Comparison of Collagenase Santyl® Ointment with Antibiotic Ointment in the Outpatient Care of Minor Partial Thickness Burns

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1.0 Synopsis

Test Article(s) / Products:	1) Collagenase Santyl Ointment 2) Bacitracin Ointment
Study Dosage / Usage:	1) Daily application to the burn, approximately 2 mm thick 2) Daily application to the burn, approximately 2 mm thick
Active Ingredients:	1) Collagenase enzymes derived from *Clostridium histolyticum* 2) Bacitracin antimicrobial peptides
Route of Administration:	Topical
Objective(s):	The primary objectives are to compare the proportion of subjects healed at 3 weeks after initiation of Collagenase Santyl ointment to Bacitracin ointment, in addition to time to healing, and incidence of burn wound infection between treatment groups. The secondary endpoint is to compare Collagenage Santyl ointment with Bacitracin ointment based on the scar assessment scores at 90 days after the initiation of treatment. and at 180 days after the initiation of treatment. Exploratory endpoints are number of burn wounds open compared to number of burn wounds closed and cutometer values at each scar assessment visit.
Study Population:	Children and adults 2 – 75 years of age, presenting with a minor burn defined as a partial thickness burn involving < 10% of the total body surface area (TBSA) that does not require hospitalization or a skin graft.

Structure:	[X] Parallel Group	Duration of Treatment:	1- 6 weeks
		Duration of Assessment:	26 weeks
	[ ] Crossover	N/A	
	[ ] Other	N/A	

Blinding:	X	None
		Observer-Blind
		Subject-Blind
		Double-Blind
Randomization:	X	Yes Group Assignment Ratio: 1:1
		No
Concurrent Control:		No Treatment
	X	Standard Care bacitracin ointment
		Placebo N/A
		Active N/A
		Other N/A

Estimated Total Sample Size:	A total of 80 subjects will be enrolled with an expectation to complete 80 subjects at 90 days after the initiation of treatment.	
Statistical Rationale Provided:	X Yes	Refer to Section 11 for Sample Size Justification.
	No	

Variable(s):	PRIMARY:	Proportion healed at 21 days after initiation of treatment, time to healing, and incidence of burn wound infection,).
	SECONDARY:	Scar appearance (Vancouver Scar Scale) at 90 days and 180 days after initiation of treatment
	EXPLORATORY:	Burn wound status (open or closed),
	SAFETY:	Local reactions, adverse events
	PK:	N/A
		Refer to Section 7 for Statistical Design.

1.1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADR	Adverse Drug Reaction(s)
AE	Adverse Event(s)
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AST/SGOT	Aspartate Transaminase
CIB	Clinical Investigator's Brochure
cm^2	Centimeters squared or square centimeters (area)
eCRF	electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent-to-Treat
LOCF	Last Observation Carried Forward
μg	Microgram
m^2	Meters squared or square meters (area)
N	Sample Size
N/A	Not Applicable
NDA	New Drug Application
QD	Once Daily
SAE	Serious Adverse Event(s)
SAS	Statistical Analysis Software, SAS® Institute, Cary, NC
TBSA	Total Body Surface Area
TM	Trademark
UPT	Urine Pregnancy Test

2. INTRODUCTION

2.1 BACKGROUND

Burns are injuries caused by heat, electricity, chemicals, radiation, or light (1). In the US about 450,000 people per year receive medical burn care through hospital emergency departments, hospital outpatient clinics, free-standing urgent care centers and private physician offices (2). Most burns only affect the epidermal and dermal tissues, but more severe burns can also involve muscle, bone, tendons and blood vessels. Burns are generally classified in terms of depth and severity. First degree burns are superficial, involve the epidermis only, and will heal spontaneously within a week or less. Second degree (partial thickness) burns extend into the dermis, are characterized by blisters, and may take up to several weeks to heal. Second degree burns can also become infected, and result in scarring and/or contractures which may require excision and skin grafting. Burns that extend through the entire dermal layer are classified as third degree burns. These burns require excision and in addition to scarring and contractures, may result in amputations.

The American Burn association has further classified burns as minor, moderate, or major depending on several factors including total body surface area (TBSA) burned, involvement of specific anatomical zones, the age of the person, and associated injuries (3). Briefly, moderate and major burns generally require hospitalization, while minor burns do not. A minor burn is one that is partial thickness involving < 15% TBSA for persons 10 to 50 years of age, or is a partial thickness burn involving < 10% TBSA for individuals younger than 10 or older than 50 years of age.

Collagenase Santyl® Ointment was approved by the FDA in 1965 and is currently distributed by Smith and Nephew as an enzymatic debriding agent indicated for use on chronic dermal ulcers and severely burned tissue (4,5). Santyl is a sterile and anhydrous product formulated in a white petrolatum base. The product contains a mixture of enzymes, most prominently collagenases that effectively attack human fibrillar collagen both from the end and the middle of the triple-helix molecule.

