Protocol Title: A Multi-center, Randomized, Open-Label, Controlled Clinical Trial Evaluating Suction Blister Grafting Utilizing a Novel Harvesting Device (CelluTome™) and Standard of Care vs. Standard of Care Alone in the Treatment of Venous Leg Ulcers.

Protocol Number:	**CELLUTOME™-VLU-013**
Version Number:	**Version 1; Rev 3**
Version Date	**February 21, 2017**
Indication:	**Venous Leg Ulcer**
Development Phase:	**Post market**
Device under study:	**CelluTome™ Epidermal Harvesting System**
Comparator:	**Standard of care**
Sponsor:	**SerenaGroup®**
Agency:	**Acelity (formerly KCI)** **San Antonio, TX**
Protocol Prepared By:	**Thomas E. Serena MD FACS**
	90 Sherman Street **Cambridge, MA 02140**

Confidentiality Statement

This protocol is provided for conducting a clinical research study. The information contained in this document is confidential and except to the extent necessary to obtain informed consent or IEC/IRB approval, cannot be disclosed unless required by governmental regulation. Persons to whom any portion of the contents of this document is disclosed must be informed that the information is confidential and may not be further disclosed by them.

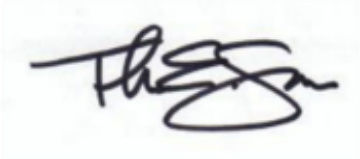
PROTOCOL APPROVAL PAGE

Protocol Number: **CELLUTOME™-VLU-13**

Version: **Version 1; Rev 3**

Date: **February 21, 2017**

PROTOCOL APPROVAL FOR USE



Feburary 21, 2017
Thomas E. Serena, MD, FACS
Chief Medical Officer
SerenaGroup®

INVESTIGATOR'S SIGNATURE PAGE

Protocol Number: **CELLUTOME™-VLU-13**

Version: **Version 1; Rev 3**

Date: **February 21, 2017**

INVESTIGATOR'S SIGNATURE

I have read the protocol specified above and agree to participate in and comply with the procedures, as outlined herein for the conduct of this clinical trial. I also agree to comply with the applicable US Food and Drug Administration (FDA) regulations and Investigational Review Board (IRB) requirements for testing on human subjects. I agree to ensure that the requirements for obtaining informed consent are met.

Principal Investigator's Signature

Date

Print Name

Site Number

SPONSOR INFORMATION

Sponsor:	**SerenaGroup® Inc.**
Funding Agency:	**Acelity (formerly KCI)** **San Antonio, TX**
Medical Monitor:	**Thomas E. Serena, MD, FACS**
Cell:	814-688-4000
Office	617-945-5225
Fax:	617-945-5823
E-mail:	serena@serenagroups.com
Regulatory Lead:	**Sharon McConnell, MS, CCRC**
Phone:	814-706-3318
E-mail:	smcconnell@serenagroups.com
Project Manager:	**Heather Connell, CCRP**
Phone:	617-383-3979
E-Mail:	hconnell@serenagroups.com
Project Lead:	**Raphael Yaakov, MS**
Phone:	617-674-1886
E-Mail:	ryaakov@serenagroups.com

LIST OF ABBREVIATIONS

TERM	DEFINITION
ABI/ABPI	Ankle/Brachial Index/Ankle Brachial Pressure Index
AE	Adverse Event
CFR	Code of Federal Regulations
CHF	Congestive heart failure
cm	Centimeter
CMO	Chief Medical Officer
CRF	Case Report Form
CV	Curriculum vitae
FDA	Food and Drug Administration
FUV	Follow-up Visit
GCP	Good Clinical Practice
HCV	Healing Confirmation Visit
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
ITT	Intent-to-Treat
PBI-P	Patient Benefit Index – Wound Version
QOL	Quality of Life questionnaire
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SoC	Standard of Care
SOP	Standard Operating Procedures
SV	Screening Visit
TTO	Time Trade-Off
TV	Treatment Visit
UADE	Unexpected Adverse Device Effects

Table of Contents

1 PROTOCOL SYNOPSIS: CELLUTOME™

Lead Investigator	Thomas E. Serena MD FACS FACHM MAPWCA on behalf of the SerenaGroup® and affiliated STRONG wound healing cooperative group centers.
Title	A Multi-center, Randomized, Open-Label, Controlled Clinical Trial Evaluating Suction Blister Grafting Utilizing a Novel Harvesting Device (CelluTome™) and Standard of Care vs. Standard of Care (SOC) Alone in the Treatment of Venous Leg Ulcers.
Introduction	Millions of Americans are afflicted with painful, open, draining sores on their lower extremities. These sores are referred to as venous leg ulcerations (VLUs). Under the best of circumstances these ulcers require weeks or months to heal. Wound care specialists often see patients who have suffered for years or faced amputation of the limb as their only option to alleviate the pain. SOC will result in healing in 50% of VLUs in 12 weeks. However, roughly half of patients suffering from venous ulcers will require advanced therapy. Epidermal grafting has been a reconstructive option for decades; however, to date there has not been a reliable and reproducible system to harvest epidermis. The CelluTome™ Harvesting System permits the harvesting of epidermal blister grafts at the patient's bedside without the need for anesthesia. The grafts can be easily transferred to the wound bed. In case studies, epidermal grafting appeared to be effective in reducing wound size and accelerating closure of VLUs.
Objectives	**Primary Objective**: The primary objective is time to heal in patients treated with epidermal grafting and SOC vs. SOC alone. **Secondary Objectives**: a) Proportion of wounds closed b) Incidence of adverse events c) Correlation between protease activity and healing d) Reduction of pain e) Cost-effectiveness (see Appendix 19.4) f) Cost-benefit (see Appendix 19.4)
Study Centers	The trial will be conducted at up to 30 SerenaGroup® and affiliated centers.
Study Endpoints	a) Time to heal at 6 and 12 weeks b) Proportion of wounds healed at 12 weeks. c) Correlation between protease activity and healing at 4 and 8 weeks d) Incidence of adverse events at 12 weeks e) Difference in pain score between baseline and 12 weeks f) Cost-effectiveness at 12 weeks g) Cost-benefit at 12 weeks
Inclusion Criteria	Potential subjects are required to meet all of the following criteria for

	enrollment into the study and subsequent randomization: 1. At least 18 years old. 2. Adequate arterial flow (Ankle Brachial Pressure Index (ABI) > 0.75. (Calculations will be made using measurements from both posterior tibial and dorsalis pedis arteries as well as both arms), OR Skin Perfusion Pressure (SPP) >30, OR biphasic PVR OR TBI > 0.60 OR TCPO2 > 30mmHg OR adequate perfusion as demonstrated on florescent angiography, LUNA®). 3. Presence of a VLU extending through the full thickness of the skin but not down to muscle, tendon or bone. The largest ulcer will be designated the index ulcer and the only one included in the study. If other ulcerations are present on the same leg they have to be more than 2 cm apart from the index ulcer. 4. Study ulcer (i.e. current episode of ulceration) has been present for at least one month prior to the initial screening visit and is excluded if it has undergone 12 months of *continuous* high strength compression therapy over its duration. 5. Study ulcer is a minimum of 2.0 cm^2 and a maximum of 25 cm^2 at the randomization visit. 6. The target ulcer has been treated with compression therapy for at least 14 days prior to randomization (run-in period). 7. Ulcer has a clean, granulating base with minimal adherent slough at the randomization visit. 8. Females of childbearing potential must be willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence). A urine pregnancy test must be administered during screening, and must be negative, for inclusion into the study. 9. Patient understands and is willing to participate in the clinical study and can comply with weekly visits and the follow-up regimen. 10. Patient has read and signed the IRB/IEC approved Informed Consent Form before screening procedures are undertaken.
Exclusion Criteria	Potential subjects meeting any of the following criteria will be excluded from enrollment and subsequent randomization: 1. Study ulcer(s) deemed by the investigator to be caused by a medical condition other than venous insufficiency. These may include, but are not limited to: fungal ulcerations, malignant ulcerations, and ulcerations due to arterial insufficiency. 2. Study ulcer exhibits clinical signs and symptoms of infection at the

	SV (screening visit) or TV1 (Treatment Visit 1). 3. Known allergy to the components of the multi-layer compression bandaging, or who cannot tolerate multi-layer compression therapy. 4. Study ulcer, in the opinion of the investigator, is suspicious for cancer should undergo an ulcer biopsy to rule out a carcinoma of the ulcer. The patient may be enrolled after a negative biopsy. 5. Patients with a history of more than two weeks treatment with immunosuppressants (including systemic corticosteroids), cytotoxic chemotherapy, or application of topical steroids to the ulcer surface within one month prior to initial screening, or who receive such medications during the screening period, or who are anticipated to require such medications during the course of the study. 6. Study ulcer has been previously treated with tissue engineered materials (e.g. Apligraf® or Dermagraft®) or other scaffold materials (e.g. Oasis, Matristem) within the last 30 days. 7. Study ulcer requiring negative pressure wound therapy or hyperbaric oxygen during the course of the trial. 8. Ulcers on the dorsum of the foot or with more than 50% of the ulcer below the malleolus are excluded. 9. Pregnant or breast feeding. 10. Known history of having Acquired Immunodeficiency Syndrome (AIDS) or with a history known to be infected with Human Immunodeficiency Virus (HIV). 11. Known uncontrolled Diabetes Mellitus, as measured by an HbA1c > 10%. 12. Ulcers that have healed more than 40% during the screening phase (run-in period) are excluded. 13. Patients on any investigational drug(s) or therapeutic device(s) within 30 days preceding screening (i.e. S1); or patient or physician anticipates use of any of these therapies by the subject during the course of the study. 14. History of radiation at the ulcer site. 15. Presence of one or more medical conditions, as determined by medical history, including renal, hepatic, hematologic, active auto-immune or immune diseases that, in the opinion of the Investigator, would make the subject an inappropriate candidate

	for this ulcer healing study. 16. Patients who are unable to understand the aims and objectives of the trial. 17. Presence of any condition(s) which seriously compromises the subject's ability to complete this study, or has a known history of poor adherence with medical treatment.
Trial Design	This study is a multi-center, randomized, open-label trial designed to evaluate the safety and effectiveness of Epidermal grafting plus SOC versus SOC alone in the healing of venous leg ulcers. The SOC therapy in this study is multi-layer compression therapy. A number of compression bandaging systems are commercially available. **The Screening Phase** (1-14 days) consists of a series of screening assessments designed to determine eligibility followed by, for those who meet the eligibility criteria (described in more detail below). At or up to 14 days before the first Screening Period Visit (S1), written informed consent from the subject will be obtained by the Investigator or suitably qualified designee before the performance of any other protocol- specific procedure. Patients who have not been treated with documented full compression therapy for the target ulcer must receive a minimum of 14 days of compression prior to enrollment. At the first Screening Period Visit (S1), the Investigator will select the study (target) ulcer. Each subject will have only one VLU selected as the study (target) ulcer. In the situation where a subject has more than one VLU at the S1 visit, the Investigator will select the largest VLU that meets the eligibility criteria of the protocol as the study (target) ulcer (no more than 2 ulcers on target limb). During the screening phase, the wound will be swabbed for protease activity prior to the application of Prisma™ Matrix Wound Dressing (Systagenix, San Antonio, TX). Prisma™ is to be used during the 2 week screening phase. Prisma™ provides a moist environment at the wound surface and provides an effective barrier against common pathogens. The rationale for permitting the use of Prisma™ in subjects is to reduce the risk of infection. For subjects who are allergic to Prisma™, any of the allowed antimicrobial dressings should be used (Appendix 19.8). The Screening Period is designed to determine whether subjects are eligible to proceed to the Treatment Period of the study. **The Treatment Phase** begins with a series of assessments designed to confirm the subjects' continued eligibility. Subjects who continue to meet eligibility criteria will be randomized to one of two groups: (1) Up to 3 applications of Epidermal grafting harvested utilizing the

	CelluTome™ system at TV1, TV5 and TV9, visits plus SOC (multi-layer compression) (**Group 1A**) (2) SOC alone (**Group 2A**). At TV 7, wound assessment will be carried out to evaluate wound healing in **Group 2A** . Subjects in **Group 2A** , whose percentage area reduction (PAR) is <40% will be allowed to have CelluTome™ treatment in addition to continuation of SOC and will constitute **Group 2C. Group 2C subjects** will receive a graft at TV7 and TV11. The remainder of **Group 2A** subjects will be termed **Group 2B** subjects. Patient codes will still remain the same to enable tracking continuity. During the Treatment Phase, the wound will be swabbed for protease activity for **Group 1A and 2A/B** subjects: TV1 (In **Group 1A** this will be prior to CelluTome™ application), TV5 and TV9 visits. Subjects will be evaluated on a weekly basis during the Treatment Phase. Effectiveness evaluations each week will include Investigator assessment of wound healing and measurements of ulcer size using digital planimetry. At TV4 and TV8, Prisma™ is to be applied to the study ulcer for **Group 1A, 2A, and 2B** subjects. **Group 2C** subjects will receive Prisma™ at TV10. Safety evaluations during the Treatment Phase will consist of adverse event assessments at each visit.
Dosage and Frequency of Administration	Epidermal grafting will be applied up to three times in **Group 1A**: TV1, TV5 and TV9. **Group 2C** subjects will receive up to two grafts: TV7 and TV11.
Duration of Study	A run-in period of 2 weeks followed by 12 weeks of active treatment.
Statistical Methods, Power, and Sample Size Calculations	Please see the Statistical Analysis Plan in the protocol.
Protease Levels	Absolute values of inflammatory proteases (Neutrophil elastase, MMP 8 and 9) will be measured at S1 before application of Prisma™, day 0 before application of Epidermal grafts and week 4 and 8 visits thereafter to determine the effect of protease levels on graft success for **Group 1A and 2A/B** subjects. The results will be blinded to the Investigators.
Key Words	Epidermal Grafts Chronic Wounds Clinical Trial Venous Leg ulcer Protease levels in chronic wounds

2 INTRODUCTION

2.1 THE PROBLEM: VENOUS LEG ULCERATION

Lower extremity ulcers pose significant clinical and economic burdens on society. Millions of Americans are afflicted with painful, open, draining sores on their lower extremities. These ulcers, referred to as venous leg ulcerations (VLUs) [1, 2,3,4,5], require weeks or months to heal under the best of circumstances. Physicians often see patients who have suffered for years or faced amputation of the limb as their only option to alleviate the pain. In fact, pain is a common feature of VLUs. A study by Phillips in 1994 found that 65% of patients with VLU complained of severe pain and 68% of patients stated that their ulcer created a negative emotional and psychological impact including feelings of fear, social isolation, anger, depression, anxiety and negative self-image[6].

VLUs are the end result of chronic venous insufficiency caused by an abnormality of the veins in the lower extremity. The venous system of the lower extremity consists of a superficial and a deep system connected by a series of perforating veins. In the normal state, valves within these veins direct blood from the superficial into the deep system which then carries the blood back to the heart. The flow in the deep system is facilitated by the pumping action of the musculature of the lower extremity during physical activity. A variety of illnesses and disease states may interrupt the normal function of the venous system. For example, patients who in the past have suffered from an episode of deep venous thrombosis may not have functioning valves within their veins. The blood then flows in a reverse fashion from the deep system to the superficial system. This reversal of flow leads to pooling of blood and fluid in the legs. The patient may first experience swelling or edema of the lower extremities followed by the hallmark signs of hyper-pigmentation, venous stasis dermatitis, hemosiderin deposits, loss of hair, thickened nails, atrophy blanch and lipodermatosclerosis. Eventually the skin ulcerates typically in the gaiter region of the leg.

2.2 THE PATHOPHYSIOLOGY: VENOUS LEG ULCERATION

The venous system in the leg is made up of two networks, the superficial and the deep venous systems.[2,3] The superficial system is composed of the axial superficial veins (the long and short saphenous veins) and their tributaries, which drain blood from the microcirculatory bed. The deep venous system is composed of the main axial veins between the muscle compartments and the venous sinuses within the calf muscles. The superficial and deep venous systems connect at the sapheno-popliteal and sapheno- femoral junctions, as well as communicating through the perforator veins. Perforator veins either connect directly to the main axial veins or link to the veins and venous sinuses within the muscles, thus indirectly draining into the main axial veins.[2,3]

Bicuspid valves in veins ensure that venous return is unidirectional towards the heart.[3,4] Valves in the perforator veins also serve to protect the superficial venous system from the high compartmental pressures present in the deep veins during contraction of the calf muscle pump. This muscle pump assists the return of blood against gravity.[5] In a leg with normal venous return, the hydrostatic pressures within the superficial and deep venous systems are both approximately 80 mmHg when a person is upright at rest.[4] However, during exercise such as walking or plantar flexion of the venous, calf muscle contraction increases pressure within the deep veins, closing the valves in the perforator veins and

propelling blood in the deep veins towards the heart.[2] Subsequent muscle relaxation following contraction causes pressure in the deep venous system to fall abruptly to a level lower than that in the perforator veins. This sudden pressure drop to between 0 and 10 mmHg ensures the valves in the superficial system open to refill the deep venous system.[4] Proper functioning of venous return is dependent on competent valves within the veins to prevent retrograde flow or reflux from the deep to the superficial venous system.[2,4]

Dysfunction in venous return occurs through:

1. Incompetent valves in the superficial, perforating or deep veins giving rise to venous reflux,
2. Outflow obstructions in the deep veins also giving rise to venous reflux, or
3. Calf muscle pump failure because of diseases influencing lower limb mobility.

The reflux resulting from valvular incompetency or outflow obstructions leads to sustained increases in venous pressure in the superficial system. This venous hypertension is the hallmark of chronic venous insufficiency (CVI).[8,9,10] Clinical manifestations of CVI include:

- Telangiectasia or spider veins
- Varicose veins, ranging in severity from submalleolar venous flare to tortuous dilatations of the axial superficial veins
- Dependent edema in the lower leg
- Atrophic blanche or smooth white scar tissue
- Hyperpigmentation caused by deposition of red blood cells into the dermis, with subsequent reddish-purple to brown discoloration of the tissue
- Eczematous skin changes, such as scaling, flaky skin
- Induration of the lower leg caused by fibrosis of subcutaneous fat, giving the appearance of an inverted bottle in its most severe presentations (so called “champagne legs”)
- Leg ulceration on the lower third of the leg, but not excluding presentations on the dorsum of the venous.

The microcirculatory cascade from venous hypertension to leg ulceration has not been fully described and understanding of the pathogenesis of VLU is incomplete. Various hypotheses have been proposed to explain experimental observations. These hypotheses include pericapillary fibrin cuff formation presenting a barrier to oxygen diffusion[11.12], white cells plugging capillaries causing tissue anoxia[13,14], and fibrin cuffs trapping growth factors.[15] More recently, ulcer pathogenesis is thought to be an inflammatory chain brought about by a chronic ischemia-reperfusion cycle.[9,16] An inflammatory cascade involving cytokines, oxygen-derived free radicals, and activated polymorphonuclear neutrophils promote the deposition of capillary cuffs trapping growth factors and cell adhesion molecules. These matrix cuffs attract and activate more white cells. The repeated activation of this cascade eventually overwhelms compensatory capacity, with balance tipped in favor of tissue destruction.[17] Although the observed cause of ulceration may in many cases be mechanical, for instance minor trauma, healing is arrested or counteracted by the ischemic-reperfusion cycle until the underlying venous hypertension is corrected.

2.3 STANDARD OF CARE: VENOUS LEG ULCERATION

The therapeutic mainstay in management of VLUs is graduated compression bandaging. Healing rates in VLUs are significantly improved by the application of compression therapy[18], but generally only approximately 45% of subjects will be healed by 12 weeks even with compression[19]. High compression (40 mmHg at the ankle) is more effective at healing venous leg ulcers than low compression.[20] High compression can be achieved using a variety of multilayer bandaging systems or compression hosiery.

Currently there are a couple of advanced products which are approved for use in VLUs. Apligraf®, a bioengineered skin product composed of living neonatal foreskin derived skin was shown to heal VLU more rapidly than compression.[21] However, even though more wounds healed than SOC, only 57% of ulcers were healed at 24 weeks. Similarly, Oasis® derived from the submucosal lining of porcine small intestine healed wounds more rapidly than SOC, but only 55% were healed at 12 weeks.[22] There are numerous other products utilized in the treatment of VLUs that do not have randomized controlled trial evidence. The goal of epidermal grafting is to accelerate this healing beyond standard care and currently available advanced products.

Compression should only be applied in the absence of significant arterial disease. The threshold normally employed for the use of compression is an ankle brachial pressure (ABI) index of greater than 0.75. An ABI is the ratio of the systolic blood pressure in the leg over the systolic blood pressure in the arm. Normal adult values range between 0.9 and 1.2. Levels lower than 0.75 are thought to indicate the presence of moderate arterial disease.

