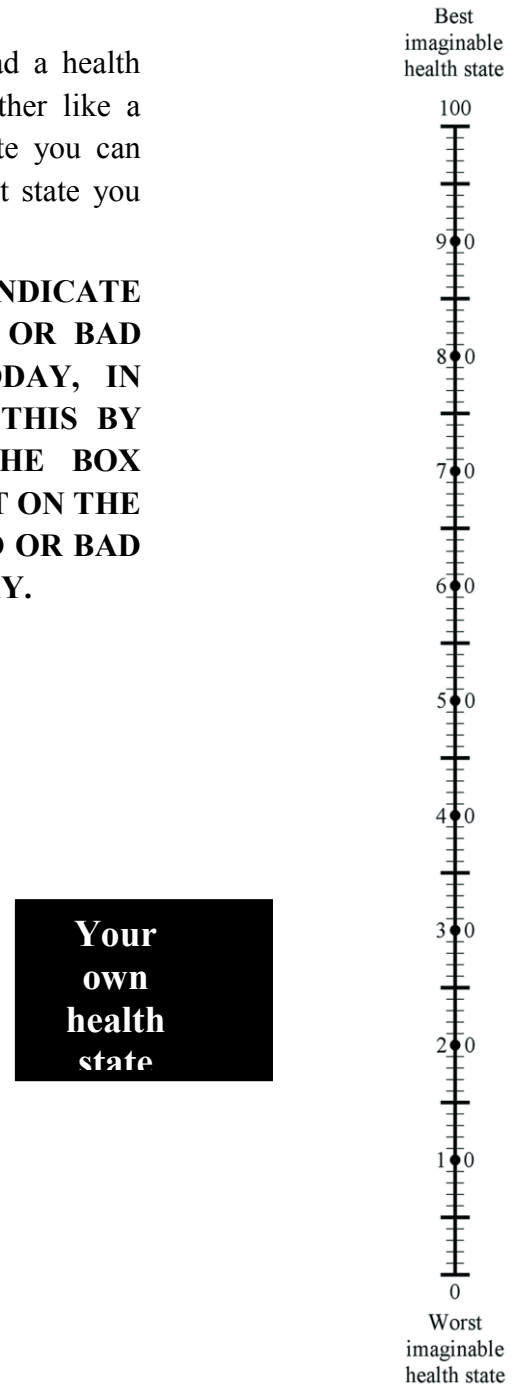
Pain/Discomfort
I have no pain or discomfort ☐
I have moderate pain or discomfort ☐
I have extreme pain or discomfort ☐

Anxiety/Depression
I am not anxious or depressed ☐
I am moderately anxious or depressed ☐
I am extremely anxious or depressed ☐

Figure 4- EQ visual analogue scale (EQ VAS)

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

WE WOULD LIKE YOU TO INDICATE ON THIS SCALE HOW GOOD OR BAD YOUR OWN HEALTH IS TODAY, IN YOUR OPINION. PLEASE DO THIS BY DRAWING A LINE FROM THE BOX BELOW TO WHICHEVER POINT ON THE SCALE INDICATES HOW GOOD OR BAD YOUR HEALTH STATE IS TODAY.



Burn Specific Health Scale-Brief

	extreme(ly)	quite a bit	moderate(ly)	a little bit	None (not at all)
1. Bathing independently					
2. Dressing by yourself					
3. Getting in and out of a chair					
4. Signing your name					
5. Eating with utensils					
6. Tying shoelaces, bows, etc					
7. Picking up coins from a flat surface					
8. Unlocking a door					
9. Working in your old job performing your old duties					
10. I am troubled by feelings of loneliness					
11. I often feel sad or blue					
12. At times, I think I have had an emotional problem					
13. I am not interested in doing things with my friends					
14. I don’t enjoy visiting people					
15. I have no one to talk to about my problems					
16. I have feelings of being trapped or caught					
17. My injury has put me further away from my family					
18. I would rather be alone than with my family					
19. I don’t like the way my family acts around me					
20.My family would be better off without me					
21. I feel frustrated because I cannot be sexually aroused as well as I					
22. I am simply not interested in sex any more					
23. I no longer hug, hold or kiss					
24. Sometimes, I would like to forget that my appearance has changed					

25. I feel that my burn is unattractive to others					
26. My general appearance really bothers me					
27. The appearance of my scars bothers me					
28. Being out in the sun bothers me					
29. Hot weather bothers me					
30. I can't get out and do things in hot weather					
31. It bothers me that I can't get out in the sun					
32. My skin is more sensitive than before					
33. Taking care of my skin is a bother					
34. There are things that I've been told to do for my burn that I dislike					
35. I wish that I didn't have to do so many things to take care of my burn					
36. I have a hard time doing all the things I've been told to take care of my					
37. Taking care of my burn makes it hard to do other things that are					
38. My burn interferes with my work					
39. Being burned has affected my ability to work					
40. My burn has caused problems with my working					

..