Collagenase enzymes are uniquely able to cleave intact collagen, thereby freeing devitalized tissue on the wound surface from its granulation base. Although collagenase is understood to act by cleaving native collagen, it has been observed that Santyl does not impede wound healing or otherwise damage healthy granulation tissue. The explanation for this is unknown. Candidate hypotheses include actions by tissue inhibitors occurring only in healthy granulation tissue, or a strictly kinetic mismatch between rates of tissue collagen production and rates of proteolysis by topical collagenases.

In addition to its safety and effectiveness in debriding the wound surface, Santyl has been reported to promote wound healing. The mechanism by which this might occur is likely to be complex, with various lines of evidence suggesting that additional mechanisms are at work beyond debridement. Observations of improved healing in patients treated with Santyl correlated with improved healing in animal burn models according to work by Zimmerman in the 1970s (6). By contrast, pancreatic enzyme preparations (Tripure Novo, Pancrazym-N) and another bacterial enzyme preparation (Fibrolan, Pfizer, Inc.) showed debridement without healing. Later studies comparing Santyl to Silvadene® Ointment in burn victims suggested both a faster rate of healing with the collagenase preparation (7,8) and a decrease in the incidence of hypertrophic scarring (9).

In the early 1990's, Saarialho-Kere and colleagues recognized that basal keratinocytes at the edge of a cutaneous wound express matrix metalloproteinase-1 (MMP-1), also known as interstitial collagenase (10). Their findings were confirmed by others (11), and more recently it has been found that non-healing wounds are marked by a lack of MMP-1 expression by leading edge keratinocytes (12). As reported by Riley (13), keratinocytes plated on biosynthesized matrices showed a 2-fold increase in proliferation and post-injury migration in the presence of clostridial collagenase. When this collagenase was added to the growth media, there was an additional doubling of growth and migration, yielding approximately 5-fold enhancement of keratinocyte proliferation and migration overall. In the same report, Riley noted that a double-blind study of full-thickness wounds on the backs of Yucatan micro pigs showed that purified clostridial collagenase improved wound healing by all parameters measured, including granulation tissue formation, inflammation, re-epithelialization, and time to wound closure. Thus clostridial collagenase has been found to affect the interaction between keratinocytes and matrix both *in vitro* and *in vivo*. Another intriguing potential mechanism *in vivo* may be the attraction of M2-type macrophages by collagen type-1 fragments (14).

The use of Collagenase Santyl Ointment for treatment of burn injuries was well described in 1972 by Zimmerman, who noted that good results were obtained in terms of both healing and reduced scarring (6). More than 30 years later, the observation of decreased incidence of hypertrophic scars following use of Collagenase Santyl Ointment on burn wounds has again been published (9). Santyl has been used in combination with antibiotics for burn wounds with good results (7), and animal studies indicate that it may be used safely without antibiotics in experimental wounds contaminated with E. coli (15). Given the continuing, if not growing desirability of a pharmacologic agent which reduces scar formation or

improves the appearance of scars (16), this clinical study has been designed to prospectively evaluate potential benefits of Santyl in partial thickness burn wounds compared to antibiotic only treatment.

A summary of known and potential risks and benefits to humans of the test articles can be found in the Package Inserts for Collagenase Santyl Ointment and bacitracin ointment.

2.2 RATIONALE

Outpatient management of partial thickness burns normally involves the application of an ointment, which may contain an antibiotic. Concerns about the promotion of antibiotic resistant staphylococci, combined with known risks of allergic hypersensitivity reactions to certain topical antibiotics, support the exploration of alternative treatments. Bacitracin ointment has been compared against white petrolatum in the management of post-procedural wounds among 922 patients with 1249 wounds (17). No significant difference was observed in infection rates (2% in the petrolatum group vs. 0.9% in the bacitracin group) or healing rates. No patients in the white petrolatum group developed allergic contact dermatitis compared with 4 patients (0.9%) in the bacitracin group. In a separate study of 142 patients, 147 Mohs micrographic surgery wounds on the ear were postoperatively treated with either gentamicin ointment or petrolatum. No significant differences were seen between treatments in the incidence of suppurative chondritis infections (4.76% in the gentamicin group; 6.67% in the petrolatum group), whereas there was a higher incidence of inflammatory chondritis in the gentamicin group (11.90% vs. 3.33% for petrolatum) (18). In the 1990s, the North American Contact Dermatitis Group (NACDG) listed bacitracin among the top 10 allergens, based on skin testing. Approximately 9% of bacitracin tests were positive.

. Bacitracin ointment is selected for this study because it is a standard treatment used by the Investigators, and is widely used in burn care. A significant cost difference exists between bacitracin and other ointments, such as Collagenase Santyl. The therapeutic intent for this study is to see if there is a cost reduction benefit as well as reduction in healing time for two standard of care treatment options for partial thickness burn wounds.

This trial does not include a rigorous evaluation of local infection, as quantitative bacteriology from biopsied tissue would be required, nor does it include a rigorous evaluation of hypersensitivity reactions, as de-challenge / re-challenge or measurement of specific humoral and cellular immune responses would be required. Instead, the appearance of the wound bed and periwound tissue will be evaluated, with

particular attention to the description of any rashes. Clinically diagnosed infections may be treated, but use of topical treatment will require that the subject exit the trial.