2.4 EPIDERMAL BLISTER GRAFTING

Epidermal grafts have been used to treat acute and chronic wounds for decades. However, in the past epidermal micrografts were created using a free hand blade or syringes which applied negative pressure to produce an epidermal blister.[23,24,25,26,27,28,29] However, these procedures are difficult to perform, can remove dermis resulting in patient discomfort and can be time consuming. The CelluTome™ epidermal harvesting system (Acelity, San Antonio, TX) is cleared by the FDA and commercially available in the United States. It harvests up to 120 epidermal blister grafts from a donor site usually on the inner thigh. The grafts are collected in a dressing that can easily be transferred to another area of the body. To date, this system has been used to treat acute and chronic wounds in Haiti and across the United States.[30,31] The results from these case series suggest that epidermal grafting is effective in accelerating the healing in VLUs. In fact, the principal investigator's experience suggests that epidermal grafting may be far superior to current advanced therapies. The epidermal harvesting procedure is described in section 19.7.

Epidermal results in minimal or no discomfort to the patient. Anesthesia is not required. The donor site heals in 3 to 4 days. In the principal investigators case series experience the only adverse event seen was irritation of the donor site secondary to the Tegaderm® dressing.[31] These adverse events resolved with

removal of the Tegaderm® There were no problems with donor site healing. Epidermal grafting is associated with a low risk of complications and potentially can accelerate closure of venous ulcers.

2.5 TREATMENT RATIONALE FOR THIS STUDY

The primary goal in treating a VLU is to obtain wound closure as rapidly as possible which in turn decreases patient discomfort, reduces the risk of infection and improves the patient's quality of life. Healing can be a lengthy and painful process for ulcers of lower extremity requiring greater than 12 weeks to achieve closure in many cases. Epidermal grafts are believed to promote healing by two mechanisms: graft take and the promotion of wound healing through the delivery of growth factors and the essential elements of tissue repair and wound healing.[30] This study is intended to establish the superior effectiveness of epidermal grafting and multi-layer compression over that of multi-layer compression alone, in the treatment of venous leg ulcers. In addition, case studies suggest that epidermal grafting may be more effective than currently approved advanced therapies.

3 STUDY OUTCOMES

Primary outcome measure is time to heal in patients treated with epidermal grafting and SOC vs. SOC alone (**Group 1A** versus **Group 2A** at 6 weeks; and **Group 1A** versus **Group 2B** at 12 weeks).

Secondary outcomes include:

1. Percentage of wounds closed at 6 and 12 weeks.
2. Correlation between protease activity and healing at 4 and 8 weeks.
3. Incidence of adverse events at 12 weeks.
4. Pain reduction between baseline and 12 weeks.
5. Cost-effectiveness (see Appendix 19.4)
6. Cost-benefit (see Appendix 19.4)

4 STUDY DESIGN

This study is a multi-center, randomized, controlled open-label study designed to evaluate the safety and effectiveness of epidermal grafting using the CelluTome™ system plus SOC (multi-layer compression) versus SOC alone in the treatment of VLUs.

The study will have two phases: Screening and Treatment.

The Screening Phase (1 -14 days) is designed to determine whether subjects are eligible to proceed to the Treatment Phase of the study and consists of a series of screening assessments designed to determine eligibility.

At or up to 14 days before the first Screening Phase Visit (S1), written informed consent from the subject will be obtained by the Investigator or suitably qualified designee before the performance of any other protocol-specific procedure.

At the first Screening Phase Visit (S1), the Investigator will select the study (target) ulcer. Each subject will have only one VLU selected as the study (target) ulcer. In the situation where a subject has more than one VLU at the S1 visit, the Investigator will select the largest VLU that meets the eligibility criteria of the protocol as the study (target) ulcer.

The wound will be swabbed for protease activity to measure the absolute values of inflammatory proteases (Neutrophil elastase, MMP 8 and 9) before the application of Prisma™ Matrix Wound Dressing (Systagenix, San Antonio, TX).

Prisma™ is to be used during the 2 week screening phase. Prisma™ provides a moist environment at the wound surface and provides an effective barrier against common pathogens. The rationale for permitting the use of Prisma™ in subjects is to reduce the risk of infection. For subjects who are allergic to Prisma™, any of the allowed antimicrobial dressings should be used (see Appendix 19.8).

Patients whose target ulcer has been treated with compression therapy for the previous two weeks are eligible to enter the treatment phase once all of the inclusion and exclusion criteria are met. If the ulcer has not received compression, the patient should be placed in compression and enrolled in the study after 14 days of compression therapy. Ulcers that have decreased in size by more than 40% during the screening period will be excluded as a "rapid healer."

The Treatment Phase (TV1 to TV13) Begins with a series of assessments designed to confirm the subjects' continued eligibility. Investigators will debride the ulcer. Subjects whose ulcers continue to meet eligibility criteria will then be randomized to one of two groups: (1) One application of epidermal microdomes at day zero and standard of care (**Group 1A**) OR (2) standard of care: multi-layer compression (**Group 2A**) (see figure on next page).

During the Treatment Phase, subjects will be evaluated on a weekly basis. Effectiveness evaluations each week will include Investigator assessment of wound healing and measurements of ulcer size using digital photos.

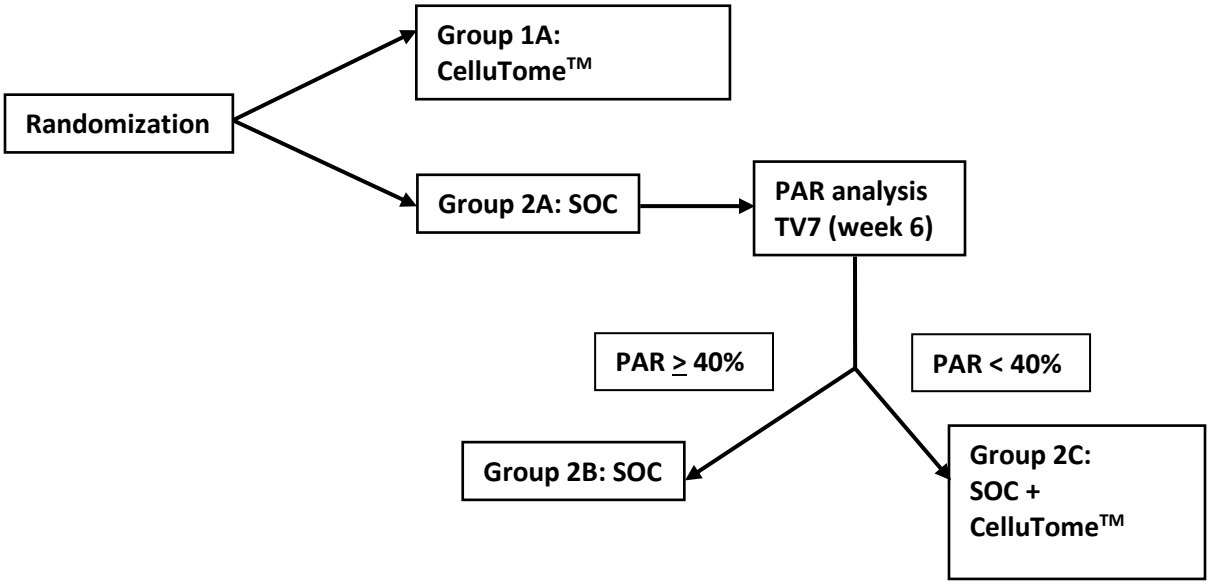
At TV 7, wound assessment will be carried out to evaluate wound healing in **Group 2A** . Subjects in **Group 2A** , whose percentage area reduction (PAR) is <40% will be allowed to have CelluTome™ treatment in addition to continuation of SOC and will constitute **Group 2C. Group 2C** will receive a graft at TV7 and TV11. The remainder of **Group 2A** subjects will be termed **Group 2B** subjects. Patient codes will still remain the same to enable tracking continuity.

During the Treatment Phase, the wound will be swabbed for protease activity for **Group 1A and 2A/B** subjects: TV1 (In **Group 1A** this will be prior to CelluTome™ application), TV5 and TV9 visits.

Subjects will be evaluated on a weekly basis during the Treatment Phase. Effectiveness evaluations each week will include Investigator assessment of wound healing and measurements of ulcer size using digital planimetry. At TV4 and TV8, Prisma™ is to be applied to the study ulcer for **Groups 1A, 2A, and 2B** subjects. **Group 2C** subjects will receive Prisma™ at TV10. Safety evaluations during the Treatment

Phase will consist of adverse event assessments at each visit.

Diagram showing progress of trial and the different subjects groups. Up to 6 weeks from randomization only **Groups 1A and 2A** exist; after 6 weeks, **Groups 1A, 2B, and 2C** exist.



5 STUDYPOPULATION

5.1 NUMBER OF SUBJECTS

The study will consist of up to 30 centers in the United States each contributing about 10 subjects per center in order to obtain 300 evaluable subjects. We anticipate a 10% drop out rate during the trial for both Groups 1A and Group 2A, and 30% of subjects in Group 2A will switch to Group 2C and therefore a total of 225 subjects will be recruited. This trial employs an adaptive design therefore the enrollment numbers may be reduced or increased based on planned interim analysis.

5.2 INCLUSION CRITERIA

Potential subjects are required to meet all of the following criteria for enrollment into the study and subsequent randomization.

1. At least 18 years old.

2. Ankle Brachial Pressure Index (ABI) > 0.75. Calculations will be made using measurements from both posterior tibial and dorsalis pedis arteries as well as both arms AND/OR Skin Perfusion Pressures (SPP) greater than 30 mmHg OR Biphasic PVR OR TBI > 0.60 OR TCPO2> 30mmHg.

3. Presence of a VLU extending through the full thickness of the skin but not down to muscle, tendon, or bone. The largest ulcer will be designated the index ulcer and the only one included in the Study. If other ulcerations are present on the same leg the index ulcer must be 2 cm or greater in distance from the wound edge of the other ulcers.

4. Study ulcer (i.e. current episode of ulceration) had been present for at least one month (30 days) prior to the initial screening visit, and is excluded if it has undergone 12 month of continuous high strength compression therapy over its duration.

5. Study ulcer is a minimum of 2.0 cm^2 and a maximum of 25 cm^2 at the TV1 Randomization visit.

6. The target ulcer has been treated with documented full compression for at least 14 days prior to randomization.

7. Ulcer has a clean, granulating base with minimal adherent slough at the TV1 Randomization visit.

8. Females of childbearing potential must be willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence). A urine pregnancy test must be administered during screening, and must be negative, for inclusion into the study.

9. Patient understands and is willing to participate in the clinical study and can comply with weekly visits and the follow-up regimen.

10. Patient has read and signed the IRB/IEC approved Informed Consent Form before screening procedures are undertaken.

5.3 EXCLUSION CRITERIA

Potential subjects meeting any of the following criteria will be excluded from enrollment and subsequent randomization:

1. Study ulcer(s) deemed by the Investigator to be caused by a medical condition other than Venous Insufficiency. These may include, but are not limited to: fungal ulcerations, malignant ulcerations, diabetic ulcerations, and ulcerations due to arterial insufficiency.

2. Study ulcer exhibits clinical signs of symptoms of infection at S1 or TV1.
3. Known allergy to the components of the multi-layer compression bandaging, or who cannot tolerate multi-layer compression therapy.

4. Study ulcer, in the opinion of the investigator, is suspicious for cancer should undergo an ulcer biopsy to rule out a carcinoma of the ulcer. The patient may be enrolled after a negative biopsy.

5. Patients with a history of more than two weeks treatment with immunosuppressants (including systemic corticosteroids), cytotoxic chemotherapy, or application of topical steroids to the ulcer surface within one month prior to initial screening, or who receive such medications during the screening period, or who are anticipated to require such medications during the course of the study.

6. Patients on any investigational drug(s) or therapeutic device(s) within 30 days preceding screening (i.e. S1); or patient or physician anticipates use of any of these therapies by the subject during the course of the study.

7. History of radiation at the ulcer site.
8. Presence of one or more medical conditions, as determined by medical history, including renal, hepatic, hematologic, active auto-immune or immune diseases that, in the opinion of the Investigator, would make the subject an inappropriate candidate for this ulcer healing study.

9. Study ulcer has been previously treated with tissue engineered materials (e.g. Apligraf® or Dermagraft®) or other scaffold materials (e.g. Oasis, Matristem) within the last 30 days.

10. Study ulcer requiring negative pressure wound therapy or hyperbaric oxygen during the course of the trial.

11. Ulcers on the dorsum of the foot or with more than 50% of the ulcer below the malleolus are excluded.

12. Ulcers that have healed more than 40% during the screening phase (run-in period) are excluded.
13. Pregnant or breast feeding.

14. Known history of having Acquired Immunodeficiency Syndrome (AIDS) or with a history known to be infected with Human Immunodeficiency Virus (HIV).

15. Known uncontrolled Diabetes Mellitus, as measured by an HbA1c > 10%.
16. Patients who are unable to understand the aims and objectives of the trial.
17. Presence of any condition(s) which seriously compromises the subject's ability to complete this study, or has a known history of poor adherence with medical treatment.

6 STUDY SCHEDULE

The study is divided into two phases: Screening and Treatment Phases. The schedules for the protocol-specified assessments and procedures in each phase are detailed below in the following sections. The Schedule of Events tables are presented on the next pages.

6.1 VISIT WINDOWS

Subject visit dates must be scheduled within the visit windows detailed on the Schedule of Events tables. One week equals 7 days.

During the Screening Phase, each visit is scheduled from the S1 visit date, with reference to the allowed visit window.

During the Treatment Phase, each visit is scheduled from the TV1 visit date, with reference to the allowed visit window. For example, the TV2 visit is scheduled for 1 week ± 2 days after the TV1 visit. TV3 is scheduled for 2 weeks ± 2 days after the TV1 visit.

SCHEDULE OF EVENTS

Visit	SVs (14 days)	TV1 (rand)	TV	TV3	TV4	TV5	TV6	TV7	TV8	TV9	TV10	TV11	TV12-13
Weeks from Randomization Date			**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11-12**
Window Period	0		±2 da	±2 days	±2 days	±2 days	±2 days	±2 day	±2 days	±2 days	±2 days	±2 days	±2 days
Assessment of eligibility	√												
Medical history	√												
Apply Prisma™	√				√				√		√[B]		
Randomization		√											
Ulcer assessments	√		√	√	√	√	√	√	√	√	√	√	√
Physical exam	√												
Vital signs	√												
Pain assessment	√		√	√	√	√	√	√	√	√	√	√	√
Study ulcer photographs	√		√	√	√	√	√	√	√	√	√	√	√
Study ulcer cleaning	√		√	√	√	√	√	√	√	√	√	√	√
Study ulcer debridement	√[A]		√	√	√	√	√	√	√	√	√	√	√
Study ulcer swab	√	√				√				√			
Assessment of compression bandaging	√		√	√	√	√	√	√	√	√	√	√	√
Epidermal graft application		√				√		√[B]		√		√[B]	
Study ulcer dressings and compression therapy	√	√	√	√	√	√	√	√	√	√	√	√	√
Study ulcer closure assessment			√	√	√	√	√	√[C]	√	√	√	√	√
Assess for concomitant medications	√	√	√	√	√	√	√	√	√	√	√	√	√
Assess for AEs and SAEs		√	√	√	√	√	√	√	√	√	√	√	√
Quality of Life, PBI-P and Time-trade off questionnaires		√				√				√			√[D]

[A] – If required per the Investigator's judgment.

[B] – **Group 2C** subjects only

[C] – Evaluation of wound area reduction since TV1. Subjects in **Group 2A** (SOC), whose study ulcer PAR is < 40%, will become **Group 2C**; the remainder of **Group 2A** subjects will become **Group 2B**

[D]- Questionnaires are to be completed for TV13 only

6.2 SCREENING PHASE

The subject will sign and date the ICF and HIPAA authorization (according to site practices) prior to any study-related procedures. A screening number will be assigned to each subject in successive order of entering the study after signing the ICF at each center, beginning with 001 at each site.

SCREENING VISIT – S1

The following procedures must be performed:

- Informed consent will be obtained prior to any study related procedures and the subject will sign a written Informed Consent Form
- Demographic information will be collected.
- Medical history obtained.
- Concomitant medications assessed.
- Vital signs taken.
- Physical examination performed.
- Pain assessment.
- Ulcer history recorded.
- Study Ulcer Assessments will be recorded.
- Assess signs and symptoms of clinical infection of the study ulcer.
- Vascular assessment.
- Clean the study ulcer using saline.
- Digital photo of the study ulcer.
- Perform swab on study ulcer.
- Debridement of the study ulcer if applicable.
- Application of Prisma™ or allowed antimicrobial dressing (Appendix 19.8).
- Apply multi-layer compression bandaging therapy.
- Assessment of subject's eligibility to continue on in the study.
- Schedule the next visit (S2 or TV1) in one week, if they are still eligible.

6.3 TREATMENT PHASE

TREATMENT VISIT 1 (TV1) – PRIOR TO RANDOMIZATION

The following procedures must be performed:

- Pain assessment

- Assess effectiveness of compression bandaging
- Check for any changes in the subject's health and update the Medical History with any changes
- Assess concomitant medications
- Assessment of study ulcer
- Check for signs of clinical infection of the study ulcer.
- Clean the study ulcer with saline.
- Digital photo of the study ulcer
- Debridement, if required to remove non-viable tissue
- If the subject is eligible, perform the following procedures (TV1) immediately.
- If the subject is not eligible, discharge the subject from the study as a screen failure.

TREATMENT VISIT (TV1) – RANDOMIZATION

- Randomize the subject
- Perform swab on study ulcer
- If randomized to the epidermal grafting arm: Harvest epidermal grafts using the CelluTome™ device then apply the grafts to the wound bed. (Procedure detailed in Appendix 10).
- If randomized to standard of care apply provided foam to the ulcer.
- Apply multi-layer compression therapy
- Complete Quality of Life, PBI-P, and Time trade off questionnaires (see Appendices 19.5, 19.6, and 19.2)
- Schedule the next study visit one week later

TREATMENT PHASE VISITS TV2-4, 6, 8, 10, 12

The following procedures must be performed:

- Pain assessment
- Assess compression bandaging
- Assess concomitant medications
- Check for any changes in the subject's health and update the Adverse Events or Serious Adverse Events pages with any events
- Study ulcer closure assessment will be determined by a blinded observer
    - If the study ulcer is 100% re-epithelialized, the subject will be scheduled for a Pre-Healing Confirmation Visit (HCV) one week later
    - If the study ulcer is healed, no other study procedures need to be completed at this visit
- Check for signs of clinical infection. If clinical diagnosis of infection has been made, then the subject can be treated with topical antimicrobials or oral antibiotics, but topical antibiotics CANNOT be used on the study ulcer. During the time that an infection is being treated,

treatment with the IP will be stopped.

- Digital photo of the study ulcer
- Apply Prisma™ or approved antimicrobial dressing at TV4, TV8 for both **Groups 1A/2A,2B**; and at TV10 for **Group 2C**.
- Apply provided foam and multi-layer compression bandaging therapy.
- The next visit will be scheduled for one week.