QuickDASH

QuickDASH outcome measure for burns in the upper extremities. The Disabilities of the Arm, Shoulder and Hand (DASH) Outcome Measure is a 30-item, self-report questionnaire designed to measure physical function and symptoms in patients with any or several musculoskeletal disorders of the upper limb. The questionnaire was designed to help describe the disability experienced by people with upper-limb disorders and also to monitor changes in symptoms and function over time. Testing has shown that the DASH performs well in both these roles.

It gives clinicians and researchers the advantage of having a single, reliable instrument that can be used to assess any or all joints in the upper extremity.

The DASH Outcome Measure contains two optional, four-item modules intended to measure symptoms and function in athletes, performing artists and other workers whose jobs require a high degree of physical performance. Because they may be having difficulties only at high performance levels–which are beyond the scope of the 30-item DASH Outcome Measure–clinicians may find the modules, which are scored separately from the DASH, useful in assessing these special patients.

The DASH Outcome Measure was jointly developed by the *Institute for Work & Health* and the American Academy of Orthopaedic Surgeons (AAOS). The project was supported by the American Association for Hand Surgery, the American Orthopaedic Society for Sports Medicine, the American Shoulder & Elbow Surgeons, the American Society for Surgery of the Hand, the Arthroscopy Association of North America and the American Society of Plastic and Reconstructive Surgeons.

The *Quick*DASH is a shortened version of the DASH Outcome Measure. Instead of 30 items, the *Quick*DASH uses 11 items to measure physical function and symptoms in persons with any or multiple musculoskeletal disorders of the upper limb. Like the DASH, the *Quick*DASH also has two four-item optional modules that are scored separately. The QuickDASH was found to be valid and repeatable in patients with upper limb burns and supports the use of the QuickDASH in this population to help assess change in functional level [100-101].

Please rate your ability to do the following activities in the last week by circling the number below the appropriate response.

	NO DIFFICULTY	MILD DIFFICULTY	MODERATE DIFFICULTY	SEVERE DIFFICULTY	UNABLE
1. Open a tight or new jar	1	2	3	4	5
2. Do heavy household chores (e.g., wash walls, floors).	1	2	3	4	5
3. Carry a shopping bag or briefcase.	1	2	3	4	5
4. Wash your back.	1	2	3	4	5
5. Use a knife to cut food.	1	2	3	4	5
6. Recreational activities in which you take some force or impact through your arm, shoulder or hand (e.g., golf, hammering, tennis, etc.).	1	2	3	4	5

	NOT AT ALL	SLIGHTLY	MODERATELY	QUITE A BIT	EXTREMELY
7. During the past week, *to what extent* has your arm, shoulder or hand problem interfered with your normal social activities with family, friends, neighbors or groups?	1	2	3	4	5

	NOT LIMITED AT ALL	SLIGHTLY LIMITED	MODERATELY LIMITED	VERY LIMITED	UNABLE
8. During the past week, were you limited in your work or other regular daily activities as a result of your arm, shoulder or hand problem?	1	2	3	4	5

Please rate the severity of the following symptoms in the last week. (circle number)	NONE	MILD	MODERATE	SEVERE	EXTREME
9. Arm, shoulder or hand pain.	1	2	3	4	5
10. Tingling (pins and needles) in your arm, shoulder or hand.	1	2	3	4	5

	NO DIFFICULTY	MILD DIFFICULTY	MODERATE DIFFICULTY	SEVERE DIFFICULTY	SO MUCH DIFFICULTY THAT I CAN'T SLEEP
11. During the past week, how much difficulty have you had sleeping because of the pain in your arm, shoulder or hand? (circle number)	1	2	3	4	5

Lower Extremity Functional Scale test (Binkley et al, 2011)

The Lower Extremity Functional Scale (LEFS) is a questionnaire containing 20 questions about a person's ability to perform everyday tasks. The LEFS can be used by clinicians as a measure of patients' initial function, ongoing progress and outcome, as well as to set functional goals. The LEFS can be used to evaluate the functional impairment of a patient with a disorder of one or both lower extremities. It can be used to monitor the patient over time and to evaluate the effectiveness of an intervention.