3. OBJECTIVE

The primary objectives are to compare Collagenase Santyl ointment with Bacitracin ointment in the proportion healed at 3 weeks after initiation of treatment, time to healing (in weeks), and incidence of burn wound infection. The secondary endpoint is scar assessment scores at 90 and 180 days after the initiation of treatment.

Exploratory endpoints are number of burn wounds open compared to number of burn wounds closed and cutometer values at each scar assessment visit.

4. TEST ARTICLES

4.1 IDENTIFICATION

Test Articles: 1) Collagenase Santyl Ointment

A sterile, enzymatic debriding agent which contains 250 collagenase units per gram of white petrolatum, USP.

2) Bacitracin Zinc Ointment

One gram is equal to 500 bacitracin units; one unit of bacitracin is equivalent to 0.026 milligrams.

4.2 USAGE

Santyl and bacitracin will be applied daily to the thickness of a nickel (2 mm) on the burn until the wound heals, with a maximum application period of six weeks.

5. SUBJECTS

5.1 SUBJECT POPULATION

Up to 80 study subjects, aged 2 – 75 years who meet all study inclusion and exclusion criteria, will be enrolled.

5.2 INCLUSION CRITERIA

Subjects will be considered qualified for enrollment if they meet the following criteria:

1. Provide written informed consent, which will consist of reading, signing, and dating the informed consent document after the Investigator, sub-Investigator or other designated study staff member has explained the study procedures, risks, and contact information. For subjects not able to provide informed consent (e.g., minors), a parent or legally authorized representative must provide consent. Assent must be provided as required by the IRB.
2. Age 2 – 75 yrs, either sex, any race.
3. Have one or more acute burns which:
    - are thermal, chemical or electrical in etiology
    - in aggregate cover <10% TBSA
    - are each ≤ 72 hrs old
    - are each no more than deep partial thickness (2^{nd} degree)
    - are not visibly infected
4. Able to take in oral fluids.
5. Able to comply with the requirement for daily dressing changes, or have a caretaker who is able to comply.
6. Willing to make all required study visits.

5.3 EXCLUSION CRITERIA

Any one (1) of the following criteria will disqualify a potential Subject from participation in the study:

1. Contraindications or hypersensitivity to the use of the test article or their components (e.g., known hypersensitivity to bacitracin).
2. Embedded foreign bodies in the burn wound which cannot be immediately removed.
3. The burned tissue includes or is within 1 cm of the eye or genitalia.
4. Severe perioral burns.
5. Airway involvement or aspiration of hot liquids.

6. Suspicion of physical abuse.
7. Burn wound requires a skin graft.
8. Outpatient management of the burn wound is not appropriate.
9. Participation in another investigational clinical study within thirty (30) days of the Screening Visit.
10. The Investigator may declare any subject ineligible for a valid medical reason.
. Current or recent (< 6 months) history of severe, unstable, or uncontrolled neurological, cardiovascular, gastrointestinal, hematological, hepatic, and/or renal disease or evidence of other diseases based upon a review of medical history that, in the opinion of the Investigator, would preclude safe subject participation in the study.

11. Test articles are cost-prohibitive for subjects

6. STUDY DESIGN

6.1 STUDY DESIGN

This is an active comparator, parallel group study in which subjects with burns meeting the study criteria will be randomly assigned to outpatient treatment with Santyl or bacitracin in a 1:1 ratio. Subjects who appear eligible will be presented with the study, and consent / assent will be obtained prior to any study procedures being conducted. The safety variables include adverse events and, specifically, burn wound infection or allergic contact dermatitis during treatment. Efficacy variables include time to complete healing and scar appearance. Both the Investigators and the subjects will contribute to these evaluations.

Enrolled, randomized subjects will be prescribed with test article (Santyl or bacitracin) and burn wound dressing supplies sufficient to last until burn wound has healed. Scar assessments will occur at 3 months and 6 months post burn healing.

Recruitment bias will exist, as any subject with a known contact dermatitis to bacitracin will be excluded. This will artificially reduce the number of allergic contact rashes in the bacitracin group, but the effect on the primary endpoint is unlikely to be significant. Study Procedure

For a summary of the required procedures by visit, refer to Table 12: *Study Plan*.

6.2 VISITS AND EXAMINATIONS

6.2.1 Visit 1 – Presentation for Evaluation and Treatment

1. Explain the purpose and nature of the study, and have the subject or the subject's legally authorized representative **read**, **sign**, and **date** the IRB-approved informed consent document. In addition, the informed consent document must be signed and dated by the individual who consents the subject. Provide a photocopy of the signed informed consent document to the subject or subject representative, and place the original signed document in the subject's medical record. If the subject is unable to read or sign the informed consent, unable to comprehend the information provided in the consent process, or is younger than the legal age of consent, the parents, legal guardian, decision making proxy or next of kin must provide written consent, and the subject must provide assent as required by the IRB.
2. Determine the etiology, extent (TBSA), age (date of burn and number of hours since burn), burn depth, burn wound perfusion measurement with laser Doppler, and location of the burn. Use the provided figure for estimation of TBSA (Appendix 13).