TREATMENT PHASE VISIT TV5 AND TV9

The following procedures must be performed:

- Pain assessment
- Assess compression bandaging
- Assess concomitant medications
- Check for any changes in the subject's health and update the Adverse Events or Serious Adverse Events pages with any events
- Study ulcer closure assessment will be determined by a blinded observer
  - If the study ulcer is 100% re-epithelialized, the subject will be scheduled for a Pre-Healing Confirmation Visit (HCV) one week later
  - If the study ulcer is healed, no other study procedures need to be completed at this visit
- Check for signs of clinical infection. If clinical diagnosis of infection has been made, then the subject can be treated with topical antimicrobials or oral antibiotics, but topical antibiotics CANNOT be used on the study ulcer. During the time that an infection is being treated, treatment with the IP will be stopped.
- Digital photo of the study ulcer
- Perform swab on study ulcer
- If in the epidermal grafting arm: Harvest epidermal grafts using the CelluTome™ device and then apply the grafts to the wound bed. (Procedure detailed in Appendix 10).
- If randomized to standard of care apply the provided foam to the wound.
- Apply multi-layer compression bandaging therapy
- Complete Quality of Life, PBI-P, and Time trade off questionnaires (see Appendices 19.5, 19.6, and 19.2)
- The next visit will be scheduled for one week

TREATMENT PHASE VISIT TV7

The following procedures must be performed:

- Pain assessment
- Assess compression bandaging

- Assess concomitant medications
- Check for any changes in the subject's health and update the Adverse Events or Serious Adverse Events pages with any events
- Study ulcer closure assessment will be determined by a blinded observer
    - If the study ulcer is 100% re-epithelialized, the subject will be scheduled for a Pre-Healing Confirmation Visit (HCV) one week later
    - If the study ulcer is healed, no other study procedures need to be completed at this visit
- Check for signs of clinical infection. If clinical diagnosis of infection has been made, then the subject can be treated with topical antimicrobials or oral antibiotics, but topical antibiotics CANNOT be used on the study ulcer. During the time that an infection is being treated, treatment with the IP will be stopped.
- Digital photo of the study ulcer
- Wound will be assessed for percentage area reduction (PAR) since TV1. Subjects in **Group 2A** whose wounds are < 40% will become **Group 2C** subjects and receive a graft at TV 7 and TV 11.
- For **Group 2C** subjects: Harvest epidermal grafts using the CelluTome™ device and then apply the grafts to the wound bed. (Procedure detailed in Appendix 10).
- Apply multi-layer compression bandaging therapy
- The next visit will be scheduled for one week

TREATMENT PHASE VISIT TV11

The following procedures must be performed:

- Pain assessment
- Assess compression bandaging
- Assess concomitant medications
- Check for any changes in the subject's health and update the Adverse Events or Serious Adverse Events pages with any events
- Study ulcer closure assessment will be determined by a blinded observer
    - If the study ulcer is 100% re-epithelialized, the subject will be scheduled for a Pre-Healing Confirmation Visit (HCV) one week later
    - If the study ulcer is healed, no other study procedures need to be completed at this visit
- Check for signs of clinical infection. If clinical diagnosis of infection has been made, then the subject can be treated with topical antimicrobials or oral antibiotics, but topical antibiotics CANNOT be used on the study ulcer. During the time that an infection is being treated, treatment with the IP will be stopped.
- Digital photo of the study ulcer
- **Group 2C** subjects will receive a graft. Harvest epidermal grafts using the CelluTome™ device and then apply the grafts to the wound bed. (Procedure detailed in appendix).
- Apply multi-layer compression bandaging therapy

- Complete Quality of Life, PBI-P, and Time trade off questionnaires (see Appendices 19.5, 19.6, and 19.2)
- The next visit will be scheduled for one week

FINAL TREATMENT PHASE VISIT (TV13)

The procedures must be performed:

- Pain assessment
- Assess compression bandaging
- Assess concomitant medications
- Check for any changes in the subject's health and update the Adverse Events or Serious Adverse Events pages with any events
- Study ulcer closure assessment will be determined by a blinded observer
- Clean the study ulcer with saline
- Digital photo of the study ulcer
- Complete Quality of Life, PBI-P, and Time trade off questionnaires (see Appendices 19.5, 19.6, and 19.2)

UNSCHEDULED VISITS

Unscheduled visits may be required in addition to the visits detailed above. Additional visits are at the discretion of the investigator. An example of an unscheduled visit is when a change in compression bandaging is required between scheduled visits. The details of these unscheduled visits with subjects will be recorded in the medical records/source documents and on the CRF.

MISSED VISITS

If a subject misses a visit, the site is to make every effort to have the subject return as soon as possible to make up the visit. Once the subject is seen, he/she is to return to his/her original weekly visit schedule. For example, if a subject was seen regularly on Mondays but missed a scheduled Monday visit and came in on Wednesday, he/she should return the next Monday to maintain his/her weekly Monday visit schedule.

6.4 SUBJECT COMPLETION AND WITHDRAWAL

SUBJECT COMPLETION

- A subject whose study ulcer has closed will be considered as having completed the study.
- A subject who completes the Treatment Phase will be considered as having completed the study.

SUBJECT PREMATURE WITHDRAWAL FROM THE STUDY

A subject who is randomized into the Treatment Period of the study but who does not complete the study through TV4 has prematurely discontinued.

All subjects have the right to withdraw at any point during treatment without prejudice. It will be documented whether or not each subject completed the clinical study. If for any subject, study treatment or observations were discontinued, the reason(s) will be recorded.

The Investigator can discontinue a subject at any time if it is considered medically necessary.
In addition, subjects WILL be withdrawn from the study, in consultation with the Medical Monitor and the Investigator, if any of the following are met:

- A subject is significantly non-compliant with the requirements of the protocol.
- A subject becomes pregnant (Note: the pregnancy will be followed up to term for safety follow-up. Relevant safety information collected after the study has completed will be reported as supplemental information.)
- A subject has revascularization surgery on the leg with the study (target) ulcer.
- A subject has a target ulcer wound bed that has exposed bone, tendon or fascia.
- The subject's study ulcer cannot be dressed with multi-layer compression.
- Two separate infections with no response to allowable treatments
- The subject's ulcer merges with an adjacent ulcer.

Premature withdrawal from the study MAY occur if:

- A subject is treated with a prohibited medication. This decision will be made by the Medical Monitor in conjunction with the Investigator as to whether premature withdrawal is warranted.

Every attempt should be made to collect follow-up information. The reason for treatment discontinuation or withdrawal from the study will be recorded in the source documents and on the appropriate page of the CRF.

Before a subject is identified as lost-to-follow up, the site should make all reasonable efforts to contact the subject. These attempts must be documented and should include at a minimum one phone call and one certified letter.

In the event that a subject is prematurely discontinued from the study at any time due to an adverse event of SAE, SAE procedures must be followed.

SCREEN FAILURES

A subject who has signed a consent form, has been assigned a screening number, but is not randomized is classified as a screen failure. Subject number, demography, and reason for screen failure will be collected.

7 STANDARD OF CARE AND CONCOMITANT MEDICATIONS

Beginning at the S1 visit, ALL subjects must have their study ulcer managed using the SOC procedures noted below.

7.1 CLEANING THE STUDY ULCER

Remove the bandage and wash the leg. At any time when the bandage is removed, the leg should be elevated for as much time as possible.

Wash the leg with potable saline or tap water. Gently irrigate the study ulcer prior to each dressing change with warm tap water or saline. Strict aseptic technique is not needed.

During the Treatment Phase, the washing and cleaning of the VLU must be done with great care on the patients receiving epidermal grafting.

7.2 DEBRIDEMENT OF THE STUDY ULCER

It is important to remove all non-viable and necrotic material from the target ulcer prior to enrolling the patient. Extensive debridement is not permitted during the treatment phase. Care must be taken not to remove the graft by debriding the ulcer. Briefly, the following procedures should be followed,

1. The target ulcer and the surrounding skin are prepped with saline solution.
2. Anesthesia, topical or injected, is applied to the ulcer as necessary to reduce patient discomfort.
3. Using clean technique. All non-viable tissue in the wound bed is to be removed from the wound bed.
4. Ideally hemostasis is achieved using direct pressure. Cautery may be employed if necessary.

7.3 COMPRESSION BANDAGING

Multi-layer compression is applied at every visit following the manufacturer's suggested procedure technique. Patients cannot be enrolled until they have received at least 14 days of compression therapy. Training in compression wrapping will be provided.

7.4 SUBJECT INSTRUCTIONS

Subjects will be instructed to elevate the study ulcer limb above their heart as much as possible. Subjects will be instructed to keep the bandaging dry and to call or visit the study site if the bandaging becomes soiled or is removed.

7.5 CONCOMITANT MEDICATIONS

The subject may be administered any necessary medications, at the discretion of the Investigator, provided such medications are not applied topically to the ulcer surface (topical medication can be applied to other surfaces around the ulcer).

All medications and therapies administered or taken by the subject beginning 30 days prior to signing the ICF and throughout the study will be recorded in the source documents and, for randomized subjects, on the appropriate page of the Case Report Form (CRF).

7.5.1 EXCLUDED MEDICATIONS AND THERAPIES

The following treatments and medications are prohibited within 30 days prior to randomized treatment and throughout the study:

- Heat lamps
- UV lights
- Whirlpool baths
- Water Piks™
- Hyperbaric Oxygen
- Jet water streams (other than gentle saline irrigation)
- Wound dressings that include growth factors, engineered tissues or skin substitutes (e.g., Regranex®, Dermagraft®, Apligraf®, GraftJacket®, OASIS®, Primatrix®, Matristem®,etc.)
- Revascularization surgery on the leg with study (target) ulcer within 3 months
- Radiation therapy to the foot
- Topical antibiotics or other non-approved topical agents (with the exception of anesthetics used during debridement).
- Patients on Trental may remain on the medication but patients should not be started on Trental during the trial.
- Systemic steroids/oral corticosteroids (note: inhaled steroids are acceptable)
- Other Immunosuppressive agents
- Autoimmune disease therapies
- Cytostatic drugs
- Any investigational treatment/medications

7.5.2 ALLOWABLE MEDICATIONS AND THERAPIES

The following treatments are allowed during the study, if in the opinion of the Investigator, they are required for proper care of the research subject:

- Use of anesthetics for debridement.
- Treatment with systemic antibiotics for acute or chronic infection; however, prophylactic use of systemic antibiotics is not allowed.
- Topical antimicrobials as listed for use to treat an infection to the reference ulcer or at the scheduled treatment weeks.
- Use of Hibiclens Skin Cleanser for cleansing of general skin, wound and the harvest site. Alternatively, chlorhexidine soap may also be used.
- Other medications/therapies that are not otherwise prohibited and, in the opinion of the Investigator, are required for proper medical care.

8 DESCRIPTION OF PROTOCOL ASSESSMENTS AND PROCEDURES

8.1 INFORMED CONSENT

Written informed consent will be obtained for this study by the Investigator or suitably qualified designee from all subjects before the performance of any protocol-specific procedure. This study will be conducted in accordance with the provisions of the Declaration of Helsinki.

In obtaining and documenting informed consent the Investigator must comply with applicable regulatory requirements and must adhere to Good Clinical Practice (GCP). Evidence of GCP training is required for all investigators. The Investigator, or designee, must fully inform subjects of all pertinent aspects of the study. Before informed consent may be obtained, the Investigator, or a person designated by the Investigator, must provide the subject ample time and opportunity to inquire about details of the study and to decide whether or not to participate in the trial. All questions about the trial must be answered to the satisfaction of the subject. Prior to the subject's participation in the trial, the written informed consent must be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

8.2 ASSESSMENT OF ELIGIBILITY

At each visit during the Screening Period, the Investigator must assess a subject's continued suitability and eligibility for the trial, especially with regards to the Inclusion and Exclusion criteria. If the subject is not suitable or eligible for the trial, then the subject will be a screen failure. Screen failure subjects may be re-entered into the study at a later time and re-screened.

8.3 RE-SCREENING

If a subject initially fails to meet inclusion/exclusion criteria and is later reconsidered for participation, the subject will be re-consented and assigned a new screening number at the time of re-screening. Subjects who fail their first screening attempt may be re-screened again (*i.e.,* up to three screenings) and may be enrolled if they are found to meet ALL inclusion and NO exclusion criteria at the second screening visit.

8.4 SUBJECT DEMOGRAPHICS, MEDICAL HISTORY, ULCER HISTORY

8.4.1 DEMOGRAPHICS

For the purposes of this study, demographic information will include:

- Dates of Informed Consent Form signature
- Date of birth
- Gender
- Race (American Indian/Alaskan Native, Asian, Black/African American, Native Hawaiian/Pacific Islander, Caucasian, or other)
- Ethnicity (Hispanic/Latino or Not Hispanic/Latino)
- Use of tobacco products

8.4.2 MEDICAL HISTORY

A medical history will be recorded during the Screening Period and will include:

- ALL ongoing medical conditions
- ALL previously resolved medical conditions related to Venous Insufficiency or Leg Ulceration or which are relevant in the opinion of the Investigator
- ANY prior medical conditions that have resolved within the last year

Events that emerge prior to the randomization visit (TV1) will be recorded in the medical history and not as Adverse Events. Aside from being used to determine subject eligibility, this information will permit the Investigator to record the nature, duration and severity of any ongoing baseline medical conditions prior to receiving investigational product treatment.

Medical histories will be recorded using the body system categories outlined below:

- Cardiovascular
- Respiratory
- Gastrointestinal
- Renal
- Hepatic
- Lymphatic
- Hematologic
- Immunologic
- Dermatologic
- Psychiatric

- Neurological
- Endocrine
- Genitourinary
- Other

For each relevant history, the following will be documented:

- Disease/disorder/condition
- Date of diagnosis
- History status (resolved or ongoing)

8.4.3 LEG ULCER HISTORY

- Duration of the current VLU

  Note: 'Duration' is defined as the length of time that the study ulcer has been open at this location since the last time it was fully closed.

- Current bandaging system used for the VLU and length of time that this has been used
- Prior treatments that have been used on the VLU
- Age when the subject developed his/her first VLU
- Total number of previous VLUs
- Location of the current VLU

  Note: 'Study ulcer location' is defined by the ulcer being on the left or right leg, by the location of the ulcer on the malleolus, low gaiter or high calf, and by the positioning of the ulcer as lateral, medial, anterior or posterior.

- Number of additional VLUs and location of each present at the screening visits
- History of VLU recurrence

  Note: 'Recurrence' is defined as the re-opening of the study ulcer after complete healing.

- History of a DVT (deep vein thrombosis) in the study leg
- Presence of hyper-pigmentation, edema, varicosities, lipodermatosclerosis, thickened nails and dermatitis.

8.4.4 PHYSICAL EXAM & VITAL SIGNS

PHYSICAL EXAMINATION

The physical examination will include routine examinations for the following:

- HEENT
- Abnormalities of the extremities
- Neurologic abnormalities
- Heart/cardiovascular abnormalities
- Musculoskeletal abnormalities

- Dermatologic abnormalities
- Any other body system for which an abnormality is noted and which, in the opinion of the Investigator, is relevant to the safety of the subject or could impact safety or efficacy results for the subject; i.e., the abnormality is clinically significant.

Each abnormality will be recorded and the Investigator will record an assessment of its clinical significance.

VITAL SIGNS

The following vital signs will be collected at S1 visit only:

- Height
- Weight
- BMI calculated.
- Seated blood pressure (take after the subject has been seated for at least 5 minutes)
- Pulse
- Temperature

8.5 VASCULAR ASSESSMENTS

8.5.1 ANKLE-BRACHIAL INDEX (ABI)

Ankle Brachial Index (ABI) is the ratio of blood pressure measured at the ankle to that measured at the arm. An ABI < 0.75 indicates that there is a high probability that arterial insufficiency is present (positive predictive value 95% in a general practice population).

It should be noted that incompressible, calcified arteries may occur in diabetes causing a falsely elevated ABI, so if the subject has other signs or symptoms that could suggest peripheral arterial disease, further investigations to determine vascular status may be warranted.

A documented record of an ABI test performed using the study ulcer (target) leg, within 3 months of the first Screening visit (S1) is acceptable for the purposes of this study otherwise this must be completed in the Screening Period.

NOTE: Compression bandaging must not be applied to subjects entered onto this study unless the ABI is confirmed to be greater than 0.75.

The technique for measuring ABI explained in Appendix 19.1.

8.5.2 SKIN PERFUSION PRESSURE

Skin perfusion pressure (SPP) can also be employed to evaluate the patient's vascular status. SPP is obtained using a Laser Doppler. Patients with SPP less than 30 mmHg have Arterial Insufficiency and are not candidates for enrollment.

8.6 PAIN ASSESSMENTS

Pain intensity of the VLU is to be assessed before any dressing changes or other wound manipulations at all screening visits, treatment visits, and at follow-up period visits.

Subject will be asked to rate his/her pain on a scale of 0 to 10 with 0 being "no pain" and 10 being the "worst pain possible".

8.7 ULCER ASSESSMENTS

8.7.1 STUDY ULCER EXUDATE ASSESSMENTS

The Investigator will determine the amount and type, if any, of study ulcer exudate. In determining the amount of study ulcer exudate, the Investigator must take into account the amount of exudate absorbed into the study ulcer dressing. The following categories will be used to quantify the amount & describe the type of ulcer exudate:

Study Ulcer Exudate Assessment	
Volume	• No exudate • Minimal amount • Light (scant) or small amount • Moderate amount • Heavy/large/copious amounts
Type	• Not applicable: no exudate present • Serous: clear or light yellow watery plasma • Serosanguinous: pink to light-red watery plasma • Sanguineous: red with fresh bleeding • Opaque exudate: green or tan

8.7.2 STUDY ULCER INFECTION ASSESSMENTS

The presence/absence of the following signs of infection at the study ulcer site will be documented at each visit. Infection of the index wound will be assessed using the STONES method developed by Woo and Sibbald.[29] The wound can be considered infected when:

Study Ulcer Infection Assessment
Three of the following signs or symptoms: • Increased surface area • Increased periwound margin temperature by more than 3°F difference between 2 mirror image sites • Exposed bone or can be probed to the bone • New areas of breakdown or satellite lesions • Presence of swelling or reddened skin in periwound area • Increased wound drainage • Unpleasant, sweet, or sickening odor present **NOTE:** A wound that can be probed to the bone should warrant work-up to eliminate osteomyelitis even if other factors are absent.

INFECTION AT STUDY ULCER SITE PRIOR TO RANDOMIZATION

If the infection occurs prior to randomization *i.e.*, prior to the first Treatment Phase visit (TV1), then the subject will be ineligible to be randomized.

INFECTION AT STUDY ULCER SITE AFTER RANDOMIZATION

If infection of the study ulcer site occurs after randomization *i.e.*, after the Randomization visit (TV1), record the infection as an adverse event, and treat as appropriate with topical antimicrobial (e.g. topical silver antimicrobial) and/or oral (but not topical) antibiotics at the discretion of the investigator.

Note: topical antibiotics to the study ulcer site are prohibited

A subject with an infected ulcer that is being treated by the Investigator will remain in the study unless the situation requires an alternative methodology that violates the protocol. Antibiotic interventions will be recorded on the Concomitant Medications Form and the event will be categorized as an Adverse Event, serious if it meets the definition of that category. All subjects who show evidence of an ulcer infection must have it reported on an Adverse Event Form.

All subjects will be instructed to contact the Investigator if signs or symptoms of infection develop prior to their next scheduled visit.

During an episode of infection, the Investigator can continue multi-layer compression. However, the dressings should be changed at least every 72 hours until infection is resolved.

8.7.3 INVESTIGATOR ASSESSMENT OF STUDY ULCER CLOSURE

"**Complete healing**" of the study ulcer is defined as 100% re-epithelialization without drainage. At each visit, the investigator will assess the wound by answering the following questions:

- Study ulcer 100% re-epithelialized?
- Drainage is absent?

Both questions must be answered "yes" for the study ulcer to be considering having reached "complete closure".

The date of complete healing is defined as the date of the first assessment of 100% re-epithelialization.

8.8 STUDY ULCER PHOTOGRAPHS

The study ulcer will be digitally photographed according to the schedules in this protocol. The digital photographic measurements will be used for surface area measurements to determine eligibility.

8.9 RANDOMIZATION

Subjects who are eligible to participate in the trial will be randomized by computer to one treatment group at the TV1 visit.

8.10 ASSESSMENT OF MULTI-LAYER COMPRESSION THERAPY

The following measurements will be taken to assess the performance of the compression bandaging during the Screening and Treatment Phase visits and at selected Follow-up visits:

1. Whether the bandage was in place at each visit (Yes/No).
2. If the bandage was not in place, the date it was removed and the reason for removal (slippage, strikethrough or other).
3. If the bandage is in place but needs to be changed at an unscheduled visit, the reason for removal/change must be recorded from the following:
    a. Maximum wear time
    b. Subject withdrawn
    c. Adverse event
    d. Slippage
    e. Primary dressing change needed
    f. Soiled
    g. Subject request
    h. Other

9 STATISTICAL ANALYSIS PLAN

SerenaGroup®

Statistical Analysis Plan (SAP): CelluTome™-VLU-013

A Multi-center Randomized Open-Label Controlled Clinical Trial Evaluating Suction Blister Grafting utilizing a Novel Harvesting Device (CelluTome™) and Standard of Care vs. Standard of Care alone in the Treatment of Venous Leg Ulcers.

Sponsor: SerenaGroup®

CONFIDENTIAL

Version 2

Trial Statistician:	**Marissa J. Carter PhD MA MAPWCA** **President** **Strategic Solutions, Inc** **307-587-5352** **mcarter@strategic-solutions-inc.com**

9.1 BRIEF TRIAL DESCRIPTION

THIS TRIAL IS A RANDOMIZED CONTROLLED TRIAL (RCT) IN WHICH SUBJECTS WITH VENOUS LEG ULCERS (VLUS) WILL BE RANDOMIZED 1:1 TO A MAXIMUM OF 3 TREATMENTS OF CELLUTOME™ 4 WEEKS (GROUP 1A) OR STANDARD OF CARE (SOC; GROUP 2A) AND FOLLOWED FOR 12 WEEKS. PRIMARY ENDPOINT IS TIME TO HEAL WITHIN 6 AND 12 WEEKS. IF WOUNDS IN GROUP 2A HAVE NOT BEEN REDUCED IN AREA BY AT LEAST 40% BY 6 WEEKS, PATIENTS WILL BE ALLOWED TO RECEIVE CELLUTOME™ TREATMENT (GROUP 2C; 2 TREATMENTS WITHIN THE REMAINING 6 WEEKS). THE REMAINDER OF THE SOC PATIENTS WILL BECOME GROUP 2B.