THE LOWER EXTREMITY FUNCTIONAL SCALE

Patient's Name: _____ Date: _____

We are interested in knowing whether you are having any difficulty at all with the activities listed below because of your lower limb problem for which you are currently seeking attention. Please provide an answer for each activity.

Today, do you, or would you have any difficulty at all with:

	Activities	Extreme Difficulty or Unable to Perform Activity	Quite a Bit of Difficulty	Moderate Difficulty	A Little Bit of Difficulty	No Difficulty
1	Any of your usual work, housework or school activities	0	1	2	3	4
2	Your usual hobbies, recreational or sporting activities	0	1	2	3	4
3	Getting into or out of the bath	0	1	2	3	4
4	Walking between rooms	0	1	2	3	4
5	Putting on your shoes or socks	0	1	2	3	4
6	Squatting	0	1	2	3	4
7	Lifting an object, like a bag of groceries, from the floor	0	1	2	3	4
8	Performing light activities around your home	0	1	2	3	4
9	Performing heavy activities around your home	0	1	2	3	4
10	Getting into or out of a car	0	1	2	3	4
11	Walking 2 blocks	0	1	2	3	4
12	Walking a mile	0	1	2	3	4
13	Going up or down 10 stairs (about 1 flight of stairs)	0	1	2	3	4
14	Standing for 1 hour	0	1	2	3	4
15	Sitting for 1 hour	0	1	2	3	4
16	Running on even ground	0	1	2	3	4
17	Running on uneven ground	0	1	2	3	4
18	Making sharp turns while running fast	0	1	2	3	4
19	Hopping	0	1	2	3	4
20	Rolling over in bed	0	1	2	3	4
	Column Totals:					

Minimum Level of Detectable Change (90% Confidence): 9 points **SCORE: _____/80**

Reprinted from Brinkley, J.Stafford, P., Lott, S., Ridle, D., & The North American Orthopedic Rehabilitation Research Network, The Lower Extremity Functional Scale: Scale development, measurement properties, and clinical application, *Physical Therapy*, 1999, 79, 4371-383, with permission of the American Physical Therapy Association

APPENDIX 13- ASA CLASSIFICATION SYSTEM

The **ASA physical status classification system** is a system for assessing the fitness of patients before surgery. These are:

ASA Physical Status 1 - A normal healthy patient

ASA Physical Status 2 - A patient with mild systemic disease

ASA Physical Status 3 - A patient with severe systemic disease

ASA Physical Status 4 - A patient with severe systemic disease that is a constant threat to life

ASA Physical Status 5 - A moribund patient who is not expected to survive without the operation

ASA Physical Status 6 - A declared brain-dead patient whose organs are being removed for donor purposes

APPENDIX 14- RANGE OF MOTION

In this study, passive ROM will be measured in which the motion is being created by the patient contracting the muscles around the joint. A goniometer will be used in this study to measure joint angles (joint range of motion).

Instructions for using a goniometer

1. Align the fulcrum of the device with the joint to be measured
2. Align the stationary arm of the device with the limb being measured
3. Hold the arms of the goniometer in place while the joint is moved through its range of motion.

The degree between the endpoints represents the entire range of motion.

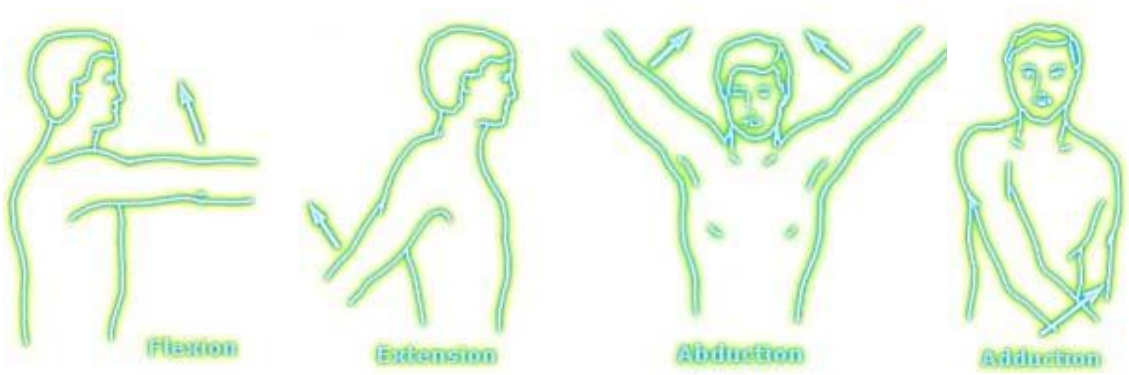
Important tips:

1. Stabilize the stationary of the body. This is the part of the body that is proximal (closer to the midline of the body) to the joint you are testing. It is important that the patient does not move his body while moving the joint; this step isolates the joint movement for a more accurate measurement.
2. Look at the reading on the goniometer before moving it from the patient's body. Ensure that you take an accurate reading of the degree of motion on the geometer, and that you consistently use the same stationary and movable landmarks on the body when measuring, to ensure consistency. Be sure to record the range of motion for the joint.

Range of Motion measurements

16.1.1.1 *Shoulder Joint*

The shoulder joint has the following normal ranges of movement: Flexion, Extension, Adduction, Abduction and Medial Rotation. Flexion, Extension, Adduction and Abduction will be measured:

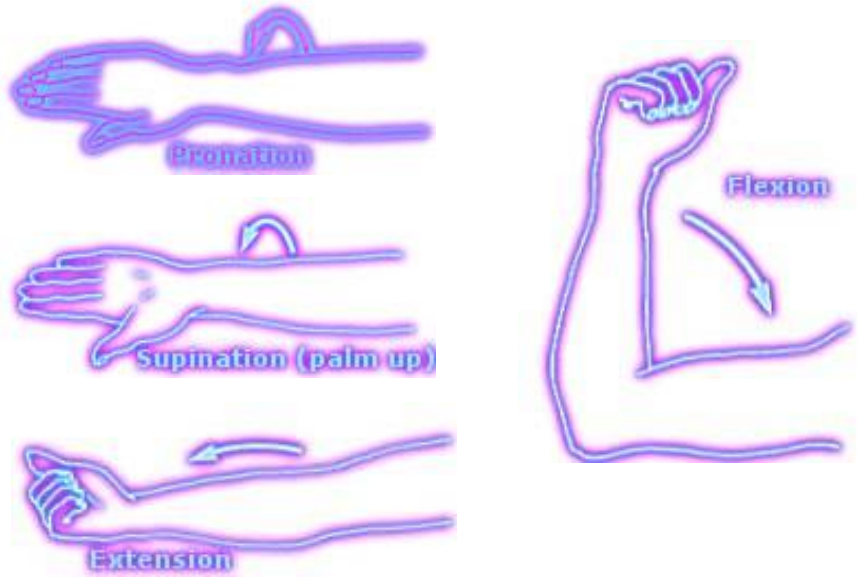


Shoulder normal ranges:

Movement	Degree	Description
Flexion (Vertical)	180°	Raise arm straight forward
Extension (Vertical)	60°	Raise arm straight backward
Abduction	90°	Bring arm up sideways
Adduction	45°	Bring arm toward the midline of the body

Elbow joint

The elbow joint has the following normal ranges of movement: Flexion, Extension, Pronation and Supination.



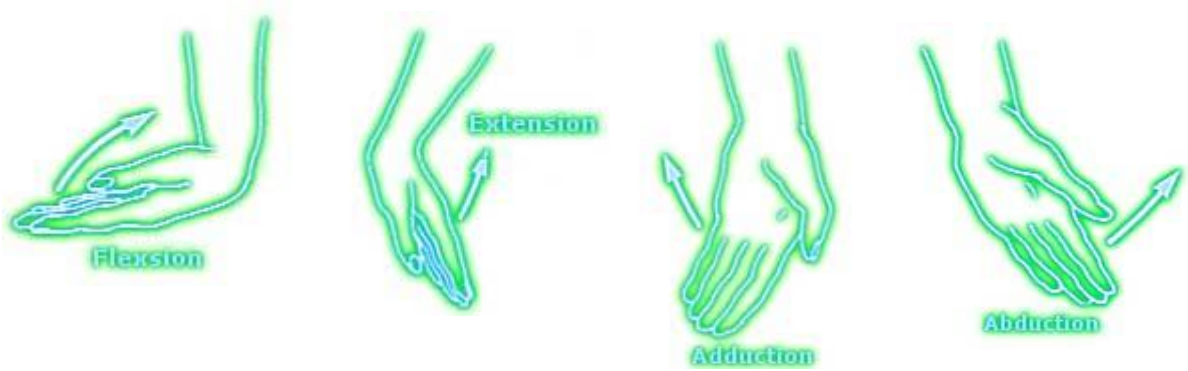
Elbow normal ranges:

Movement	Degree	Description
Flexion	150°	Bring lower arm to the biceps
Extension	180°	Straighten out lower arm
Pronation	90°	Turn lower arm so palm faces down
Supination	90°	Turn lower arm so palm of hand faces

		up

Wrist joint

The wrist joint has the following normal ranges of movement: Flexion, Extension, Adduction, Abduction and Circumduction. Flexion, Extension, Adduction and Abduction will be measured:



Wrist normal ranges:

Movement	Degree	Description
Flexion	80-90°	Bend wrist so palm nears lower arm
Extension	70°	Bend wrist in opposite direction
Abduction	20°	Bend wrist so thumb nears radius
Adduction	30-50°	Bend wrist so pinky finger nears ulna

Palm and Fingers

Normal ranges of the palm & fingers joints will include the following joints (see Figure 5):

- The fingers IP joints;
  - Distal Interphalangeal (DIP) joints
  - Proximal Interphalangeal (PIP) joints
- The thumb joints;
  - Proximal Interphalangeal (PIP) joint
  - Metacarpophalangeal (MTP) joint

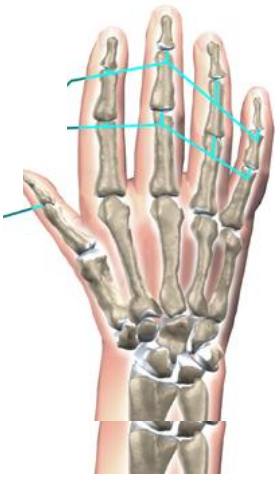


Figure 5- Hand fingers joints

Joints normal ranges:

Movement	Degree	Description
Thumb IP	Hyperextension/Flexion	0/80
Thumb MTP	Hyperextension/Flexion	0/55
Finger DIP joints	Extension/Flexion	0/60
Finger PIP joints	Extension/Flexion	0/100
Finger MCP joints (see Figure 6)	Hyperextension/Flexion	10/90

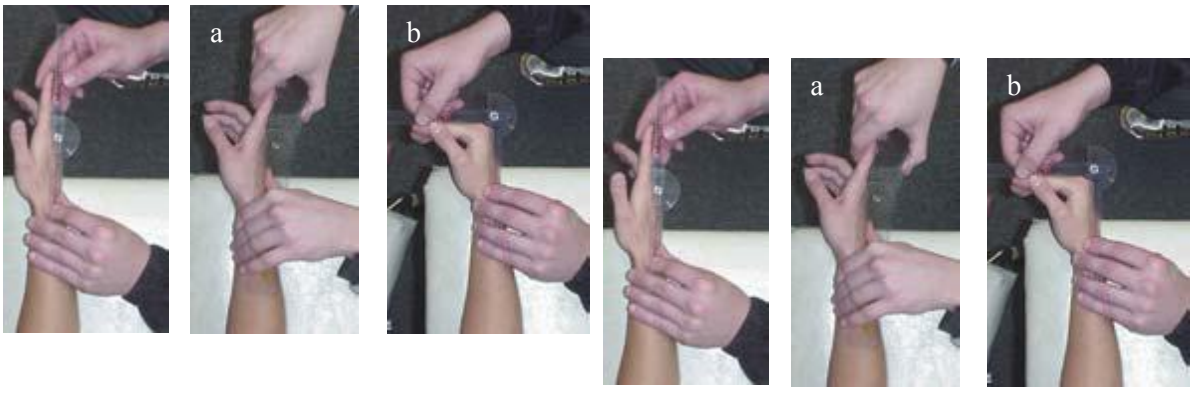
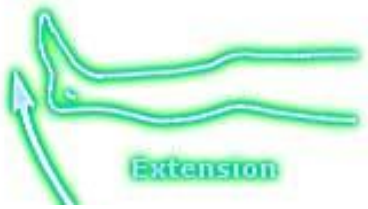


Figure 6- Finger MCP hyperextension (a) and flexion (b)

Knee joint

The knee joint has the following normal ranges of movement: Flexion and Extension.