   If there are multiple burn wounds on a subject, the TBSA for all burn wounds combined must be <10%. Each burn wound must meet study criteria, only the data from the largest burn wound ("target wound") will be recorded in the case report forms and utilized for data analysis. If two or more burn wounds are within 1 cm of each other, the burn wounds can be evaluated as if they were a single burn wound.
3. Photograph the burn per standard of care.
4. Determine the Fitzpatrick skin type (refer to Section 13).
5. Obtain demographic information and medical history, including information on all concomitant medications.
6. Perform any required procedures for standard burn care (e.g., debridement, pain relief, tetanus vaccination).

7. Obtain baseline measures of the burn wound bed, including the area (in cm^2) of the target wound. These measures include exudate type and amount, odor, presence of eschar, presence of epithelial islands, epithelialization of the burn, peri-burn skin characteristics, pain, itching, and state of the burn wound (must be "open"). Refer to Section 13 for details.
8. If the subject meets all of the inclusion criteria, the subject should be randomized.

   If the subject does not meet all of the inclusion, the subject will be considered a screen failure.
9. Ensure that the subject has enough test article and dressings to last the subject for 10 days. Dispensed supplies may include sterile tongue depressors (for test article application), Xeroform gauze, gauze, paper tape (as needed), and compression bandage/garment.
10. A staff member should apply the test article and the dressings to the burn wound while demonstrating to the subject (or caregiver) the proper application of ointment and dressing(s).

    Instruct the subject (or caregiver) that:

    a. Only the ointment and dressings prescribed should be applied to the burn wound during the study.
    b. Application of the study ointment and changing the dressings must occur each day.
    c. Test articles are not to be used on any other person or to treat any other wound.
11. Instruct subject (or caregiver) to refrain from using excluded medications, to treat as instructed, and to return to the outpatient burn center for Visit 2 in 7 (± 2) days. Make any necessary arrangements for that visit. Remind subject (or caregiver) to bring all study supplies with them to Visit 2.

6.2.2 Visits 2 to 6 [weekly (±2 days) visits after 1st visit]

1. Query subject (or caregiver) regarding any changes in general health and the use of concomitant medications.
2. If any adverse events are observed or reported, they must be recorded as instructed in Section 9, *Adverse Events*.
3. Assess the burn wound. The assessments include exudate type and amount, odor, presence of eschar, presence of epithelial islands, peri-burn skin characteristics & rash, pain, and itching (refer to Section 13).

   If it is decided that topical antibiotics will be initiated to treat a burn wound infection, the subject must be discontinued from the study. The use of systemic antibiotics does not require subject discontinuation.
4. Evaluate re-epithelialization of the burn wound. Complete the visit based on the status of the burn wound as outlined below.

   If the burn wound is not completely re-epithelialized ("open"), complete the following steps:

   1. A staff member should apply the test article and the dressings to the burn wound while demonstrating to the subject (or caregiver) the proper application of ointment and dressing(s).

      Instruct the subject (or caregiver) that:

      a. Only the ointment and dressings prescribed should be applied to the burn wound during the study.
      b. Application of the study ointment and changing the dressings must occur each day.
      c. Test articles are not to be used on any other person or to treat any other burn wound.
   2. Instruct subject (or caregiver) to refrain from using excluded medications, to treat the burn wound as instructed, and to return to the office for the next sequential visit.

If the burn wound is completely re-epithelialized ("closed"), complete the following steps:

1. Photograph the burn wound per standard of care.
2. Only moisturizer (Aveeno with Oatmeal) and compression dressing may be used on the scar until the next scheduled study visit. Moisturizer should be used two times per day and as needed. Compression should be used at all times (approximately 23 hours per day).
3. Instruct the subject (or caregiver) to continue avoiding any prohibited concomitant medications and to return for the next evaluation at day 90 (±2 weeks). Any scheduled study visits between the current visit and first scar assessment visit not be completed.
4. Instruct subject to return to clinic for follow up scar assessment visits 7-8.

6.2.3 Scar Assessment visits 7 to 8 [90 (± 2 weeks), and 180 (±2 weeks), Days After Visit 1]

1. Query subject (or caregiver) regarding any changes in general health and the use of concomitant medications.
2. If any adverse events are observed or reported, they must be recorded as instructed in Section 9, *Adverse Events*.
3. Per SOC photograph the scar at all visits or at any other visit if the subject is discontinuing.
4. Provide subject (or caregiver) with the Subject Scar Assessment Scale and ask them to complete an assessment without input from study personnel beyond simple instructions and explanations of terms (refer to Section 13).
5. Ask the subject to rate the pain and itch of the scar (refer to Section 13).
6. The Investigator or sub-investigator completes the Vancouver Scar Scale (refer to Section 13).

7. Once wound healing is confirmed, the subject (or caregiver) will use compression and moisturizer daily 4 weeks post wound healing. Moisturizer should be used two times per day and as needed. Compression should be at all times (approximately 23 hours per day).

   The subject (or caregiver) will use compression and moisturizer for 6 months after wound healing is confirmed only if instructed by the investigator.

8. At all visits, remind the subject (or caregiver) to avoid any prohibited concomitant medications and to return for the next evaluation per the protocol visit schedule.