9.1.1 GENERAL

Descriptive statistical methods will be used to summarize the data from this study with hypothesis testing performed for primary and secondary endpoints. These include number of subjects (n), mean, median, standard deviation, minimum, and maximum for continuous data, and frequencies and percentages for categorical data. All data collected during the study will be reported and analyzed.

Unless specified otherwise, all statistical testing will be two-sided and performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted using PASW 19.

9.2 RESEARCH HYPOTHESES

Primary endpoints

H1: Time to heal

Time to heal after 12 weeks of CelluTome™ + SOC (**Group 1A**; maximum of 3 CelluTome™ applications) versus 12 weeks of SOC (**Group 2B**) will be equal for both groups or time to heal after 6 weeks of CelluTome™ + SOC (**Group 1A**; maximum of 2 CelluTome™ applications) versus 6 weeks of SOC (**Group 2A**) will be equal for both groups. Formally, H0: T1–T2=0; HA: T1–T2=D1 ≠ 0, where T1 is the time to heal for **Group 1A**, T2 is the time to heal for **Group 2A or 2B** depending whether T2 is 12 or 6 weeks, D1 is the difference (T1–T2) assuming the alternative hypothesis, and statistical test used is log rank applied to a Kaplan-Meier analysis. If either result is statistically significant, results will be adjusted using Cox regression and appropriate covariates.

Secondary endpoints

H2: Proportion of wounds healed

The proportion of wounds healed at 12 weeks after 12 weeks of CelluTome™ + SOC (**Group 1A**; maximum of 3 CelluTome™ applications) versus 12 weeks of SOC (**Group 2B**) will be equal for both groups. Formally, H0: P1–P2=0; HA: P1– P2=D1≠0, where P1 is the proportion of wounds healed for **Group 1A**, P2 is the proportion of wounds healed for **Group 2B**, D1 is the difference (P1–P2) assuming the alternative hypothesis, and statistical test used is chi square.

H3: Incidence of Adverse Events (AEs)

The incidence of adverse events (AEs) by 12 weeks after 12 weeks of CelluTome™ + SOC (**Group 1A**; maximum of 3 CelluTome™ applications) 12 weeks of versus SOC (**Group 2B**) will be equal for both groups. Formally, H0: I1–I2=0; HA: I1– I2=D1≠0, where I1 is the incidence of AEs for **Group 1A** on a per subject basis , I2 is same metric for **Group 2B**, D1 is the difference (I1–I2) assuming the alternative hypothesis, and statistical test used is the Mann-Whitney test.

H4: Correlation of wound area with protease activity at 4 weeks

The Pearson correlation coefficient (or Spearman's rho in the case of severe non-normality) calculated

from the mean change in wound area and protease activity between baseline and 4 weeks after 4 weeks of CelluTome™ + SOC (**Group 1A**) versus 4 weeks of SOC (**Group 2A**) will be equal for both groups. Formally, H0: r1–r2=0; H1: r1–r2=D1 ≠ 0, where r1 is the Pearson correlation coefficient for subjects in **Group 1A** calculated from difference in mean wound area and difference in mean protease activity between baseline and 4 weeks, r2 is the is the same specified metric for subjects **Group 2A**, D1 is the difference (r1–r2) assuming the alternative hypothesis, and the statistical test used is Steiger's Z test with the assumption that r will have the same sign for both groups.

H5: Correlation of wound area with protease activity at 8 weeks

The Pearson correlation coefficient (or Spearman's rho in the case of severe non-normality) calculated from the mean change in wound area and protease activity between baseline and 8 weeks after 8 weeks of CelluTome™ + SOC (**Group 1A**) versus 8 weeks of SOC (**Group 2B**) will be equal for both groups. Formally, H0: r1–r2=0; H1: r1–r2=D1 ≠ 0, where r1 is the Pearson correlation coefficient for subjects in **Group 1A** calculated from difference in mean wound area and difference in mean protease activity between baseline and 8 weeks, r2 is the is the same specified metric for subjects in **Group 2B**, D1 is the difference (r1–r2) assuming the alternative hypothesis, and the statistical test used is Steiger's Z test with the assumption that r will have the same sign for both groups.

H6: Reduction in pain score at 12 weeks

The change in mean pain score between baseline and at 12 weeks after 12 weeks of CelluTome™ + SOC (**Group 1A**) versus 12 weeks of SOC (**Group 2B**) will be equal for both groups. Formally, H0: μ1–μ2=0; H1: μ1–μ2=D1 ≠ 0, where μ1 is the mean difference in pain score for subjects in **Group 1A** between baseline and 12 weeks, μ2 is the is the same specified metric in **Group 2B**, D1 is the difference (μ1–μ2) assuming the alternative hypothesis, and the statistical test used is the paired t test if data are normal and the Mann-Whitney test on a non-paired basis if data are non-normal.

H7: Proportion of wounds healed at 12 weeks (Groups 2B versus 2C)

The first hypothesis to be tested is that the proportion of wounds healed in **Group 2C** (12 weeks of SOC + 2 CelluTome™ applications) is no more than 15% lower than the proportion of wound healed in **Group 2B** (12 weeks of SOC). Noninferiority is established if the lower limit of the (1-2α) x 100% CI (confidence interval) for the difference (**Group 2C** – **Group 2B**) is above -15% where the equivalence margin, δ, is 15%. If non-inferiority is not established because the proportion of wounds healed in **Group 2C** is much higher than in **Group 2B**, the superiority hypothesis testing used in H2 will be utilized.

H8: Incidence of Adverse Events (AEs) (Groups 2B versus 2C)

The incidence of adverse events (AEs) by 12 weeks after 12 weeks of CelluTome™ + SOC (**Group 2C**; maximum of 2 CelluTome™ applications) versus 12 weeks of SOC (**Group 2B**) will be equal for both groups. Formally, H0: I1–I2=0; HA: I1– I2=D1≠0, where I1 is the incidence of AEs for **Group 2C** on a per subject basis , I2 is same metric for **Group 2B**, D1 is the difference (I1–I2) assuming the alternative hypothesis, and statistical test used is the Mann-Whitney test.

9.3 SAMPLE SIZE CALCULATIONS

A two-sided logrank test with an overall sample size of 225 subjects (113 in the control group and 112 in the treatment group) achieves 86.1% power at a 0.05 significance level to detect a hazard ratio of 1.74 when the proportion of wounds healed in the control group is 0.5. The study lasts for 12 weeks with all subjects beginning the study together (no accrual periods). The proportion dropping out of the control group is 0.033 per week. The proportion dropping out of the treatment group is 0.0083 per week. The proportion switching from the control group to another group with a hazard ratio equal to that of the treatment group is 0.

9.4 RANDOMIZATION

Randomization will be stratified by age of wound at the first screening visit (1-6 months and > 6 months).

The randomization algorithm will employ blocks of 2 and 4, with random selection of block sizes (Pass 11).

9.5 INTERIM OPERATIONS (WHILE RECRUITMENT IS CONTINUING)

Safety

The following variables will be analyzed for the first 40 subjects after each subject has completed the study (or been withdrawn):

- Initial assignment (**Group 1A or 2A**)
- Subsequent assignments (Groups 2B and 2C)
- Site
- Ulcer area at randomization (TV1) through TV13
- Patients withdrawn
- Patients who experience infection (and visit number(s))

The report should show accrued data in aggregate and by site. The report should be available in Excel format.

If for some reason severe imbalances develop between groups or at sites, a new randomization scheme will be implemented with additional or modified stratification based on the most affected variables.

9.6 INTERIM ANALYSIS

One interim safety and efficacy analysis will be performed when approximately 110 subjects have been enrolled (approximately 50% of subjects). The total number of AEs and SAEs will be calculated, and the proportion of healed wounds tabulated for all groups (at 12 weeks). New sample size calculations will be performed based on the proportion of healed wound data (time to heal). No statistical analysis will be conducted.

TRIAL STOPPING RULES

The Medical Monitor may unblind, or appoint an independent assessor to unblind interim data analyses to determine whether the study should be stopped if any of the following occur:

- If the primary outcome likely has reached statistical significance and there is agreement to analyze the secondary outcomes regardless of whether they are likely to achieve statistical significance or not.
- If the primary outcome has likely not reached statistical significance and based on futility boundary analysis, the numbers of additional subjects needed to reach significance based on the rate data are too high to realistically extend the trial.
- ≥ 25% of ulcers have deteriorated (meaning an increase in area from baseline to 12 weeks).
- A significant proportion of patients (> 25%) in either group continue to experience infections despite Prisma/antimicrobial treatment or other measures
- Excessive non-planned withdrawal of subjects

List of variables for interim analysis

- Site ID
- Subject ID
- Treatment assignment
- Gender
- Pain score at baseline and 12 weeks
- Wound healed (Y/N) by 12 weeks (after 12 weeks have elapsed from date of randomization)
- When wound healed (days from randomization)
- Ulcer area at baseline and 6/12 weeks
- AEs (by category; have separate category for infected wounds) and SAEs at 12 weeks (count by subject, and description/category where applicable)
- Withdrawn subjects or subjects that have been lost to follow-up (count and by assignment with reason where applicable)
- Number of subjects in **Groups 2B and 2C**.

PATIENT POPULATIONS

The populations defined for the final analysis will include the intent-to-treat (ITT), per protocol (PP), and safety populations.

INTENT-TO-TREAT POPULATION

The ITT population will be analyzed for primary and secondary endpoints up to 12 weeks; however, Index Ulcers that have been healed are still subject to confirmed wound closure for 2 weeks later (up to 14 weeks for ulcers initially healed in the 12th week).

PRE PROTOCOL POPULATION

The PP population for the 12-week study will comprise all subjects for which no protocol violations occurred and in which for both groups' complete data are available for all primary and secondary endpoints, and all covariates needed to conduct adjusted analyses.

SAFETY POPULATION

The safety population will be all subjects who have received at least CelluTome™ treatment or have completed the first visit (randomization, TV1).

9.7 STATISTICAL METHODS

MISSING DATA

Missing data may be the result of missed visits, subjects lost to follow-up or who have died, or data not available (e.g., smoking status).

Missing primary endpoint data within 12 weeks from start of study will not be imputed for ITT populations as these will constitute right-censored data in the time to heal analysis. The last visit for which data are available will constitute the start of censoring (healing outcome). For secondary endpoints within 12 weeks from start of study, the last observation carried forward (LOCF) principle will be used for ITT populations.

For adjusted analyses of primary and secondary endpoints, missing data for covariates used to adjust analyses may be imputed in separate models when the missing data for each covariate is ≤ 30% and it can be reasonably demonstrated that the missing data for each covariate are MCAR.

FINAL ANALYSIS

Analysis can be started when database lock for the trial is initiated. In regard to statistical modeling, the initial list of independent variables will be examined for correlation between pairs. In the event that

correlation (however calculated) exceeds 0.7, a selection will be made between the 2 variables to avoid possible issues of co linearity and variance inflation.

The following analysis will be conducted:

1. Flow chart (subjects)
2. Overall trial statistics (enrollment dates, site numbers, numbers of subjects)
3. Demographics between groups at baseline (no statistical testing but identification of likely trial imbalances)
4. Count of subjects in each group
5. Primary endpoints within 6 and 12 weeks (time to heal; previously defined by groups) will be analyzed by Kaplan-Meier and log rank test (unadjusted); and then Cox regression to adjust for the following variables (with and without covariate imputation):

    - Site
    - Year enrolled (if trial lasts 2 years or more)
    - Treatment group
    - Initial area at randomization (cm^2)
    - Initial wound depth (cm)
    - Age of wound at enrollment (days)
    - Smoking status
    - Prior VLU
    - Pain score at randomization
    - Patient age
    - Gender
    - Ethnicity/race
    - VCS score
    - Diabetes
    - Patient on pain medications
    - Smoking status
    - BMI
    - Infection episodes

6. Secondary endpoints will be analyzed as follows:

    - Proportion of wounds healed at 12 weeks will be analyzed by chi square (unadjusted); this endpoint may also be analyzed using logistic regression to adjust for the following variables (with and without covariate imputation) if needed:

        - Site
        - Year enrolled (if trial lasts 2 years or more)
        - Treatment group
        - Initial area at randomization (cm^2)
        - Initial wound depth (cm)
        - Age of wound at enrollment (days)
        - Smoking status
        - Prior VLU
        - Pain score at randomization

- Patient age
- Gender
- Ethnicity/race
- VCS score
- Diabetes
- Patient on pain medications
- Smoking status
- BMI
- Infection episodes
  Note: unadjusted testing for **Group 2C** versus **2B** will be non-inferiority, followed by superiority testing if the former fails and proportion of wounds healed in **Group 2C** is much higher than in **Group 2B**.

- A summary of the incidence of adverse events (count by patient):

  - AE (category)
  - SAE (category; brief description; resolution; should include death)
  - When event happened in relation to start of treatment (days)
  - Associated patient ID, site ID

  Incidence of AEs will be analyzed by Mann-Whitney test on a per subject basis.

- Correlation of changes in wound area with changes in protease activity will be first analyzed by calculating the Pearson correlation coefficient for each group or Spearman's rho if data are grossly non-normal. For each subject the change in protease activity is calculated by subtraction of the value at 4 or 8 weeks from the value at baseline; reduction in wound area is calculated similarly. Differences in r will be analyzed using Steiger's Z test with the assumption that r will have the same sign for both groups. If significant differences are found between groups, further analysis using GLM doubly multivariate repeated measures (change in wound area and protease activity between baseline and 4 and 8 weeks) to adjust for the following variables (with and without covariate imputation) may be undertaken (ordinal re-categorization will be done if data are severely non-normal):

  - Site
  - Year enrolled (if trial lasts 2 years or more)
  - Treatment group
  - Initial area at randomization (cm^2)
  - Initial wound depth (cm)
  - Age of wound at enrollment (days)
  - Smoking status
  - Prior VLU
  - Pain score at randomization
  - Patient age
  - Gender
  - Ethnicity/race
  - VCS score

- Diabetes
- Patient on pain medications
- Smoking status
- BMI
- Infection episodes

- Reduction in pain score (baseline – Week 12) will be analyzed by paired t test if the dependent variable data are not grossly non-normal or the Mann-Whitney test if they are grossly non-normal (unadjusted).

To create the flow chart, the following numbers will be required for each group: eligible (n), screened (n), screen failure (n, with categorical reasons), randomized (n), completed 12 weeks study (n), withdrawals or lost to follow-up after randomization (n, with categorical reasons).

These variables are required for the final analysis:

- Site ID (alphanumeric)
- Patient ID (alphanumeric)
- Year enrolled (numeric; date, year only)
- Group assignment (nominal factor, [1A, 2A, 2B, 2C]; note: subjects in 2A can also have 2B or 2C assignments as well)
- Lost to follow up (binomial factor [1,0; 1 = yes]; estimated date lost to follow-up; associated patient ID; categorical reason for lost to follow-up)
- Death during study (binomial factor [1,0; 1 = yes]; estimated date when death occurred)
- Gender (nominal factor [1, 0; male = 1])
- Subject age at S1 (continuous variable with breakpoint at > 90 years if > 90)
- Ethnicity/race (multinomial factor)
- Smoking status at S1 (ordinal factor: [1, 2, 3; never, ever, current])
- Wound count (Poisson distribution variable; count of VLUs at patient level at randomization)
- Index Ulcer location (multinomial factor)
- Prior VLU at S1 (binomial factor [1,0; 1 = yes])
- Wound age at S1 (continuous variable, days; S1)
- Pain score (continuous variable, 0-10, TV1 and 13)
- VCS score at S1 (continuous variable, 0-24)
- BMI at S1 (continuous variable)
- Diabetes at S1 (nominal factor [1, 0; yes = 1])
- Subject on pain medication at S1 (nominal factor [1, 0; yes = 1])
- Wound area (continuous variable, cm^2; weekly, weeks TV1-13)
- Wound depth (continuous variable, cm; weekly, weeks TV1-13)
- Outcome healed at 12 weeks (binomial factor, [1,0; 1 = yes])
- Time to heal (continuous variable, 0-84, days after randomization for healed wounds)
- Initial wound healed within 12 weeks and confirmed closed about 2 weeks later (binomial, [1,0; 1 = yes])
- Ulcer recurrence by 12 weeks (binomial factor, [1,0; 1 = yes]; estimated days after randomization when event occurred)
- Infection episode (Poisson variable, weeks TV1-13)
- AE (Poisson distribution variable, count at patient level; date of onset)

- TEAE (Poisson distribution variable, count at patient level for each of 4 categories; date of onset)
- SAE (Poisson distribution variable, count at patient level; date of onset; resolution; brief description)

ADJUSTMENT FOR FEWR

Adjustment to control for the family wise error rate (FEWR) (also known as multiplicity of testing) will use the Hochberg step-up procedure thus: Let $p1 \geq p2 \geq \ldots pK$ be the ordered p-values and H1, H2, …, HK be the corresponding null hypotheses. Reject Hk and all Hj for $j \leq k$ if $pk \leq \alpha/k$. The adjusted p-values are pak = min {p1, 2p2, …kpk} for k = 1, 2, …, K.

10 ADVERSE EVENTS (DEFINITIONS AND REPORTING)

The Investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE, as provided in this protocol. During the study when there is a safety evaluation, the Investigator or site staff will be responsible for detecting, documenting and reporting AEs and SAEs as detailed in this Section of the protocol.

10.1 ADVERSE EVENTS

An adverse event (AE) is defined as any unfavorable or unintended sign, symptom, or disease that occurs or is reported by the patient to have occurred, or a worsening of a pre-existing condition. An adverse event may or may not be related to the treatment regimen. All AEs and intercurrent illnesses must be recorded in the subject's medical records and on the CRF.

Adverse events will be defined as those events that occur after signing the informed consent until the final study visit. A description of the AE along with the onset date, end date, severity, action taken, treatment, outcome, likely cause, and relationship to the study products will be recorded in the CRF.

Local and systemic AEs associated with each treatment arm will be tabulated and compared. AEs will be elicited through direct questioning, subject reports, and physical examination.

An abnormal laboratory test result is not by itself considered to be an AE unless the Investigator considers the finding of clinical significance that should be reported in such a manner.

10.2 REPORTING OF ADVERSE EVENTS

The Investigator is responsible for assessing the relationship of the adverse event to the treatment, and the seriousness and expectedness of the adverse event at the time of occurrence. A medically qualified person appointed by the Sponsor will also assess this, once the Sponsor has been notified of an AE. All adverse events that occur during the trial will be documented on the supplied adverse event forms.

AEs/SAEs reported during the study, or SAEs reported within 30 days of the end of the study, should be followed to resolution of the AE/SAE or, within thirty days from the end of the study. A final assessment of the outcome will be made at that time.

Each AE will be categorized as "serious" or "not serious" based on the definition of an SAE. An SAE is defined as an AE resulting in at least one of the outcomes:

- Results in death
- Is life threatening (the subject is at immediate risk of dying from the adverse experience)

- Requires subject hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse device effect when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

10.3 ADVERSE EVENT SEVERITY ASSESSMENTS

The guidelines outlined in CTCAE v4 will be used for severity assessments. Note: the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

AE SEVERITY GRADING SCALE

Severity Grade	Description
Mild (1)	Awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks.
Moderate (2)	Discomfort enough to cause interference with usual daily activity. It may warrant therapeutic intervention.
Severe (3)	Incapacitating; inability to perform usual activities and daily tasks; significantly affects clinical status; requires therapeutic intervention.
Life-threatening (4)	Emergency treatment required life-threatening, death.

10.4 ADVERSE EVENT CAUSALITY ASSESSMENTS

Adverse events will be assigned a relationship (causality) to the treatments. The Investigator will be responsible for determining the relationship between an AE and the treatment. The type of event, organ system affected, and timing of onset of the event will be factors in assessing the likelihood that an AE is related to the treatment. Relationship of AEs to study products will be classified as follows:

- Not Related: No relationship exists between the AE and the treatment. The event is attributed to a pre-existing medical condition or an intercurrent event unrelated to the study product.
- Possibly Related: Follows the treatment, but may have developed as a result of an underlying clinical condition or treatments/interventions unrelated to the study product.
- Probably Related: Follows the treatment, but is unlikely to have developed as a result of the subject's underlying clinical condition or other treatment or other interventions.
- Definitely Related: Follows the treatment and physical evidence shows a convincing relationship to the treatment.