Knee normal ranges:

Movement	Degree	Description
Flexion	130°	Touch calf to hamstring
Extension	15°	Straighten out knee as much as possible

Ankle joint

The ankle joint has the following normal ranges of movement: Plantar Flexion, Dorsi Flexion, Inversion and Eversion. Dorsi and Plantar flexion will be measured in this study:

Knee normal ranges:

Movement	Degree	Description
Flexion	45°	Bend ankle so toes point up
Extension	20°	Bend ankle so toes point down

APPENDIX 15- BLINDED ASSESSOR MEMO- SAMPLE

In accordance with study protocol, for patients who are randomized to one of the topical arms, NexoBrid or Gel Vehicle, eschar removal assessment will be done by assessor blinded to the treatment arm. This assessor will evaluate eschar removal in all study arm (but will be blinded only to the topical arms) and for all procedures until complete eschar removal.

In addition, weekly evaluations of wound closure and long term cosmesis and function will be done by a second assessor blinded to all treatment arms.

The following procedures and assessments will be performed by the blinded assessors at the following time-points:

First blinded assessor:

Eschar Removal assessment- For the topical arms (NexoBrid/Gel Vehicle), eschar removal assessment will be performed immediately following removal of the soaking dressing (6 hours after start of 1st and 2nd treatment and after any additional procedure until complete eschar removal. The assessment will include photograph, wound depth assessment and clinical assessment of the extent of eschar removal.

Eschar removal in the SOC arm will be evaluated by the same assessor, but he will not be blinded to the SOC arm.

Second blinded assessor (blinded to all treatment arms):

Weekly follow up visits and wound closure confirmation visit-

The following procedures will be performed by the blinded assessor:

- All wounds should be photographed.
- Clinical assessment of % of target wound area epithelialized and/or closed by graft and assessment of % donor site epithelialized,
- Assessment of the percent 'take' of any graft should be recorded 2-7 days post-grafting procedure. A photograph should be taken at the time of assessment

Long term follow up visits-

The following procedures will be performed by the blinded assessor:

- All wounds should be photographed.
- The following questionnaires will be filled by the blinded assessor:
  - Patient and Observer Scar Assessment Scale (POSAS)
  - A modified Vancouver Scar Scale (MVSS)
- Range of Motion measurements (injured and non-injured joints)

In order to maintain the blinding, the sponsor would like your site to implement measures, as best as possible, to ensure that the blinded assessors will not be aware of the treatment assignments for the duration of the study.

The first blinded assessor (eschar removal assessor) should not be involved with product application. The second blinded assessor (wound closure and long term assessor) should not be involved with any eschar removal procedure, PK and Immunogenicity blood sampling should not be taken at the same time of the blinded assessment.

The topical (NexoBrid/Gel Vehicle) treatment allocation will not be accessible through the subject's electronic case report forms. Paper source documents that could unblind the assessor will be kept in a separate folder, appropriately labeled. The blinded assessor should not review subject's data that will include randomization reports, PK and Immunogenicity blood collection, drug accountability records, and any other data that could unblind the assessor for the topical arms. In addition, all Investigational Products (used and unused) should be stored, dispensed, and administered out of the sight of the blinded assessor.

Treatment allocation to the SOC group cannot be reconciled from all eCRFs and SD. The weekly and long term follow up set of source documents worksheets are in separated sections and not reviling the treatment arm.

The second blinded assessor who evaluates the weekly and long term effects of the treatment should not review other sections of source worksheets and will not access to eCRFs.

The Sponsor's medical team will be blinded to the topical arms treatment, and can provide support blindly when needed during the eschar removal treatment.

You are being required to sign this memo to acknowledge and certify that you will make all attempts to comply with these requirements.

Acknowledgement (To be signed at the start of the study):

I acknowledge that I fully understand my role in maintaining the blind and assure that I will not access subject's data except for the information relevant to the conduct the blinded assessments listed above.