6.2.4 Unscheduled Visits

Unscheduled exams may be conducted at the discretion of the Investigator with all obtained information recorded in the source documents and on the Unscheduled/Interim Visit pages within the CRF.

6.2.5 Concomitant Medications

A concomitant medication is any drug or substance administered from Day 1 of the study through the last study visit. Any product applied topically to the burn wound (except as specified by the protocol) and/or any product intended or claiming to alter the formation or appearance of a scar (e.g., silicone, steroids, Vitamin E, etc) may not be used during the study. The use of concomitant medication must be recorded on the subject's CRF. Adverse events related to administration of these medications must be documented on the appropriate CRF.

6.2.6 Concomitant Therapy

A concomitant therapy is any therapy administered from Day 1 of the study through the last study visit. Any therapy intended or claiming to alter the formation or appearance of a scar (e.g., use of lasers, cryotherapy, radiation, surgical intervention, etc) may not be used during the study.

The use of concomitant therapies must be recorded on the subject's CRF. Adverse events related to administration of these therapies must be documented on the appropriate CRF.

6.3 DISCONTINUED SUBJECTS

6.3.1 Definition and Procedures

Discontinued subjects are those who voluntarily discontinue participation, who are withdrawn for safety reasons, or who have missed 2 or more consecutive study visits or 3 or more consecutive test article doses so as to be ineligible for continued study participation. Subjects who discontinue the study prior to completing all of the regularly scheduled visits should complete the visit procedures for the visit at which the subject is being discontinued. If appropriate, the Investigator should also advise the subject of subsequent therapy and/or procedures necessary for their medical condition.

6.3.2 Delinquent Subjects

In the case of subjects who persistently fail to return for scheduled visits, three attempts must be made to contact the subject (or caregiver) for the purpose of scheduling an Exit Visit. The third attempt should be via registered mail, return receipt requested. If a subject is unable to return to clinic for the 90 or 180 day assessments the Subject Scar Assessment may be collected over the phone.

6.4 SUBJECT PREGNANCY

Women of child-bearing potential are not excluded from the study, nor is birth control required. Both Santyl and bacitracin are FDA approved products and will be used per their respective package inserts; neither product has any pregnancy precautions. If a woman becomes pregnant during the study, she is not automatically discontinued from study participation. She and the investigator will determine if the potential benefits outweigh the potential risks of continued study participation.

6.5 STUDY METHODS AND MEASUREMENTS

Redcap will be used for data collection. The data to be collected, and the methods of collection, are described below. The exact variables to be measured are detailed in the Appendix section.

6.5.1 Burn Wound Bed Appearance and Rash Evaluation

At each weekly visit, the Investigator will note the appearance of the burn wound bed and surrounding tissue. The variables are exudate, odor, presence of eschar, presence of epithelial islands, and peri-burn skin characteristics. If a rash is observed, the rash will be evaluated separately.

6.5.2 Pain and Itching

Pain and itching will each be measured on a scale of 0-10. The subject will be asked to rate pain and itching as a whole number. The anchor points for pain are 0=no pain and 10=worst pain imaginable. The anchor points for itch are 0= no itch and 10= worst itch imaginable.

NOTE: Data from the pain and itch scales will be recorded in the case report forms for subjects who are age 12 and older.

6.5.3 Burn Wound Closure

At each weekly visit, the Investigator will assess epithelialization (as a percentage of the burn wound). When the burn wound is fully re-epithelialized (i.e., 100%), without drainage and without need of a dressing, the burn wound will be considered "closed".

6.5.4 Laser Doppler

A commercially available instrument (PeriScan, Perimed AB) will be used to measure the perfusion of the burn wound. The data will be obtained per the manufacturer's instructions.

6.5.5 Vancouver Scar Scale

Beginning when the burn wound has fully re-epithelialized, the Investigator or sub-Investigator will assess the appearance of the scar using the Vancouver Scar Scale. The scale and descriptions are found in Section 13.

6.5.6 Subject Scar Assessment Scale

Beginning when the burn wound has fully re-epithelialized, the subject (or caregiver) will assess the appearance of the scar using the Subject Scar Assessment Scale. The scale and descriptions are found in Section 13.

NOTE: Data from the subject scar assessment scale will be recorded in the case report forms for subjects who are age 12 and older.

6.6 SUBJECT COMPENSATION

Subjects will be compensated $25.00 USD for their time at the confirmation of wound healing visit (last weekly visit) and upon completion of Visits 7 to 8.

7. STATISTICAL DESIGN

7.1 EVALUABILITY

All subjects who receive study medication will be evaluable for the safety analysis. All subjects who are randomized to receive study medication will be evaluable for the intent-to-treat analysis.

Unless specified otherwise, SPSS® Version 10.0 or higher will be utilized to perform the statistical analyses of efficacy and safety measures.

Categorical variables will be summarized in general using frequencies and percentages, whereas continuous variables will be summarized in general using descriptive statistics of number of observations (n), mean, standard deviation (SD), minimum (Min), median, and maximum (Max).