- Unknown: Follows the treatment, but unable to determine the relationship to the treatment.

11 SERIOUS ADVERSE EVENTS

A Serious Adverse Event (SAE) is any untoward medical incident that occurs during the course of the trial beginning after informed consent has been executed and extending until 30 days after the final study visit.

A SAE is defined as any AE that:

- Results in death
- Is life threatening (the subject is at immediate risk of dying from the adverse experience)
- Requires subject hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse device effect when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.1 REPORTING OF SERIOUS ADVERSE EVENTS

The Investigator is required to report all SAEs that occur during the study. Once the Investigator becomes aware of an SAE, he/she must report the SAE to SerenaGroup® and the Project Lead within 24 hours of discovery:

Medical Monitor	Thomas E. Serena MD FACS (814) 688-4000

A written SAE report must follow and must include a full description of the event and all supporting documentation available at the time (e.g., lab reports, electrocardiogram [ECG] reports, etc.). Additional follow-up information as it becomes available must be reported to the Sponsor.

The Investigator is also responsible for reporting all SAEs to the appropriate Institutional Review Board (IRB) in accordance with local laws and regulations. The Investigator is responsible for maintaining documentation in the study file that indicates the IRB has been properly notified.

11.2 SAE FOLLOW-UP

All SAEs will be monitored for a minimum of 30 days until they are resolved, have stabilized, or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es).

12 DIRECT ACCESS TO SOURCE DATA/DOCUMENTATION

Subjects will be identified on CRFs by a unique subject identification number and on source documents by name and date of birth. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject.

The monitors, auditors, personnel authorized by the Sponsor, the local IRB, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research and will be given direct access to source data and documentation (e.g., medical charts/records, printouts etc.) for source data verification, provided that subject confidentiality is maintained in accordance with local requirements. Access to electronic medical records may be governed by institution policy and each site will be required ensure access while remaining compliant with institutional requirements.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 ACCEPTABILITY OF CASE REPORT FORMS

CRF must be completed for each subject who has signed an informed consent form. For subjects who are screen failures, this would be limited to the screen failure CRF page. All source documents and CRFs will be completed as soon as possible after the subject's visit.

13.2 MODIFICATION OF PROTOCOL

The Investigator will not modify or alter this protocol without first obtaining the concurrence of the Sponsor. Approval by the Investigator's IRB must also be obtained prior to implementation of the change, with two exceptions:

1. When necessary to eliminate apparent immediate hazard to the subject; or
2. When the modification does not involve the subject's participation in the trial.

An amendment may also require modification of the informed consent form. The Investigator will provide an approval letter for the amendment and revised informed consent form, if applicable, to the Sponsor. An amendment must be in writing and it must be dated by both the Sponsor and the Investigator. If necessary, the Sponsor will submit protocol amendments to FDA and other appropriate regulatory authorities and notify other Investigators using this protocol.

13.3 REPORTING PROTOCOL DEVIATIONS

The Investigator is obligated to follow the protocol without departure from the requirements written in the protocol. If the Investigator deviates from the protocol requirements, the Sponsor will make the determination as to whether the subject will continue in the study. The Sponsor also has the right to discontinue the subject for protocol violations. The IRB may also have to contacted if safety to the subject or if the scientific soundness of the study is involved. All protocol deviations must be documented in the CRFs.

14 ETHICS AND REGULATORY REQUIREMENTS

This study is to be conducted in accordance with the specifications of this protocol and in accordance with principles consistent with Declaration of Helsinki, Good Clinical Practice (GCP), 21 CFR 312, ICH E6, HIPAA regulations in 45 CFR Part 164, and the Belmont Principles of respect for persons, beneficence, and justice. No protocol changes will be implemented without the prior review and approval of the IRB, except where it may be necessary to eliminate an immediate hazard to a research subject. In such a case, the change will be reported to the IRB as soon as possible, according to IRB regulations. Additionally, all study products used in this study are manufactured, handled and stored in accordance with applicable Good Manufacturing Practices (GMP) and the products provided for this study will be used only in accordance with this protocol.

14.1 INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE

The Principal Investigator will provide the Institutional Review Board/Independent Ethics Committee (IRB/IEC) with all appropriate materials as required by their IRB, including but not limited to the clinical study protocol, informed consent form, and any advertising materials. The study will not be initiated until the IRB provides written approval of the aforementioned documents and until approval documents have been obtained by the Principal Investigator and Sponsor or Sponsor designee. The Investigator will not participate in the decision. If the Investigator is an IRB or IEC member, documentation must be provided indicating recusal from the approval process. Appropriate reports on the progress of this study by the Principal Investigator will be made to the IRB/IEC as required by local and applicable government regulations and in agreement with policy established by the Sponsor. The Investigator is required to

maintain an accurate and complete record of all written correspondence to and received from the IRB/IEC, and must agree to share all such documents and reports with the Sponsor.

No changes from the final approved protocol will be initiated without the IRB/IEC's prior written approval or favorable opinion of a written amendment, except when necessary to eliminate immediate hazards to the subjects or when the change involves only logistics or administration.

14.2 INVESTIGATOR'S RESPONSIBILITIES

The Investigators are responsible for performing the study in full accordance with the protocol and the current revision of the Declaration of Helsinki, the Good Clinical Practice: Consolidated Guideline, approved by the ICH, and any applicable national and local laws and regulations. Information regarding any study centers participating in this study that cannot comply with these standards will be documented.

14.3 SUBJECT INFORMED CONSENT REQUIREMENTS

Written and oral information about the study in a language understandable by the subject will be given to all subjects by the Investigator and/or designee. Written informed consent will be obtained from each subject before any procedures or assessments that would not otherwise be required for the care of the subject are done and after the aims, methods, anticipated benefits, potential hazards, and insurance arrangements in force are explained and the subject has been given sufficient time to ask questions and consider participation in the study. It will also be explained to the subjects that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. It is permissible for a third person (e.g., a family member) to be present during the explanation of the study.

The written Informed Consent Form (ICF) is to be in compliance with Code of Federal Regulations (CFR) 21 Part 50.27 and Good Clinical Practice (GCP) guidelines. The Sponsor and/or designated CRO will approve the ICF and all amendments to the ICF prior to submission to the IRB/IEC. A copy of the ICF to be used will be submitted by the Investigator to the IRB/IEC for review and approval prior to the start of the study. Each study site must provide the Sponsor with an unsigned copy of IRB/IEC-approved ICF along with applicable documentation to support this approval. The original signed ICF is retained in the subject's study records, and a copy is provided to the subject. A second copy may be filed in the subject's medical record, if allowed by institutional policy.

15 DATA HANDLING AND RECORD KEEPING

15.1 RECORDING AND COLLECTION OF DATA

The primary source document for this study will be the subject's medical record. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study.

Applicable source data will be manually transcribed to approved case report forms (CRF). The investigator is ultimately responsible for the accuracy of the data transcribed on the forms. All source documents and CRFs will be completed as soon as possible after the subject's visit.

The Investigator will review CRFs to indicate that, to his/her knowledge, they are complete and accurate. If further changes are made after this, the Investigator will need to again sign the Investigator signature page. Designated source documents will be signed and dated by the appropriate study personnel. The Investigator must agree to complete and maintain source documents and CRFs for each subject participating in the study.

All research data will be entered, either electronically or manually, into a computerized database, designed in accordance with 21 CFR Part 11 and based on protocol requirements defined by the Sponsor in association with the PI and clinical data manager.

The Investigator will maintain a confidential list of study subjects which will include each subject's study number, name, date of birth and unique hospital identification number if applicable. This list will be kept by the Investigator and will not be collected by the Sponsor. A notation will be made in the subject's case history/medical chart that he/she is participating in a clinical study and has provided a signed and dated informed consent form (ICF) as well as the required site HIPAA authorization (if separate from the ICF). The Investigator must also maintain a separate screening log of all the subjects screened for participation in the study; it should include gender; age; eligibility status; reason for ineligibility, if applicable; and study allocated subject number, if applicable.

15.2 CLINICAL DATA MANAGEMENT

The Sponsor will be responsible for the processing and quality control of the data. Data management will be carried out as described in the Sponsor's standard operating procedures (SOPs) for clinical studies.

15.3 ARCHIVING

All study documentation at the Investigator site and Sponsor site will be archived in accordance with ICH GCP E6 and the Sponsor's quality standards and SOPs.

In accordance with new Medical Device Directive [MDD 2007/47/EC], study records must be retained by the Investigator for 15 years following the conclusion or termination of the study. Study records should

not be destroyed without prior written agreement between the Sponsor and the study Investigator. At the completion of the study, details of the archival process must be provided to the Sponsor. Study records are subject to inspection by applicable health and regulatory agencies at any time.

Records to be retained by the Investigator include, but are not restricted to:

- Source data and the primary records upon which they are based (e.g., subject's progress notes, adverse event data, test results, and any other diagnostic procedures required to evaluate the progress of the study).
- Signed protocols and protocol amendments
- Laboratory results, ranges and certifications
- Product (e.g., Investigational product, Standard of Care supplies) and accountability records
- Study personnel signature log
- Monitoring logs
- Correspondence to and from the Sponsor, designee and IRB
- Investigator and sub-investigator CVs
- Signed informed consent and HIPAA consent forms
- Subject screening and randomization log
- Serious adverse event reports
- Institutional Review Board approval and re-approval letters
- Wound tracings and images
- Subject diary pages
- Completed quality of life questionnaire
- Other documents pertaining to the conduct of the study

These documents must be maintained and kept on file by the Investigator so that the conduct of the study can be fully documented and monitored.

16 PUBLICATION PLAN

Manuscripts and abstracts will be prepared by both the Sponsor. The results of the study may be published in scientific literature and may also be used in submissions to regulatory authorities. It is the intent of the Sponsor, and the Principal Investigator to publish or present the study results together with the other sites, unless specific permission is obtained in advance from the Sponsor to publish separate results. Co-authorship with any of the Sponsor's personnel will be discussed and mutually accepted upon submission of a manuscript or publication.

All information concerning the Sponsor's operations (such as patent applications, formulae, manufacturing processes, basic scientific data, or formulation information supplied to the Investigator and not previously published) is considered confidential by the Sponsor and shall remain the sole property of the Sponsor. The Investigator agrees not to use it for other purposes without written consent.

PUBLICIATION AND DISCLOSURE

Because this is a multi-center trial, site and investigator shall not independently publish, publicly disclose, present or discuss any results of or information pertaining to site's and Investigator's activities conducted under this agreement until such a multi-center publication is released under Sponsor's direction; provided, however, that if a publication is not released within eighteen (18) months after completion of analysis of all study data from all studies conducted within the multi-center trial, site and Investigator shall have the right to publish the results of and information pertaining to site's and Investigator's activities conducted under this protocol and the clinical trial agreement. Site and Investigator agree to submit any proposed manuscript, presentation or other public disclosure regarding the study to Sponsor for review at least thirty (30) days prior to submitting such proposed manuscript to a publisher or delivering or making such presentation or other public disclosure to any third party. Within thirty (30) days of its receipt, Sponsor shall advise site and/or Investigator, as the case may be, in writing of any information contained therein that is confidential information (other than research results included in a proposed manuscript) or that may impair sponsor's ability to obtain patent protection. Sponsor shall have the right to require site and/or Investigator, as applicable, to remove specifically identified confidential information (but may not require removal of research results from a proposed manuscript) and/or to delay the proposed submission or delivery of the proposed manuscript or presentation, or other public disclosure, for an additional sixty (60) days to enable Sponsor to seek patent protection. Site and Investigator shall not publish, publicly disclose, present or discuss any results of or information pertaining to site's and Investigator's activities prior to completion of the trial, even if the multi-center trial or the study is terminated before its completion and the final clinical study report is signed off, or (b) with respect to any endpoints or analyses other than those specified in this protocol.

17 MONITORING PLAN

17.1 SITES

17.1.1 SITE SELECTION

SerenaGroup® will be responsible for site selection.

17.1.2 INVESTIGATORS MEETING

The purpose of the Investigator's meeting is to review the salient features of the study and what will be required of the investigators. In addition, the need for strict adherence to the research protocol and research principles in general will be discussed. The meeting will be conducted by the Lead or Principal Investigator and other personnel as necessary. Regional meetings may be held in place of a national meeting if a large number of sites are expected for the study.

At the end of the meeting, if the site investigator still wishes to continue, a site initiation visit will be scheduled.

17.1.3 SITE INITIATION VISIT (SIV)

The purpose of the visit is to ensure that all the required study documentation is in place and that study site personnel are sufficiently prepared to conduct the clinical trial according to the study protocol, and ICH-GCP. The visit will include review of the study protocol, subject recruitment, informed consent procedures, case report form (CRF) completion, investigational device (ID), study specific procedures, monitoring and source documentation, adverse event reporting and review of essential documents maintained in the Investigator Site File.

SIV

The following topics will be reviewed with key site personnel during the SIV:

<u>Study Specific introductory information</u>

- Background
- Standard treatments
- Study design
- Rationale for study
- Study objectives

<u>Investigational Products</u>

- Presentation, use, ordering, delivery, dispensing, handling, storage and disposal of CelluTome™ units and harvesters.
- Physician training on use of the CelluTome™ epidermal harvesting system.

Study design and schedule of events

- CelluTome™ manufacturers suggested use.
- Review of protocol and procedures
- Inclusion/exclusion criteria, including study eligibility after the 2-week screening period
- Efficacy and safety endpoints
- SAE and Adverse Event documentation and reporting

Subject Recruitment

- Informed consent procedure and documentation
- Screening and enrolment procedures
- Procedure for randomization
- Procedure for follow up / withdrawal and criteria for withdrawal

Case Report Form

- Methods of completion of trial documentation
- Data Clarification
- Monitoring queries
- Resolution process

Study specific procedures

- Preparation of ulcer area
- Wound measurement.
- ABI measurement AND/OR SPP measurement
- Photography
- Swab collection for proteases/
- Protease collection procedure (Serena Technique®)
- Dressing application / Compression wrapping

Investigator Site File (ISF)

- Signed protocol
- Signed letter of agreement
- Regulatory approvals

ICH-GCP

- Confirmation that all staff have completed GCP training
- Investigator responsibilities
- Sponsor responsibilities
- Source Documentation
- Protocol Compliance
- Protocol Deviations/Violations

STUDY SPECIFIC MATERIALS

The site will be provided with study specific materials:

- Protocol
- Source documentation
- Protease swabs
- Camera and Memory Card
- Measuring grids, if applicable.

SIV DOCUMENTATION

All attendees must sign the SIV "Local Site and Trial Personnel Training Form" attendee log. SIV training will be documented in the monitor's visit report and filed in the trial master file (TMF). A pre-visit letter listing the agenda items and a post-visit letter will be sent to the principle investigator (PI) at the site and filed in the ISF, copies will be filed in the TMF.
An "Initiation Checklist" will be completed at the SIV to confirm that all essential study related procedures, trial products and responsibilities have been discussed at the visit. The PI will sign and date the Initiation Checklist, and "Protocol receipt."
Following the SIV the medical monitor or designee will notify the site that it is ready to start recruiting.

17.2 MONITORING VISITS

FREQUENCY OF MONITORING VISITS

CRFs will be accessed and monitored remotely through a secure server. The monitor will schedule at least one on-site routine monitoring visit during the active phase of the study. This will be arranged as soon as practical. This visit is intended to ensure that the study is conducted according to the protocol, verifies subject's eligibility and, if applicable, SAE forms have been completed appropriately.
This may be adjusted should there be concerns with the site such as significant number of protocol violations or non-compliance with GCP, in such circumstances further on site monitoring visits may be required.
Telephone contact may also take place to discuss the status of the site. These must be recorded in a telephone contact report and filed in both ISF and TMF.

The frequency of on-site monitoring visits may increase due to any of the following, including but not limited to:

- Detection of significant protocol violations.
- Protocol non-compliance.
- Change in site staff requiring training.
- Significant and persistent non-compliance with GCP.
- Protocol amendment that introduces a significant change requiring training.

- Rapid enrolment.
- Poor enrolment.
- Unusually high AE or SAE counts
- Improper standard of care
- Poor data management.

17.3 MONITORING DOCUMENTATION

- Documents Created with Regard to the Monitoring Visit
    - Pre-visit Letter
    - Monitor Visit Report
    - Post Visit Follow Up Letter
    - Any other Site Correspondence

- Monitoring Logs
    - Informed Consent Log
    - CV checklist Log
    - GCP checklist Log
    - Adverse Event Log
    - Protocol Violation/Deviation Log
    - SAE Log
    - Termination / Withdrawal Log

- Monitoring Reports
    - Study Initiation Visit Report
    - Routine Monitoring Visit Report
    - Close Out Monitoring Visit Report

- Site Logs and Tracking Forms
    - Investigational Device Accountability Log
    - Screening / Enrolment Log
    - Delegation of Staff Duties Log

17.4 CLOSE OUT VISIT

Each site will receive a close out visit. During the visit, the monitor shall perform the following activities including but not limited to:

- Review and address unresolved action items from previous monitoring visit(s).
- Review regulatory documents for completeness including a final review of signed informed consent documents.

- Verify that all general and ethics/regulations/R&D correspondence is present in the ISF.
- Dispose of all unused investigational devices (if applicable) in accordance with standard procedures for the site.
- Verify that all queries have been resolved and copies are filed in the appropriate CRF binder.
- Sign the Monitoring Visit Log.
- Review Investigator Responsibilities with the Principal Investigator; including archive.
- Discuss trial publication.

The ISF will be thoroughly reviewed at the close out visit. The monitor will use the ISF checklist to verify that all essential documents are filed at the site and with the Sponsor in the TMF. Updated documents will be collected for inclusion in the TMF. Any document(s) that the site does not have will be sent to the site with the close out visit follow-up letter. A follow-up letter will be sent to the site by the monitor, which summarizes the visit, addresses protocol violations, highlights site action items, and provides information about study material retention.

17.5 SOURCE DOCUMENTATION VERIFICATION REQUIREMENTS

Source Data Verification will be performed through-out the study.

Definition:
Source documents are the ORIGINAL documents or records where raw/source data concerning a patient (subject) have been first recorded. These documents include but are not limited to the following:

- Patient (subject) files (e.g. medical notes, physician's notes, nurse's notes, dictations, death certificates, etc).
- Clinical laboratory reports and other laboratory reports (e.g. chest x-ray, haematology, microbiology, urinalysis, ultrasounds, etc).
- ID related records, signed and dated informed consent documents, parts of the case report form, and contact report forms (e.g. telephone logs, appointment books, etc).

17.6 INFORMED CONSENT AND AUTHORIZATION

The following points will be validated for all screened and enrolled subjects:

- The informed consent document has been personally signed and dated by the subject.
- The informed consent has been taken by a physician / PI / Research nurse who is delegated to perform this procedure on the delegation of duties log.
- The informed consent process is documented in the source notes.
- The correct approved informed consent document was used for consenting.
- The original signed and dated consent document is maintained with the subject's file notes.

- A copy of the signed consent document has been provided to the subject and a copy has been filed in the subjects medical / source data notes.
- The informed consent was obtained prior to any study related procedures being performed.
- Validation of the above procedures will be documented on the consent form log.

17.7 SERIOUS ADVERSE EVENTS AND ADVERSE EVENTS

At each monitoring visit the site monitor will verify the following:

- Serious and non-serious adverse events are recorded in the source document and accurately reflected on the case report form.
- All participant withdrawals due to an adverse event are accurately reflected on the case report form.
- That the investigator site has reported any serious adverse events (SAEs) to SerenaGroup® in accordance with the protocol.

17.8 CONCOMITANT MEDICATION DOCUMENTATION

At each monitoring visit the site monitor will verify the following:

- All medication usage including over-the-counter products and products related to the administration of general anaesthesia, regional block or local anaesthesia are documented in the source document and accurately reflected in the CRF.
- Use of any exclusionary medications per protocol are documented in the source document and accurately reflected in the CRF.

17.9 INCLUSION AND EXCLUSION CRITERIA VERIFICATION

At each monitoring visit the site monitor will verify the following:

- That each participant (subject) was eligible to enter the study by checking specific inclusion / exclusion criteria against medical history and source documents.
- All inclusion criteria must be checked “yes” and all exclusion criteria must be checked “no”.

17.10 DEVICE ACCOUNTABILITY AND RANDOMIZATION REQUIREMENTS

At each monitoring visit the site monitor will verify the following:

- The CelluTome™ has been used according to the study schedule and administered in accordance with the protocol

17.11 PHOTOGRAPHS

At each monitoring visit the site monitor will verify the following:

- That the site staff has been trained to use the camera.
- Ensure that the photographs are of a suitable quality and include the following:
    - In-focus.
    - Study specific information with the subject's initials, visit number, visit date.
    - A measurement scale is visible in the photograph.