Eschar removal assessor:

Printed Name of Blinded Assessor Signature Date_____

Wound closure, cosmesis and function assessor:

Printed Name of Blinded Assessor Signature Date_____

Certification (To be signed at completion of study):

I certify and confirm that I did not review subject's data except for the information relevant to the conduct of the blinded assessments.

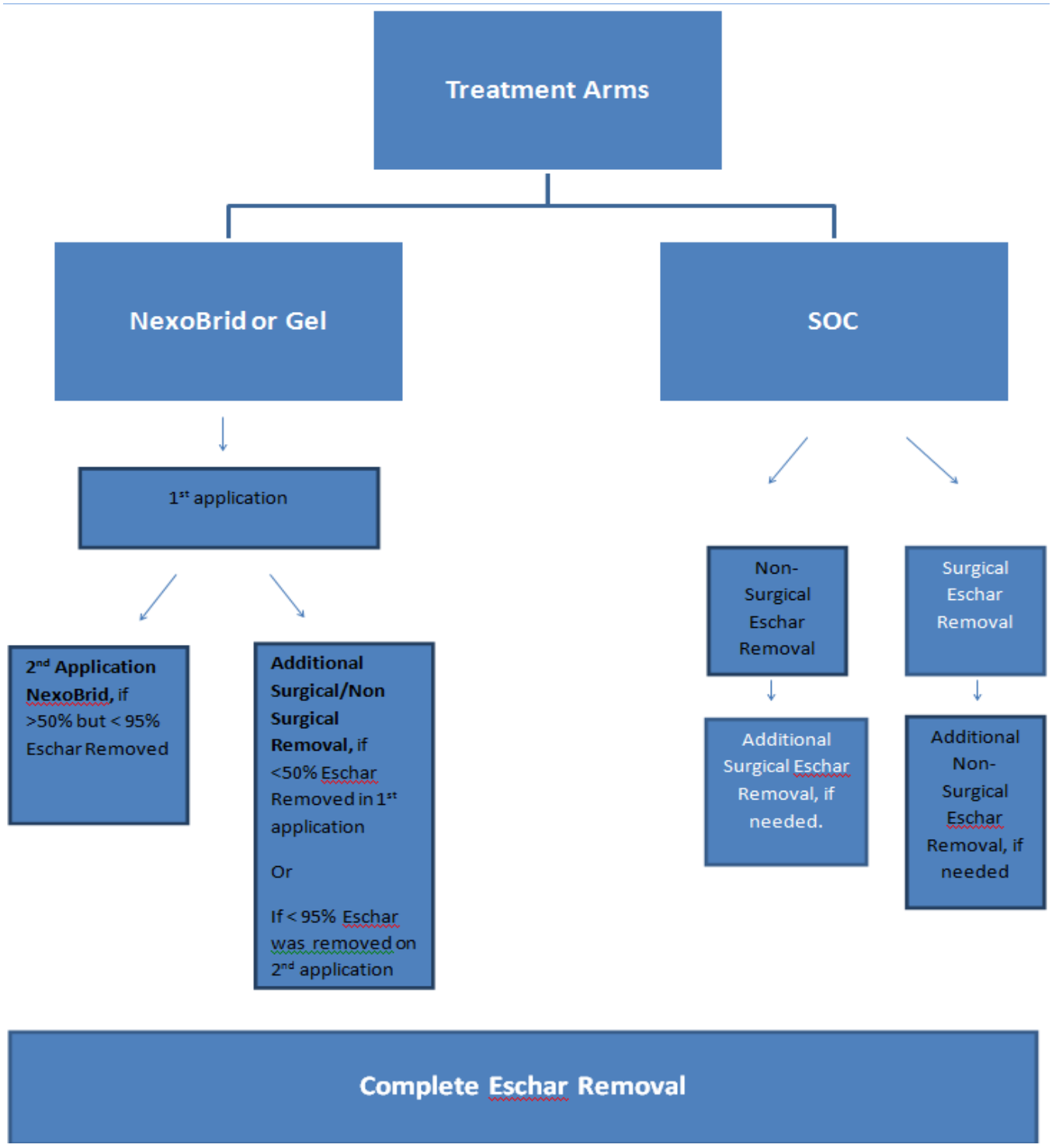
Eschar removal assessor:

Printed Name of Blinded Assessor Signature Date_____

Wound closure, cosmesis and function assessor:

Printed Name of Blinded Assessor Signature Date_____

APPENDIX 16- TREATMENT DIAGRAM



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