Hypothesis testing, unless otherwise indicated, will be performed at the 5% significance level. All P values will be rounded to four decimal places; P values less than 0.0001 will be presented as '<0.0001' in all tables. All group comparisons from analysis of variance (ANOVA) and/or analysis of covariance (ANCOVA) models will be based on Type III sums of squares. All confidence intervals will be two-sided with 95% coverage.

Missing or invalid data of efficacy assessments will be handled by individual scales or subscales on the basis of each individual assessment. Missing data for individual items will be assessed for each assessment of each scale and imputed with the mean score of the corresponding assessment and rounded up to the nearest integer if the number of items with missing data or invalid is less than or equal to 20% of total item number. Otherwise, the assessment score will be set to missing.

Considering that a subject's burn wound may be healed and/or a subject may discontinue from the study prior to the end of the study period, missing values at each weekly visit due to either burn wound healing or early termination or any other reasons will be imputed in this study prior to deriving the efficacy endpoints for evaluation, using the technique of last-observation carried forward (LOCF). By so doing, a burn wound that heals sooner will be given more weight for efficacy.

7.1.1 Primary Efficacy

The primary objectives of the study include the proportion of subjects with the burn wound healed after 3 weeks of treatment, time (in weeks) to healing, and incidence of burn wound infection.,

Both the proportion of subjects with burn wound healed after 3 weeks of treatment and the time to heal between the two treatment groups will be analyzed using ANOVA. Also, the proportion of burn wounds healed at each of the 5 assessments (visit 2,3,4,5, and 6) between the two treatment groups will be analyzed through a mixed-effect ANOVA.The incidence of burn wound infection observed over the study will be compared between the two treatment groups, using a t-test.

The analysis of the primary efficacy evaluations will be performed for the ITT population.

7.1.2 Secondary Efficacy

The secondary efficacy endpoint is to evaluate the effect of daily Collagenase Santyl ointment versus daily Bacitracin ointment on scar appearance at 90 days and at 180 days after the initiation of treatment. The secondary efficacy endpoint is defined as the VSS total score obtained at Visit 7 (Day 90) and at Visit 8 (Day 180).

The treatment effect of Collagenase Santyl ointment versus Bacitracin ointment on the VSS total scores will be obtained at each of the two post-healing assessment time points, using analysis of variance (ANOVA) for the ITT population. The mixed-effect model will utilize SPSSPROC MIXED to perform the analysis and define treatment (Santyl vs. Bacitracin) and assessment time point (4 visits: Visits 5, 6, 7 and 8) as well as their interaction as fixed effects, and subject as a random effect. The option of variance components will be used to define the covariance structure of the model.

In addition, a model-based t-test comparisons between Santyl vs. Bacitracin will be performed for each of the 2 assessment time points (or visits).

The null hypothesis states that there are no differences between the two treatments, with the alternative of non-zero differences. The type I error rate for rejecting a null hypothesis will be set at an alpha level of 0.05.

Prior to conducting the analysis, those VSS total scores that are obtained at the early termination visit will be assigned to the next scheduled study visit.

The analyses of the primary efficacy evaluations will also be performed for the PP population.

7.1.3 Exploratory Efficacy

Burn wound status (closed or open) at each study visit will be compared between the two treatments, using ANOVA for the combination groups of the burn wound perfusion and Fitzpatrick skin type.

7.2 SAFETY

Safety variables include adverse events (AE). Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) adverse event dictionary. Frequency of treatment emergent AE will be calculated for each body system, by preferred term, by treatment, for number of subjects and proportion reporting the event for the safety population. The severity of the AE and the relationship to study medication will be summarized for each body system and preferred term by treatment. Withdrawals, in general, and withdrawals due to AE will be summarized for each body system and preferred term by treatment.

8. SAMPLE SIZE JUSTIFICATION

A previous study of Santyl vs. white petrolatum in normal volunteers with dermatome wounds enrolled 28 subjects, each of whom served as their own control (paired wounds, treated differently). At 9 months follow-up, the Santyl treated wounds had lower total scores on the Vancouver Scar Scale, suggesting the efficacy of Santyl ointment, although the difference between Santyl and its petrolatum vehicle was not significant ($P = 0.09$, Wilcoxon signed-rank test).

The current study uses a parallel-group design and non-uniform injuries on different individuals, which are more likely to lead to a larger variance as compared to the previous study. The injuries are non-uniform and different in nature compared to the dermatome study. Assuming a moderate effect size of 0.750 for the present study, the sample size of 80 (40 per group) will yield a statistical power of approximately 70% to detect a difference between the two treatment groups at the significance level of 0.05 (2-sided). Based on a two-sample t-test, the current study is comparable in power to the described previous study.

9. ADVERSE EVENTS

9.1 GENERAL INFORMATION

An adverse event (AE) is any untoward medical occurrence in a patient administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to this medicinal product.

Adverse drug reactions (ADR) are noxious and unintended responses to a medicinal product, meaning that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. A reaction, in contrast to an event, is characterized by the fact that a causal relationship between the drug and the occurrence is suspected.