17.12 PROTOCOL ADHERENCE/VIOLATIONS/DEVIATIONS/SERIOUS BREACHES

A breach of GCP and/or protocol is defined as **serious** when it is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects of the trial; or the scientific value of the trial.

A protocol violation is a serious failure to comply with the study protocol and may result in the exclusion of the subject from the study.

SerenaGroup® (sponsor) shall review all protocol violations/deviations submitted by the site and/or monitor and documented in the monitoring report. At each monitoring visit the site monitor will verify the following:

- Protocol Adherence.
- Protocol violations/deviations (e.g. missing procedures or evaluations outside of scheduled visits) – these should be recorded in the source document and discussed with the site to prevent future occurrence.
- Document protocol violations and deviations in the monitoring report (which is filed in the TMF) and Investigator Follow-up Visit letter (which is filed in the TMF and ISF).
- Verify the Independent Ethics Committee (IEC) reporting requirements on Protocol Violations and document such requirements in the monitoring report and follow up visit letter and file in TMF and ISF.

17.13 CASE REPORT FORM (CRF) COMPLETION

All CRFs must be completed as per training. The header information at the top of each CRF must be complete and accurate. The monitor will check the visit schedule to ensure that the required CRFs have been completed. The monitor shall check all CRFs for appropriate initials and/or signatures and verify that they can be found on the delegation of duties log. The site and monitor should refer to the CRF completion instructions, to assist them in decreasing queries and the manner in which the CRF forms should be completed.

17.14 EARLY TERMINATION DOCUMENTATION

Any subject who discontinues study treatment early must have the reason documented in the source document and a termination form must be completed.

17.15 MANAGING POOR PERFORMING OR NON-COMPLIANT SITES

If a site is not conducting the study per protocol or Clinical Trial Directive, ICH guidelines or GCP regulations, the monitor should discuss this with SerenaGroup®. If the non-compliance impacts directly on the safety of subjects, study endpoints or timelines, the medical monitor should be involved in the discussion. Corrective action (e.g. extra training,) should be considered. Repeated poor performance may result in the site being shut down.

17.16 FRAUD AND MISCONDUCT

Suspicion that any data provided may not be valid should be reported immediately to SerenaGroup® and the medical monitor.

Examples of trends that may cause suspicion of fraud include but are not limited to:

- Similar handwriting on CRF/source documentation to documentation completed by subjects such as consent forms or quality of life questionnaires.
- No original source documents only copies.
- No adverse events.
- Perfect data with no issues such as missed visits etc.
- A pattern of data for a given site which does not match the pattern of data generated at other sites (adverse events, trends in lab data, repetition of data, and frequency of withdrawals).
- Visit adherence schedule too exact for all subjects.

18 REFERENCES

1. Vowden K, Goulding V, Vowden P. Hand-held Doppler assessment for peripheral arterial disease. J Wound Care 1996;5(3):125-127.

2. Nelzen O, Bergqvist D, Lindhagen A. Leg ulcer etiology - a cross sectional population study. J VascSurg 1991;14(4):557-64.

3. Baker SR, Stacey MC, Jopp-McKay AG, Hoskin SE, Thompson PJ. Epidemiology of chronic venous ulcers. Br J Surg 1991;78(7):864-7.

4. Schmid-Schobein GW, Takase S, Bergan JJ. New advances in the understanding of the pathophysiology of chronic venous insufficiency. Angiology 2001;52(Suppl 1):S27-S34.

5. Cullum N, Fletcher AW, Nelson EA, Sheldon TA. Compression bandages and stockings in the treatment of venous ulcers. Cochrane Database Syst Rev 2000;CD000265:DOI: 10.1002/14651858.

6. Antignani PL. Classification of chronic venous insufficiency: a review. Angiology 2001;52(Suppl 1):S17-S26.

7. Negus D. Leg ulcers: A practical approach to management. 2nd ed. Oxford: Butterworth Heinemann, 1995.

8. Ballard JL, Bergan JJ, editors. Chronic venous insufficiency. Diagnosis and treatment. London: Springer-Verlag, 2000.

9. Olivencia JF. Pathophysiology of venous ulcers: surgical implications, review and update. DermatolSurg 1999;25:880-885.

10. Nelzen O, Bergqvist D, Lindhagen A, Hallbook T. Chronic leg ulcers: an underestimated problem Nelzen O, Bergqvist D, Lindhagen A. Venous and non-venous leg ulcers: clinical history and appearance in a population study. Br J Surg 1994;81(2):182-7.

11. Callam MJ, Ruckley CV, Harper DR, Dale JJ. Chronic ulceration of the leg: extent of the problem and provision of care. Br Med J (Clin Res Ed) 1985;290(6485):1855-66.

12. Coleridge Smith PD, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. BMJ 1988;296:1726-1727.

13. Bergan JJ, Schmid-Schonbein GW, Coleridge Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. New Engl J Med 2006;355:488-498.

14. Browse NL, Burnand KG. The cause of venous ulceration. Lancet 1982;ii:243-245.

15. Coleridge Smith PD. Pathogenesis of chronic venous insufficiency and possible effects of compression and pentoxifylline. Yale J Biol Med 1993;66:47-59.

16. Agren MS, Eaglstein WH, Ferguson MW, Harding KG, Moore K, Saarialho-Kere UK, et al. Causes and effects of the chronic inflammation in venous leg ulcers. ActaDermVenereolSuppl (Stockh) 2000;210(Suppl):3-17.

17. Browse NL. The cause of venous ulceration. Lancet 1982;2(8292):243-5.

18. Browse NL. Venous ulceration. BMJ 1983;286:1920-1922.

19. Cornwall JV, Dore CJ, Lewis JD. Leg ulcers: epidemiology and aetiology. Br J Surg 1986;73(9):693-6.

20. Cullum N, Fletcher AW, Nelson EA, Sheldon TA. Compression bandages and stockings in the treatment of venous ulcers. Cochrane Database Syst Rev 2000;CD000265:DOI: 10.1002/14651858.

21. Falanga V, Et al Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Arch Dermatol 1998 134(3) 293-300.

22. Mustoe EN, Haraway GD, HoddeJP, King D. Effectiveness of an extracellular matrix graft (Oasis Wound Matrix) in the treatment of venous leg ulcers: a randomized clinical trial. J Vasc Surg 2005 May 41(5);837-43.

23. Biswas A, et al. The micrograft concept for wound healing: strategies and application. J Diabetes Sci Technol. 2010;4(4):808-819.

24. Hsieh CS, et al. Five years' experience of the modified Meek technique in the management of extensive burns. Burns. 2008;34(3):350-354.

25. Ichiki Y, et al. Successful treatment of scleroderma-related cutaneous ulcer with suction blister grafting. Rheumatol Int. 2008;28(3):299-301.

26. Costanzo U, et al. Autologous suction blister grafting for chronic leg ulcers. J EurAcadDermatolVenereol. 2008;22(1):7-10.

27. Njoo MD, et al. A systematic review of autologous transplantation methods in vitiligo. Arch Dermatol. 1998;134(12):1543-1549.

28. Patel NS, et al. Advanced treatment modalities for vitiligo. Dermatol Surg. 2012;38(3):381- 391.

29. Li J, et al. Suction blister epidermal grafting using a modified suction method in the treatment of stable vitiligo: a retrospective study. Dermatol Surg. 2011;37(7):999-1006.

30. Serena T, et al. Experience with a novel epidermal harvesting device in Haiti. Manuscript in progress.

31. Serena T. et. al. Case studies utilizing CelluTome™ a novel epidermal harvesting device in chr onic wounds. Manuscript in progress.

19 APPENDICES

19.1 ANKLE-BRACHIAL INDEX (ABI)

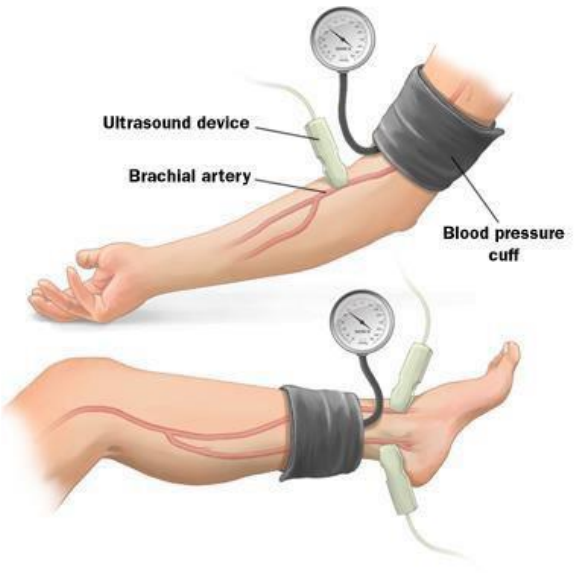
Technique: Place the patient in the supine position, with the arms and legs at the same level as the heart, for a minimum of 10 minutes before measurement

Select an appropriately sized blood pressure cuff for both the ankle and the arms (figure 1); the cuff width should be, at a minimum, 20% greater than the diameter of the extremity. The ankle cuff should be placed on the leg between the malleolus and the calf. Enough room should be left below both cuffs to permit placement of the ultrasound gel, so that the Doppler device can adequately detect the brachial, dorsalis pedis, and posterior tibial arteries.

Obtain the brachial systolic pressures of both arms. Use the higher of the arm pressures in the ABI calculation. Obtain the pressure in the dorsalis pedis and posterior tibial arteries for the extremity with the target ulcer. Use the highest pressure for the ABI calculation.

Ankle-Brachial Index = Highest ankle pressure/Highest brachial pressure.

Care should be taken to cover the ulcer during the ABI measurement. In addition, patients should be informed that they may experience discomfort during the test secondary to the pressure exerted by the cuff in the area of skin breakdown.



19.2 TIME TRADE OFF EVALUATION

A time-tradeoff (TTO) measurement using an electronic system called Wound-Trade© will be elicited from patients to facilitate the assessment of cost-effectiveness (cost-utility). Value for improving patient quality of life is typically quantified using cost-utility analysis. A cost-utility analysis is defined as a type of cost-effective analysis that compares different procedures and outcomes relative to a person's quality of life. It is the most sophisticated form of economic analysis and typically incorporates utility values. Utility values measure the preference for a health state and range from 0.0 (death) to 1.0 (perfect health). When the change in utility measures conferred by a health care intervention is multiplied by the duration of the benefit, the number of quality-adjusted life-years (QALYs) gained from the intervention is ascertained. This methodology incorporates either the improvement in quality of life and/or length of life, or the value, occurring as a result of the intervention. In wound care, patients' experiences of living with wounds are dominated by symptom management, with all studies showing that the wound and its treatment have a profound effect on quality of life.

WOUND-TRADE©

Wound-Trade© is a system developed and created by Dr. Walton Sumner, MD, Associate Professor, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri and Mr. Ronald Shannon, MPH, Health Economist and Epidemiologist, Global Health Economic Projects, LLC, Clifton Park, New York. Dr. Wound-Trade© is implemented on a laptop computer, which facilitates the development of customized utility interviews. The automated format facilitates a consistent presentation of questions and enables a discounted, risk-adjusted, quality of life health utility index score through classic time-trade-off methodology. An individual is given a hypothetical choice: he/she can live for x-years in perfect health followed by death, or he/she can live y years with a particular chronic condition such as a chronic wound, where y > x. The number of healthy years x is then varied until the person is indifferent between the two outcomes. The health utility index in this example equals x/y. For example, suppose that an individual feels that fifteen years of perfect health is worth twenty years of life with a chronic wound. Using the time trade-off approach, this individual is giving a chronic wound a health utility index of0.75. The health utility index can be used in a cost-utility equation (cost-effectiveness based on quality-adjusted life years) from a new medical treatment or technology:

$$\frac{\text{Cost}_{\text{new}} - \text{Cost}_{\text{old}}}{\text{No. of QALYs}_{\text{new}} - \text{No. of QALYs}_{\text{old}}}$$

RISKS

There are no risks to the patient related to their health or well-being. The scenarios in Wound-Trade© are imaginary and do not interfere with their current therapy as guided by the hospital wound center.

INTERVIEW TIME

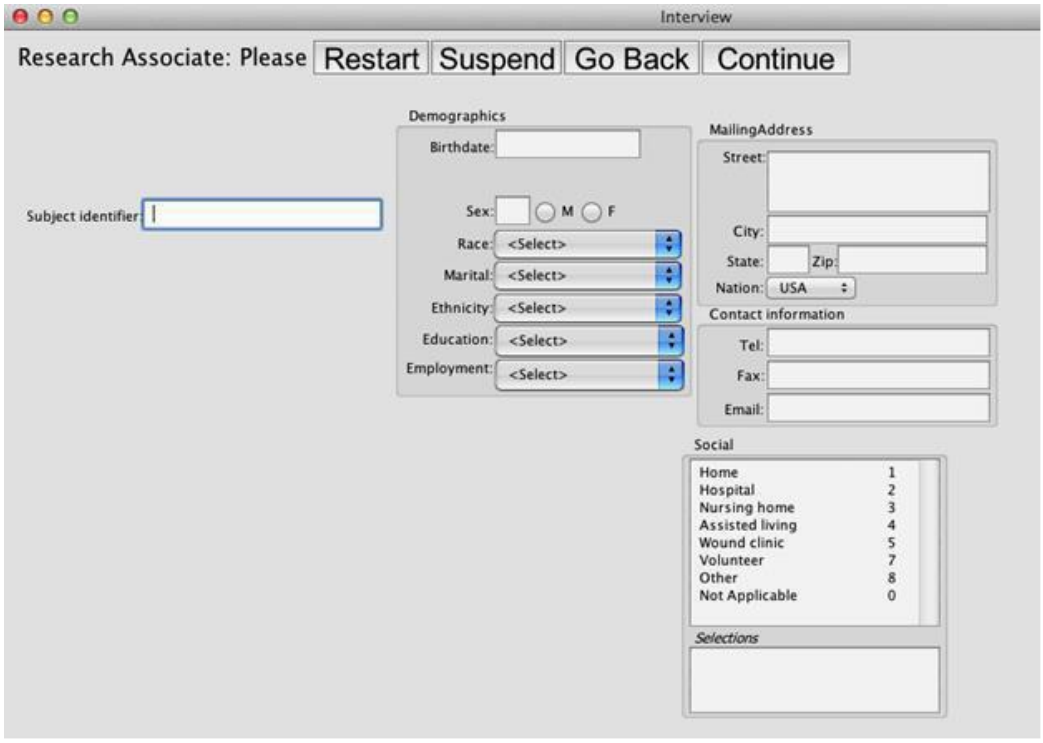
The interview process can take as little as a few minutes to 15 minutes to complete. A research assistant will be available to assist the patient with process but will not influence the decisions made by the patient.

WOUND-TRADE SCREEN SHOTS AND INTERVIEW PROCESS

A. Introductory Screen

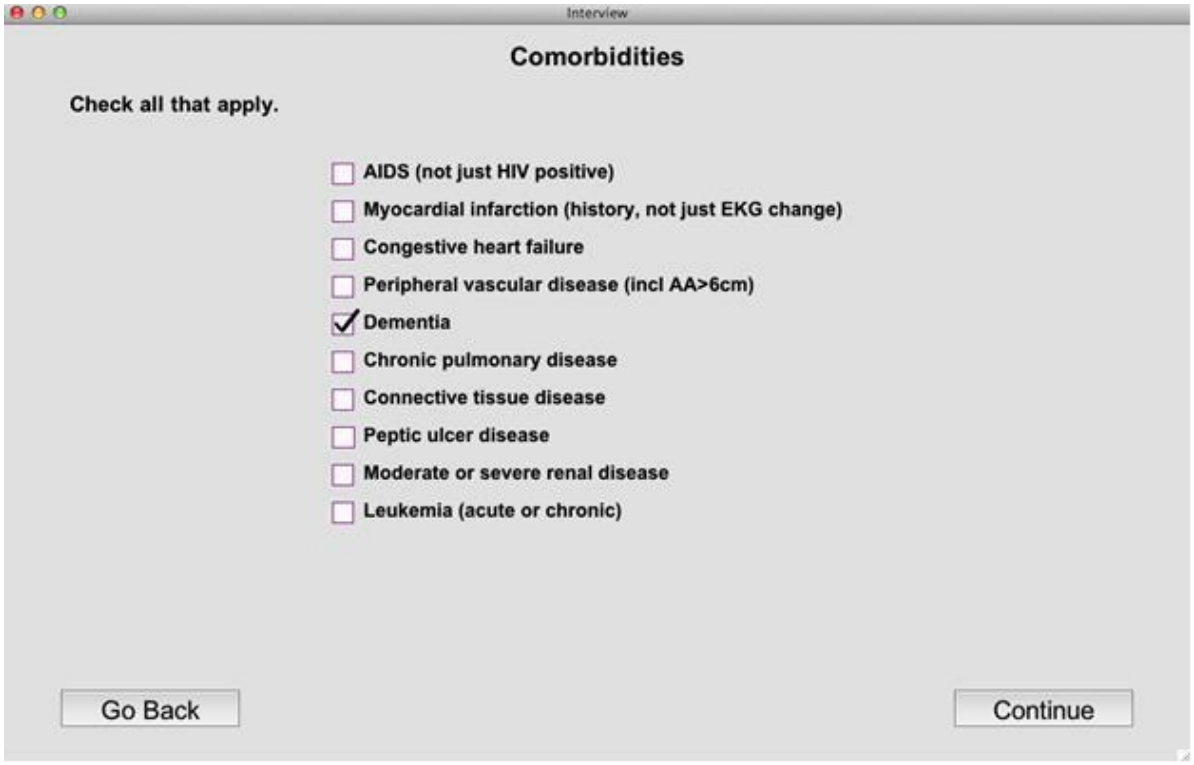
Researcher will enter the following information:

1. Enter a patient number in subject identifier field;
2. Enter birth date as month/day/ year exactly like this: 06/25/1955;
3. Select either M for Male or F for Female;
4. For the remainder of information it is not necessary to enter it but you can if you wish;
5. Hit continue button to go to next page