Adverse Event CRF must be used to document all adverse events that occur during the course of the study. For each recorded event, the CRF must include the event's onset and resolution date (if the event has resolved), the frequency and severity of the event, a summary of any action taken as a result of the event (both in regard to any treatment given and to any change in dosing with the test article), and an assessment of the event's relationship to the test article.

a Serious Adverse Events that are suspected to be related to the test article and are unexpected (not identified in the product-package insert), should be reported to IRB and to the manufacturer of the test article within 24 hrs of the investigator's knowledge as described below in section 10.4

9.2 NON-SERIOUS ADVERSE EVENTS

A non-serious adverse event is defined as a change from baseline (pre-treatment) in a subject's medical health that is not life-threatening, does not require hospitalization, does not prolong a current hospitalization, and is not disabling.

9.3 SERIOUS ADVERSE EVENTS

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death

- Is life-threatening (***NOTE***: The term "life-threatening" in the definition of "serious" refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or results in prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a medically important event or reaction

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious such as important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

9.4 REPORTING OF SERIOUS ADVERSE EVENTS

A Serious Adverse Event Form must be completed for all serious adverse events to the Institutional Review Board according to their requirements.

9.5 ADVERSE EVENT SEVERITY AND CAUSALITY ASSESSMENT

Events should be classified as mild, moderate, or severe, regardless of whether or not the events are considered to be serious or non-serious. The classification should be based on the following definitions:

- **Mild** – An event is mild if the subject is aware of, but can easily tolerate the sign or symptom
- **Moderate** – An event is moderate if the sign or symptom results in discomfort significant enough to cause interference with the subject's usual activities
- **Severe** – An event is severe if the sign or symptom is incapacitating and results in the subject's inability to work or engage in their usual activities

In addition, the Principal Investigator will determine adverse event causality according to the following definitions and with due consideration to conditions and AE normally associated with the population under study:

- **Not Related** – An event is considered to be not related to the use of the test article when the event is DEFINITELY UNRELATED or UNLIKELY to have any relationship to the use of the test article.

- **Related** – An event is considered to be related to the use of the test article when there is a POSSIBLE, PROBABLE, or DEFINITE relationship between the adverse event and the use of the test article.

For example, a single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens - Johnson syndrome) has a reasonable possibility of association with the test article. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the test article, may have a reasonable possibility of association. Adverse events which are known to be common in the population under study would require additional evidence (e.g., strong temporal relationship, recurrence on re-challenge, unusual severity, and/or increasing frequency) before being considered possibly related.

Adverse events which are known to occur frequently in the population defined by this protocol include: burn wound infection, pain and itch of the burn wound, and contact dermatitis.

9.6 FOLLOW-UP OF SUBJECTS WITH ADVERSE EVENTS

For subjects who are experiencing ongoing unresolved adverse events at the time of their study completion or early discontinuation from the study, it is recommended that the Investigator schedule an appropriate follow-up visit in order to determine the outcome of the event.

10. INVESTIGATOR OBLIGATIONS

10.1 THE PRINCIPAL INVESTIGATOR WILL COMPLY WITH CURRENT GOOD CLINICAL PRACTICE (GCP), AND WITH ALL APPLICABLE REGULATORY REQUIREMENTS. .

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12. STUDY PLAN

Table 1-: Study Procedures by Visit

	Visit 1	Visits 2-6	Visits 7-8
Informed Consent	X		
Medical History/Demographics	X		
Concomitant Medications	X	X	X
Inclusion/Exclusion	X		
Determine Fitzpatrick skin type	X		
Determine burn depth (laser Doppler)	X		
Pain and itch assessment	X	X	X
Randomize	X		
Evaluate burn wound appearance	X	X	
Evaluate burn wound for closure	X	X*	
Apply daily treatment	X	X	
Photograph burn wound	X	X	X
Cutometer measurement			X
Subject scar assessment			X
Vancouver Scar Scale			X
Record Adverse Events	X	X	X
• If wound closed, subject does not return until Visit 7.			

13. APPENDICES

13.1.1

13.1.2 Fitzpatrick skin types

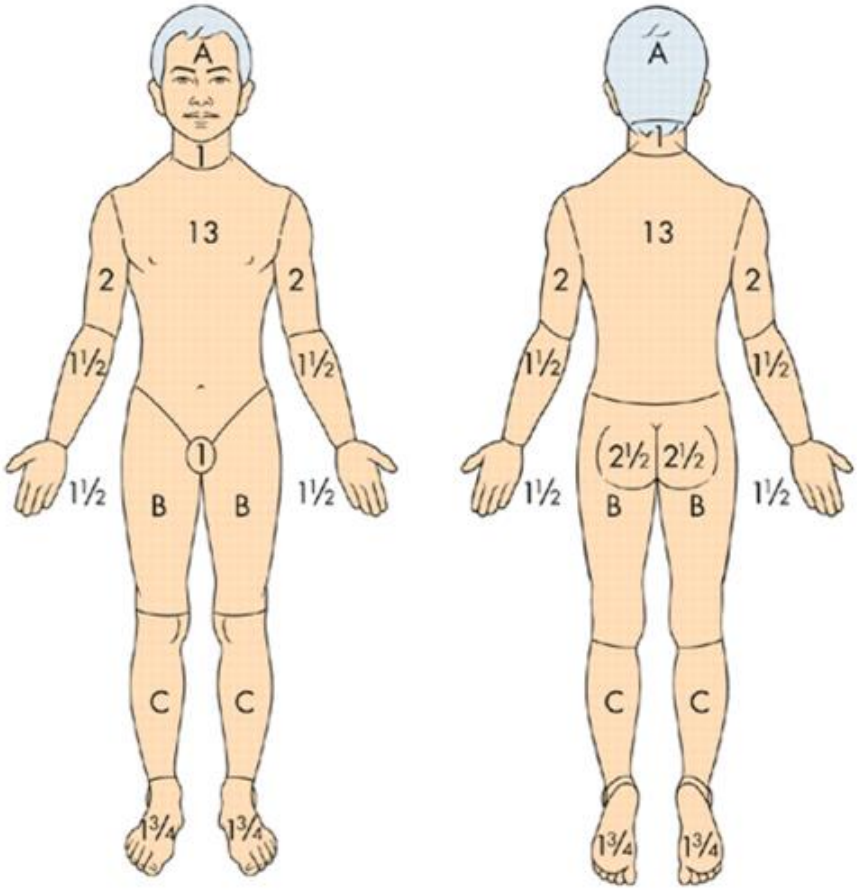
Figure 13.1.1-1: Fitzpatrick Skin Type Chart

Type 1	Red & blonde hair, blue eyes, burns easily, never tans, freckles, very fair skin.	High risk for skin cancer, may scar if slow to heal.
Type 2	Fair sandy/red hair, green or blue eyes, burns easily, tans with difficulty, some freckles.	High risk for skin cancer, may pigment with trauma and may scar if slow to heal.
Type 3	Brown, fair, sandy hair; green, hazel, blue eyes. Slow to burn, will tan.	High risk for all pigmented skin conditions; moderate risk for skin cancer; higher potential for scarring.
Type 4	Dark brown hair; green, hazel, brown eyes. Slow to burn--tans easily.	High risk for trauma, heat & chemically caused pigmentation, and moderate risk for all other pigmented skin conditions. High risk for scarring.
Type 5	Dark and black hair; brown and dark brown eyes. May never burn.	Very high risk for trauma, heat & chemically caused pigmentation, and lower risk for solar pigmented skin conditions. High risk for scarring (keloid).
Type 6	Black hair, dark brown eyes. May never burn.	Very high risk for trauma, heat & chemically caused pigmentation, and lower risk for solar pigmented skin conditions. Very high risk for scarring (keloid).

13.1.3 Burn Chart

A replica of the following diagram will be used to depict the location and shape of the burn. It may be used as an aid to calculating BSA, if other methods are not available. (from Fenlon S , Nene S Contin Educ Anaesth Crit Care Pain 2007;7:76-80)

Figure 13.1.2-1: Lund and Browder Chart



Area	Age 0	1	5	10	15	Adult
A = half of head	9½	8½	6½	5½	4½	3½
B = half of one thigh	2¾	3¼	4	4½	4½	4¾
C = half of one lower leg	2½	2½	2¾	3	3¼	3½

13.1.4 Burn Wound Appearance

Exudate amount	☐ copious ☐ large ☐ moderate ☐ small ☐ scant ☐ none
Exudate type	☐ foul purulent ☐ fresh blood ☐ green ☐ sanguinous ☐ serosanguinous ☐ yellow
Odor	☐ none ☐ mild ☐ strong
Presence of eschar	☐ present ☐ not present
Presence of epithelial islands	☐ present ☐ not present
Epithelialization of the burn wound	expressed as a percent of the burn wound area re-epithelialized
Burn wound closure	☐ closed ☐ open
Peri-burn skin	☐ cyanosis ☐ ecchymosis ☐ edema ☐ erythema ☐ intact ☐ maceration ☐ pallor ☐ rash – if present, complete rash evaluation per Appendix 18.1.4.

13.1.5 Rash Evaluation

Periwound rash	1 ☐ None 2 ☐ Macular or multiforme 3 ☐ Confluent erythema 4 ☐ Papular or nodular 5 ☐ Vesicular 6 ☐ Pustular

13.1.6 Subject Scar Assessment Scale

No, not at all	1	2	3	4	5	6	7	8	9	10	Yes, very much
Was the scar painful during the last weeks?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Was the scar itching during the last weeks?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

Like normal skin	1	2	3	4	5	6	7	8	9	10	Worst Difference
Is the color of the scar different from your normal skin?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Is the pliability of the scar different from your normal skin?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Is the thickness of the scar different from your normal skin?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Is the scar surface more uneven than your normal skin?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

Best possible scar	1	2	3	4	5	6	7	8	9	10	Worst possible scar
What is your general impression of the scar at this moment?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

13.1.7 Vancouver Scar Scale

1.	Vascularity	Normal	0
		Pink	1
		Red	2
		Purple	3
2.	Pigmentation	Normal	0
		Hypopigmentation	1
		Hyperpigmentation	2
3.	Pliability	Normal	0
		Supple	1
		Yielding	2
		Firm	3
		Ropes	4
		Contracture	5
4.	Height	Flat	0
		< 2 mm	1
		2-5 mm	2
		> 5 mm	3