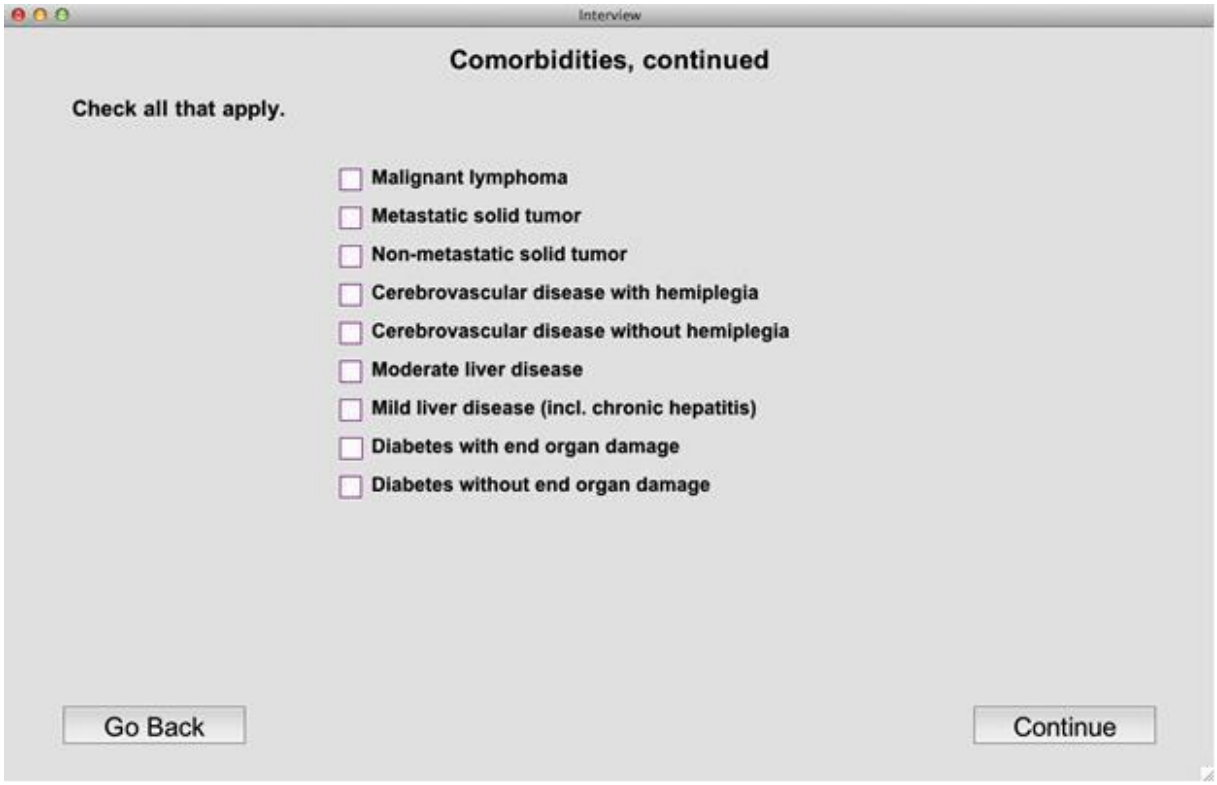


B. Comorbidities

6. Check all that apply and hit continue:

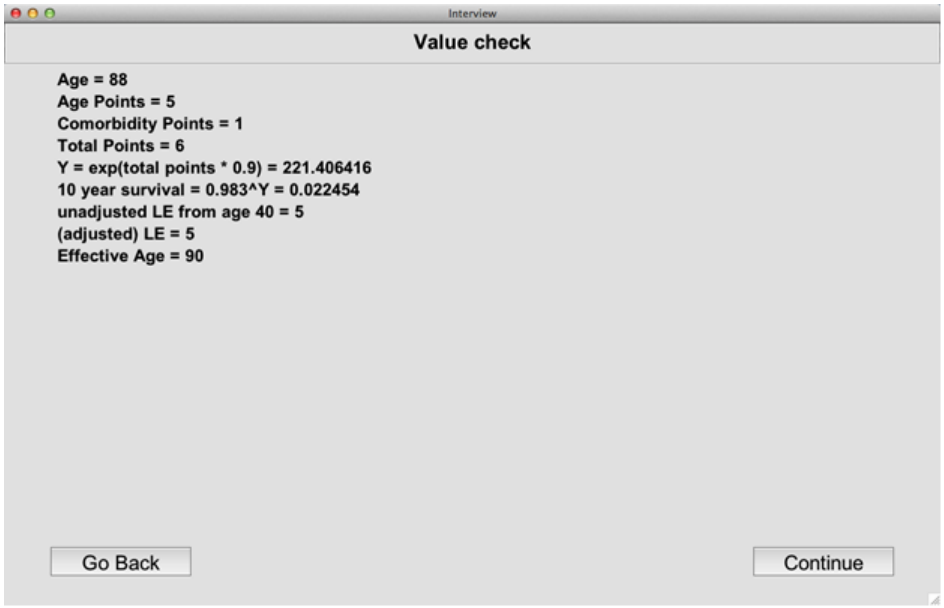


6. Check all that apply and hit continue:



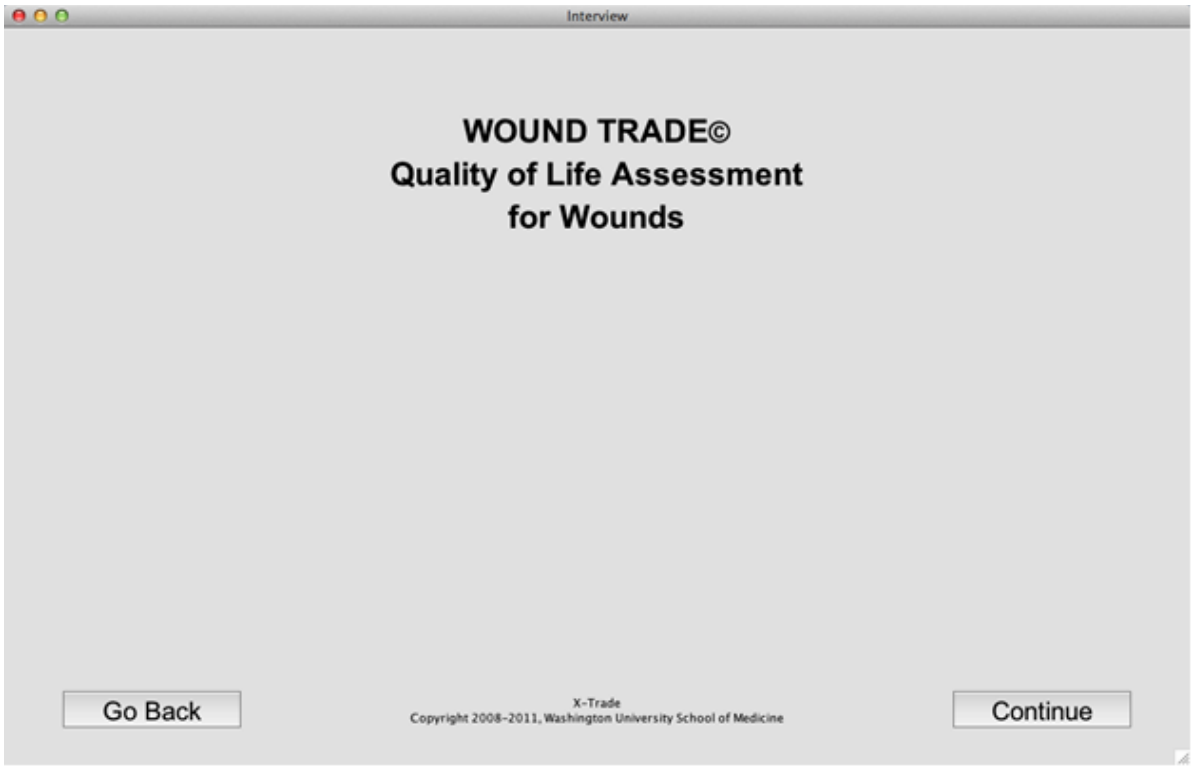
C. Value Check

This page reflects the calculation used to determine the number of years of life expectancy for a person of their current age and existing comorbidities. The adjusted life expectancy is used in the forthcoming time trade-off questions. This page is for the research assistant.

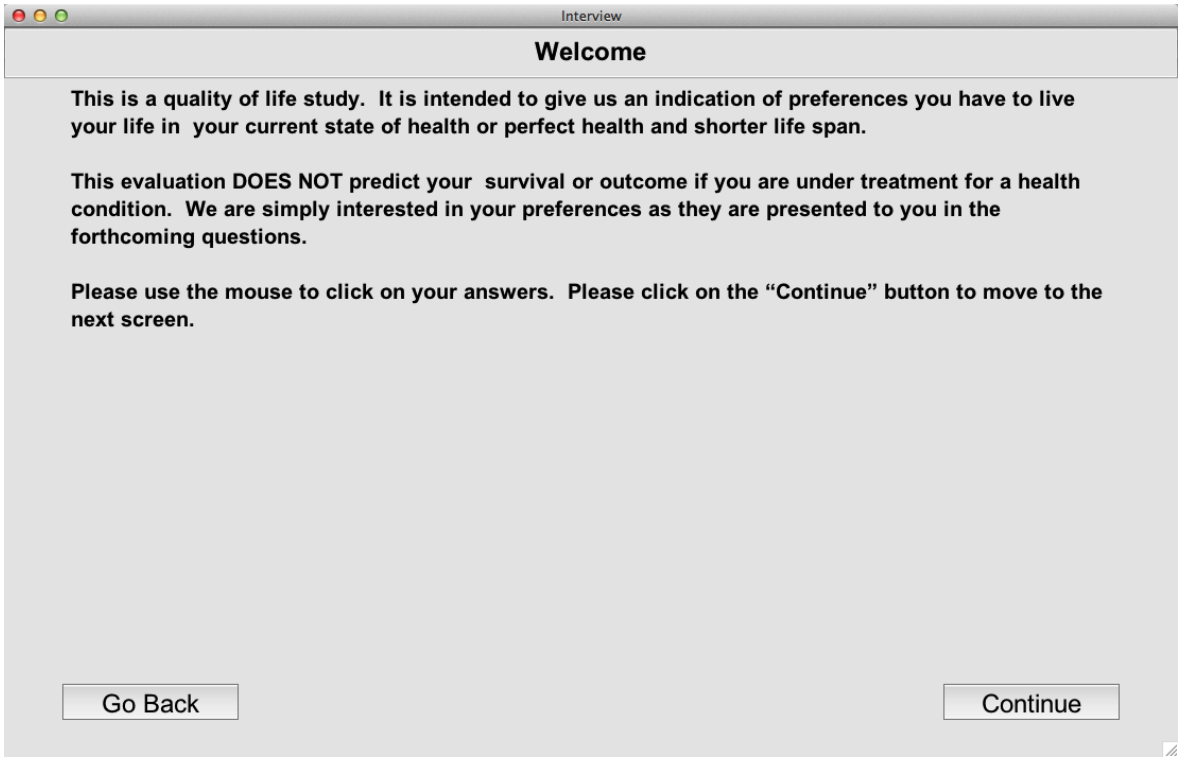


D. Introductory Patient Screens

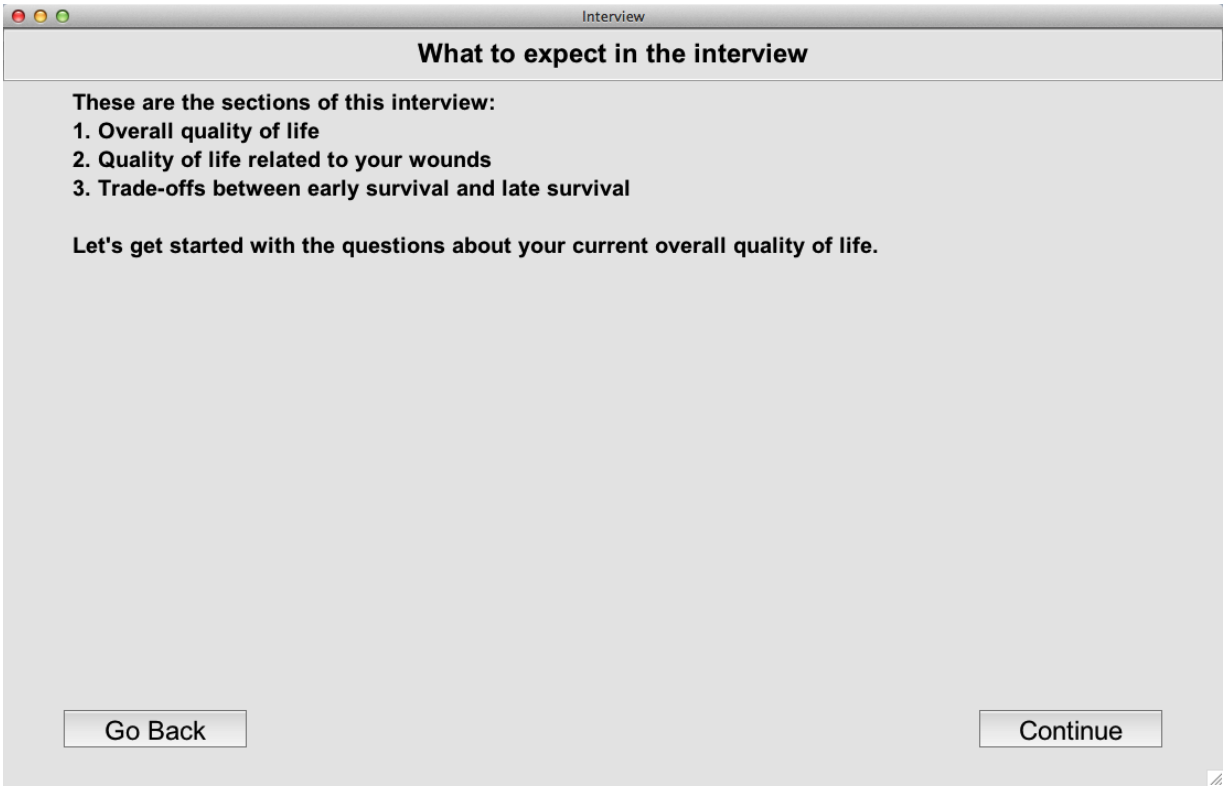
1. Hit continue button;



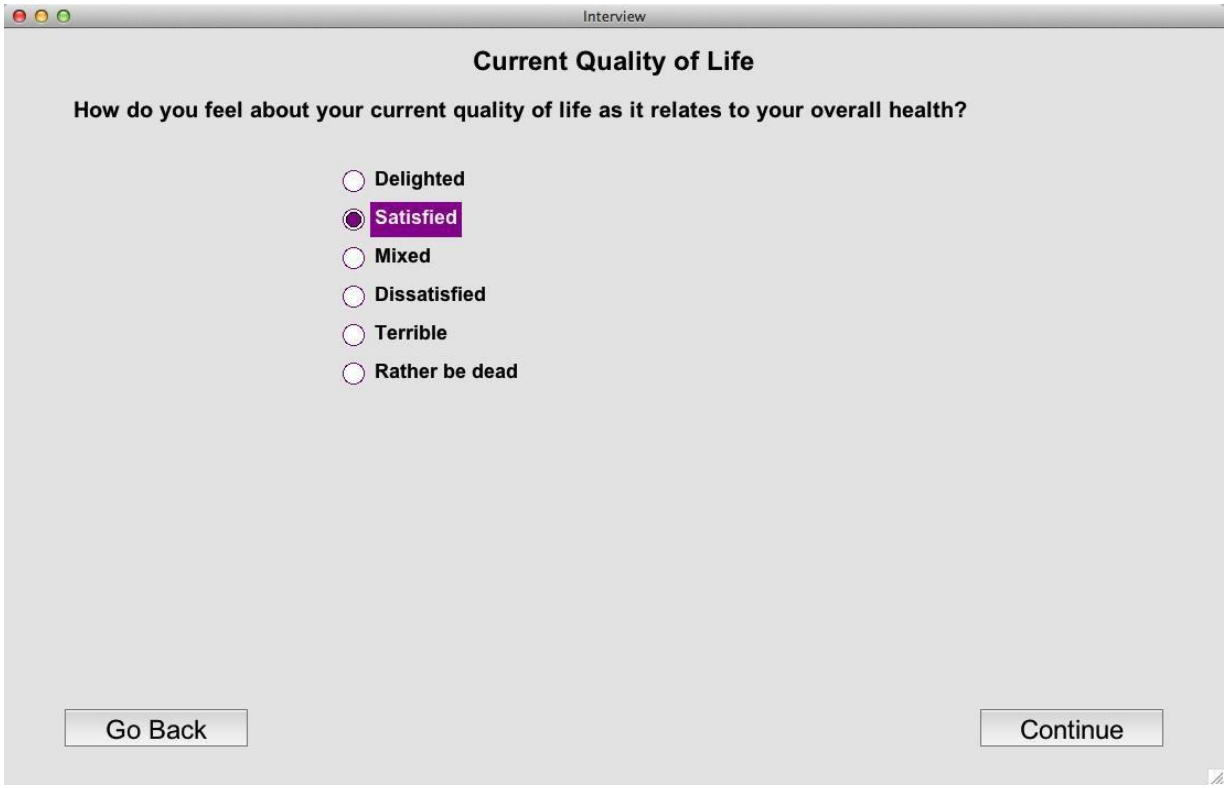
1. Patient will read and then hit continue button;



1. Patient will read and then hit continue button;

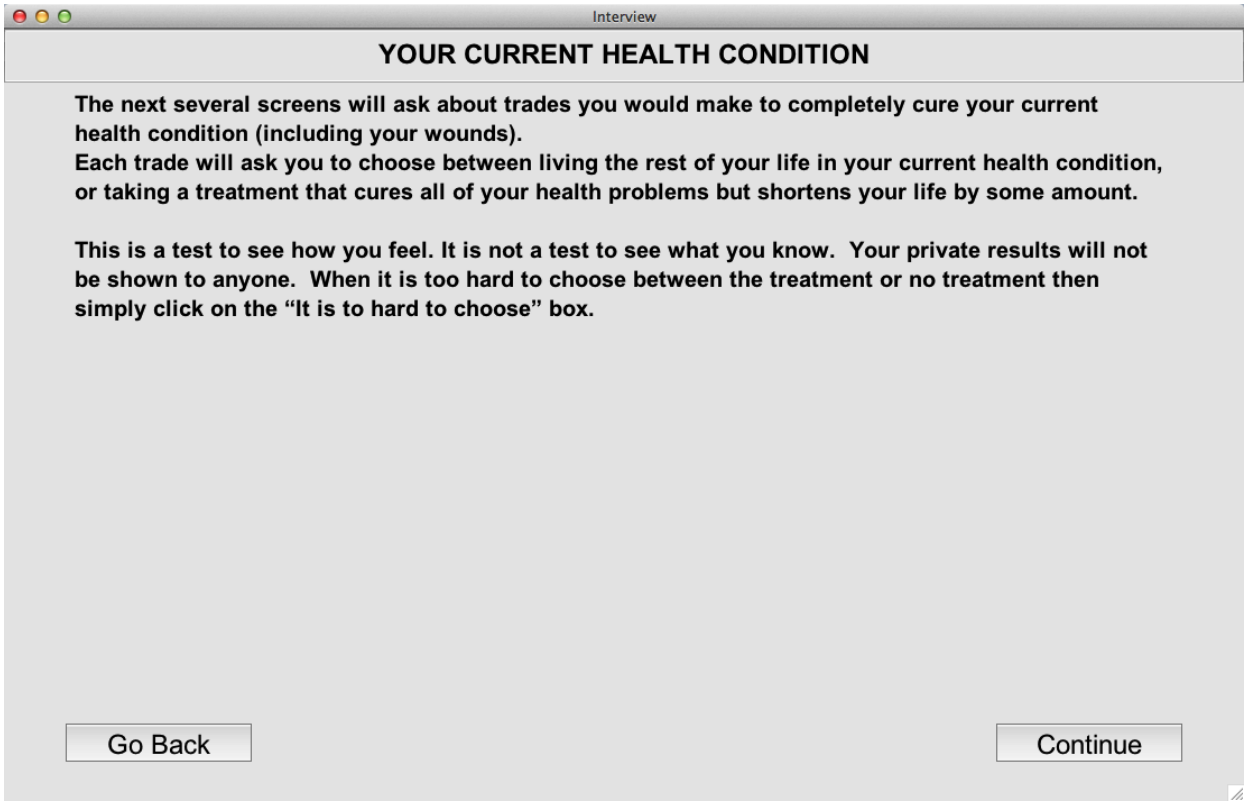


1. Patient will read and then hit continue button;

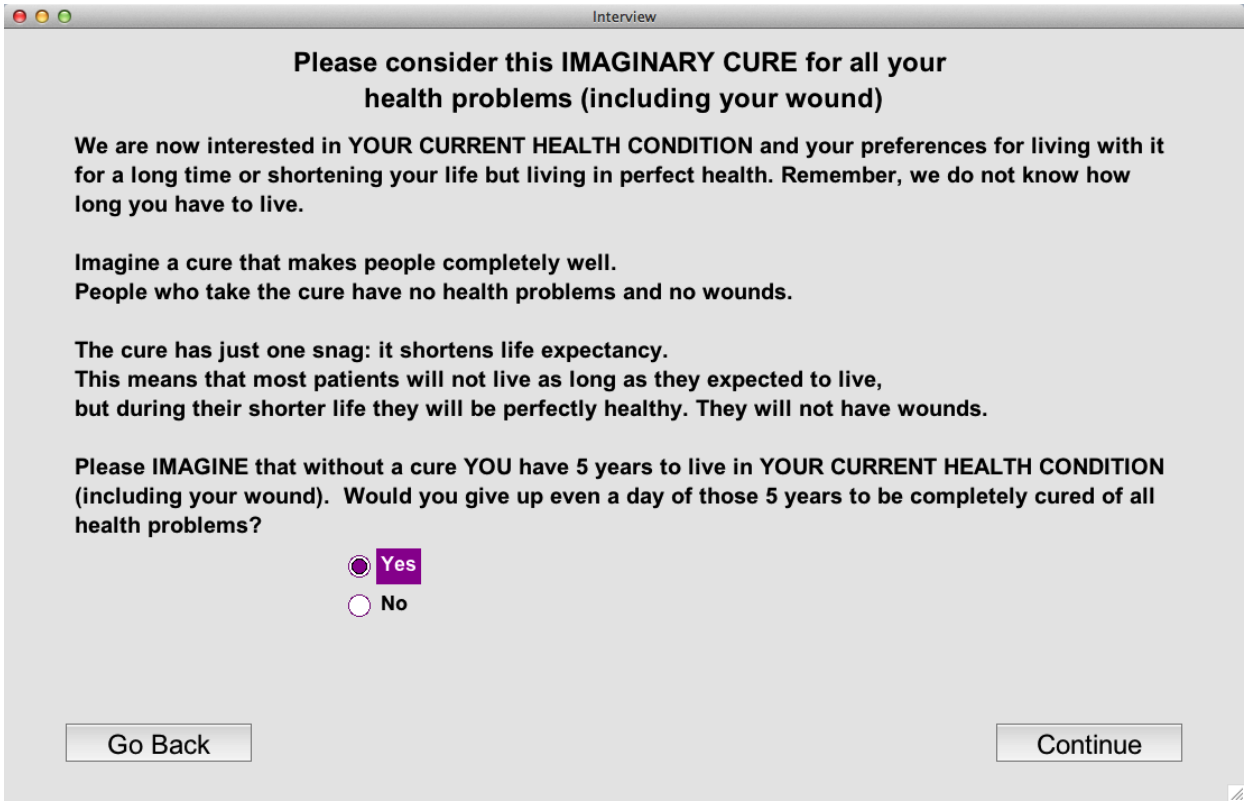


2. Patient will read and then select their choice;
3. Hit continue;

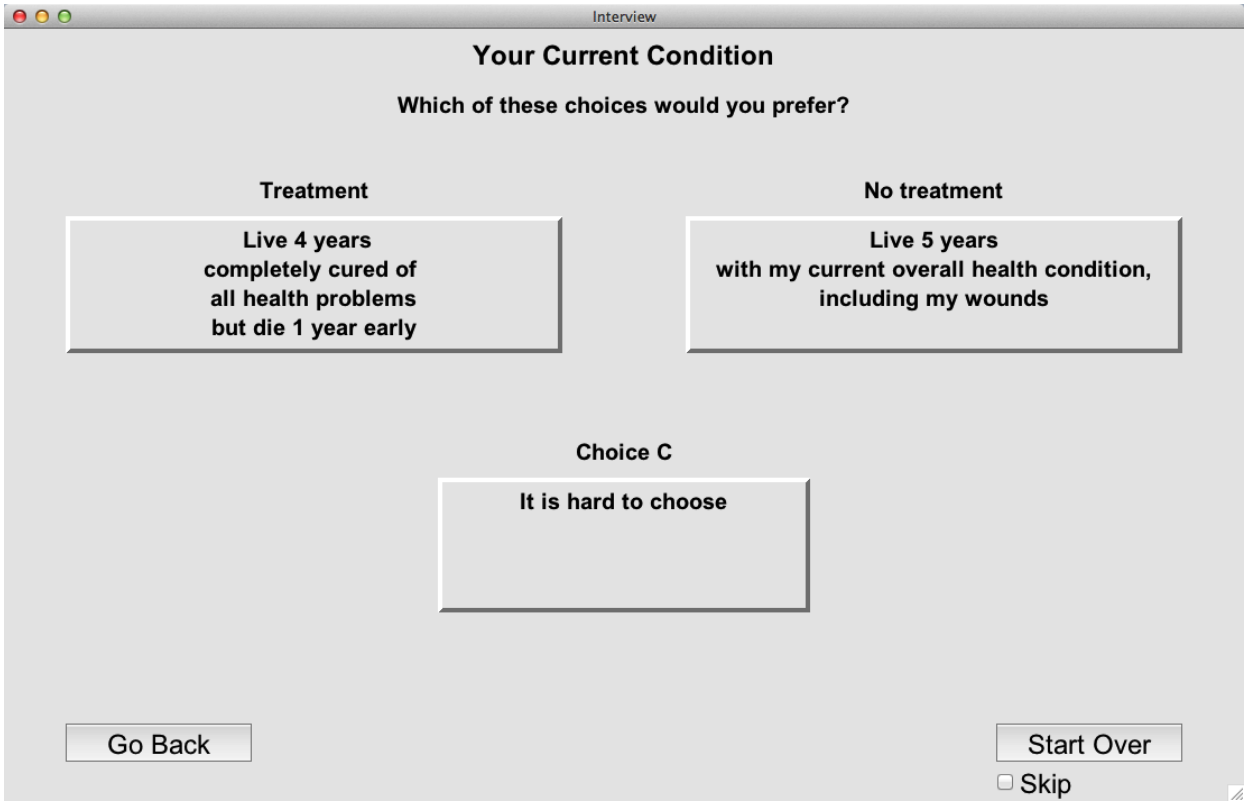
4. Patient will read and then hit continue. Questions may be answered by research assistant;



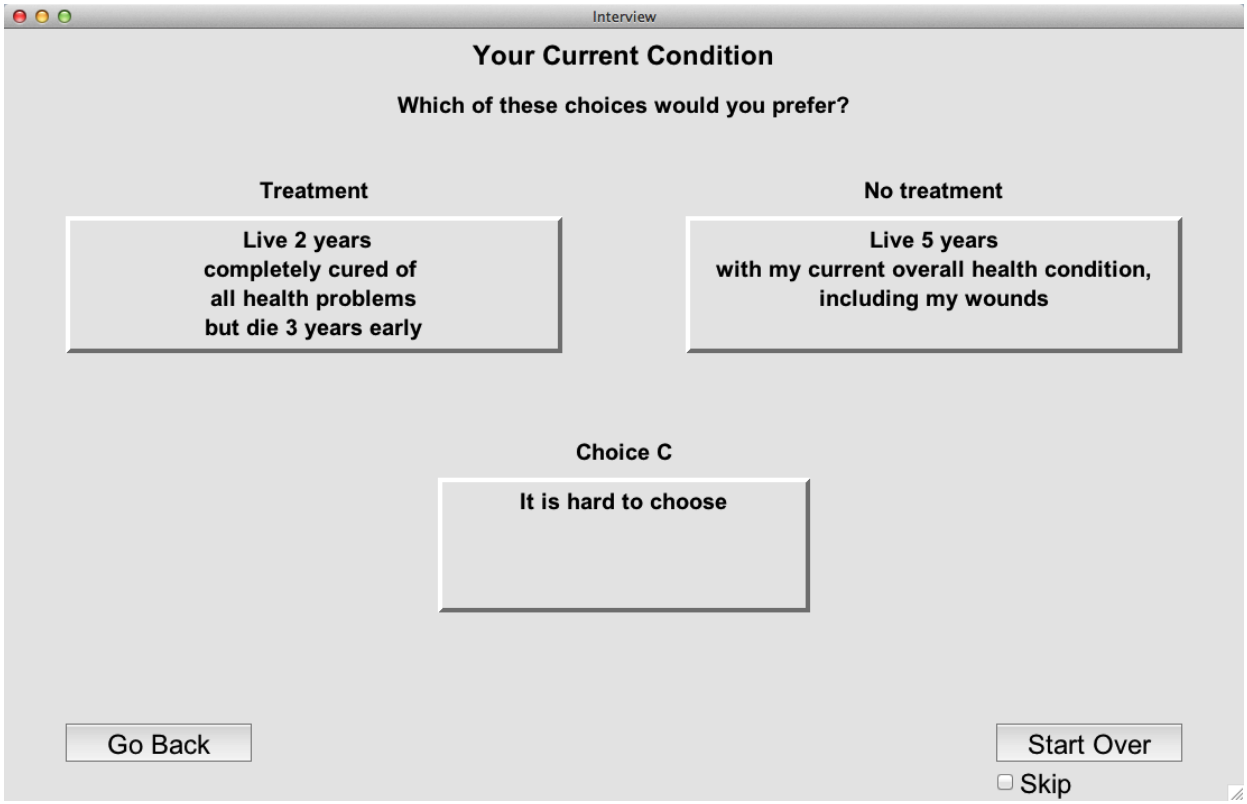
5. Patient will read, ask questions to research assistant if necessary and then choose yes or no;
6. If they hit yes then they will go on to trade questions;
7. If no, then patient will go to next section focusing on their wound and not their general health;
8. Either way they hit continue;



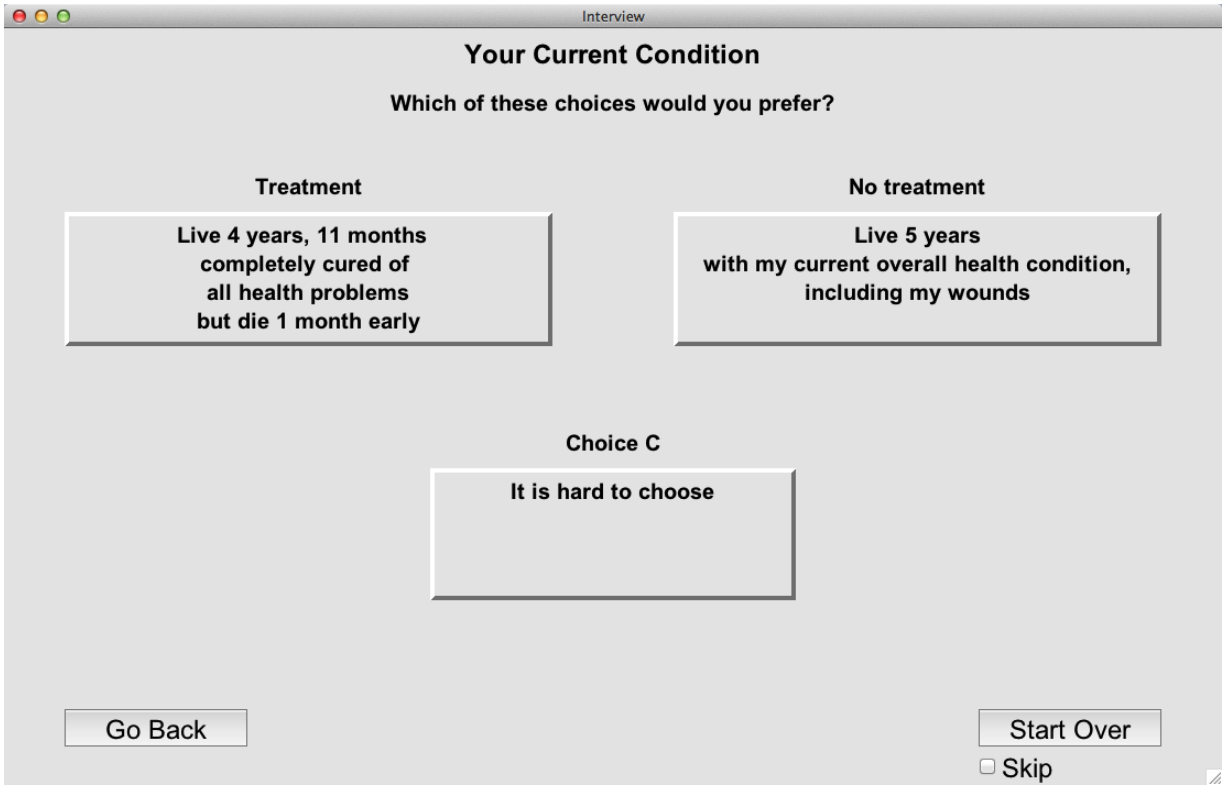
9. Assuming they choose yes, the patient will click on the box of their preference. In this scenario the patient may choose "No treatment" which indicates they would rather live 5 years in their current overall health condition including their wounds rather than live 4 years completely cured of all health problems but die 1 year early (The screen will transition to new scenario - goto next page);



10. The trade questions continue after the patient chooses “No treatment”. A new scenario evolves whereby the patient will make another choice. For sake of demonstration the patient selects “Treatment”. Their preference is 2 years completely cured of all health problems but die 3 years early over “No treatment”. A new screen comes forward (next page);

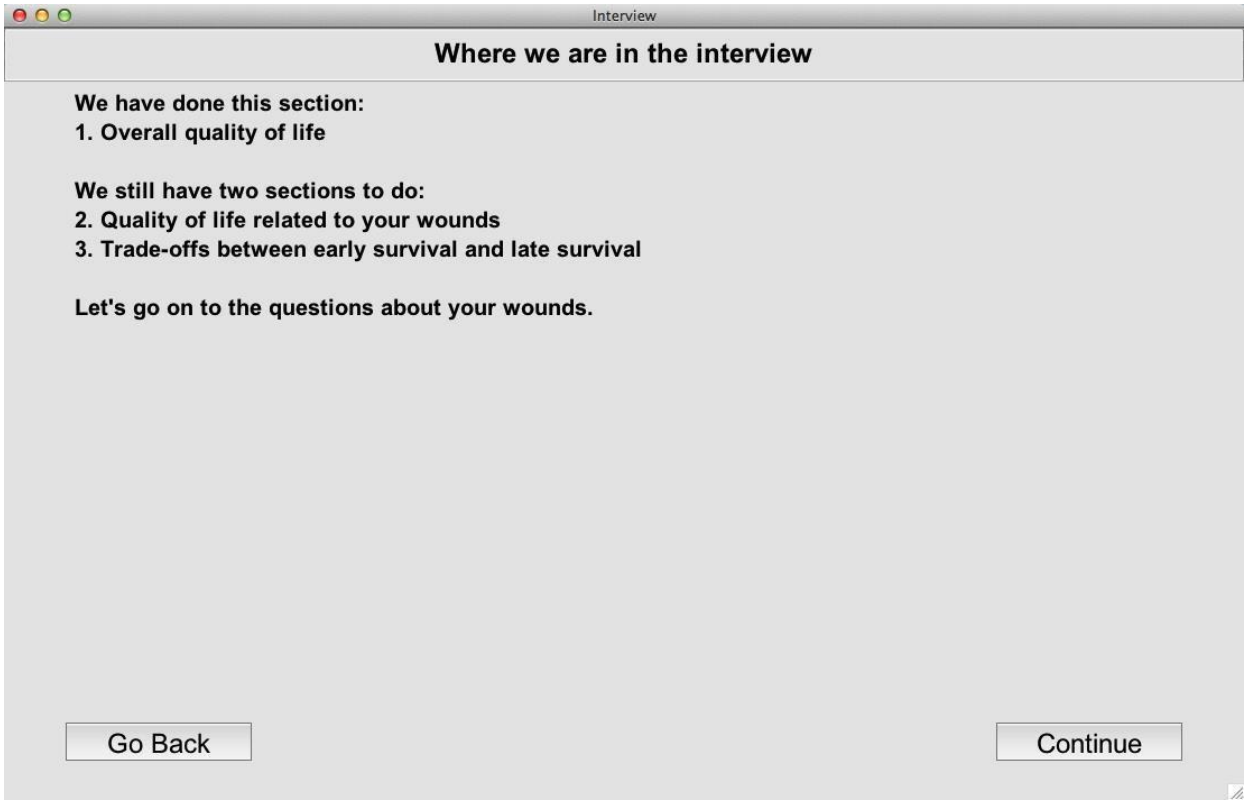


11. The trade continues until patient cannot decide between Treatment or No treatment. They click on Choice C which is hard to choose. This trade is done for general health but with a wound. A new scree will will evolve (next page);



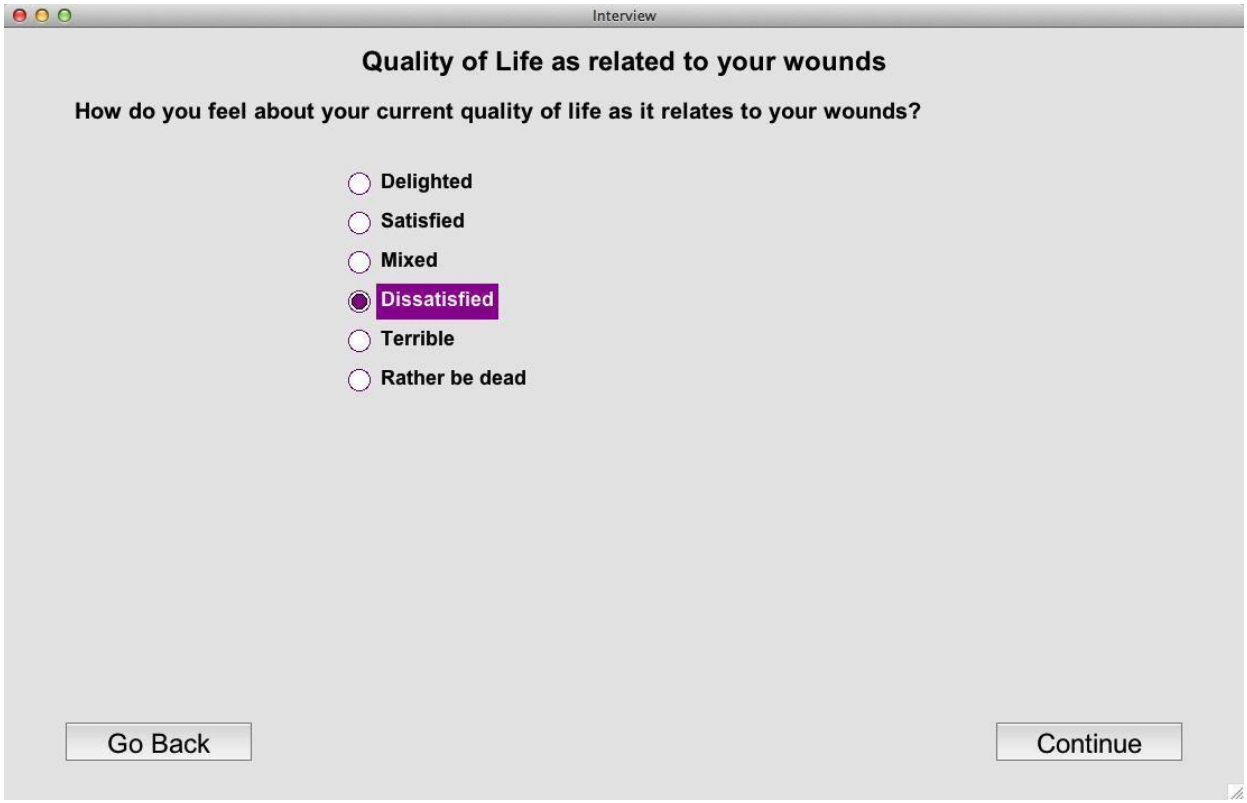
12. Status of interview;

13. Hit continue to go to wound focus;



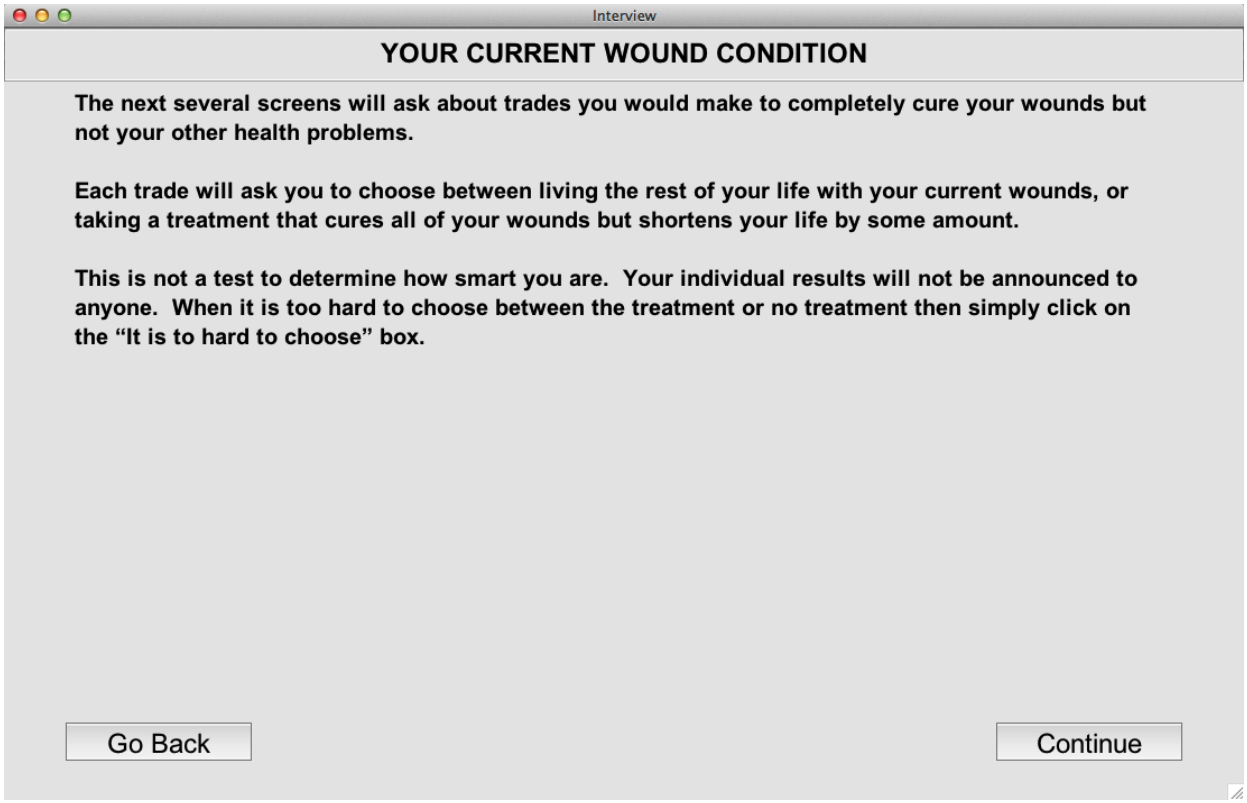
14. Patient answers question related to their wound;

15. Hit continue;

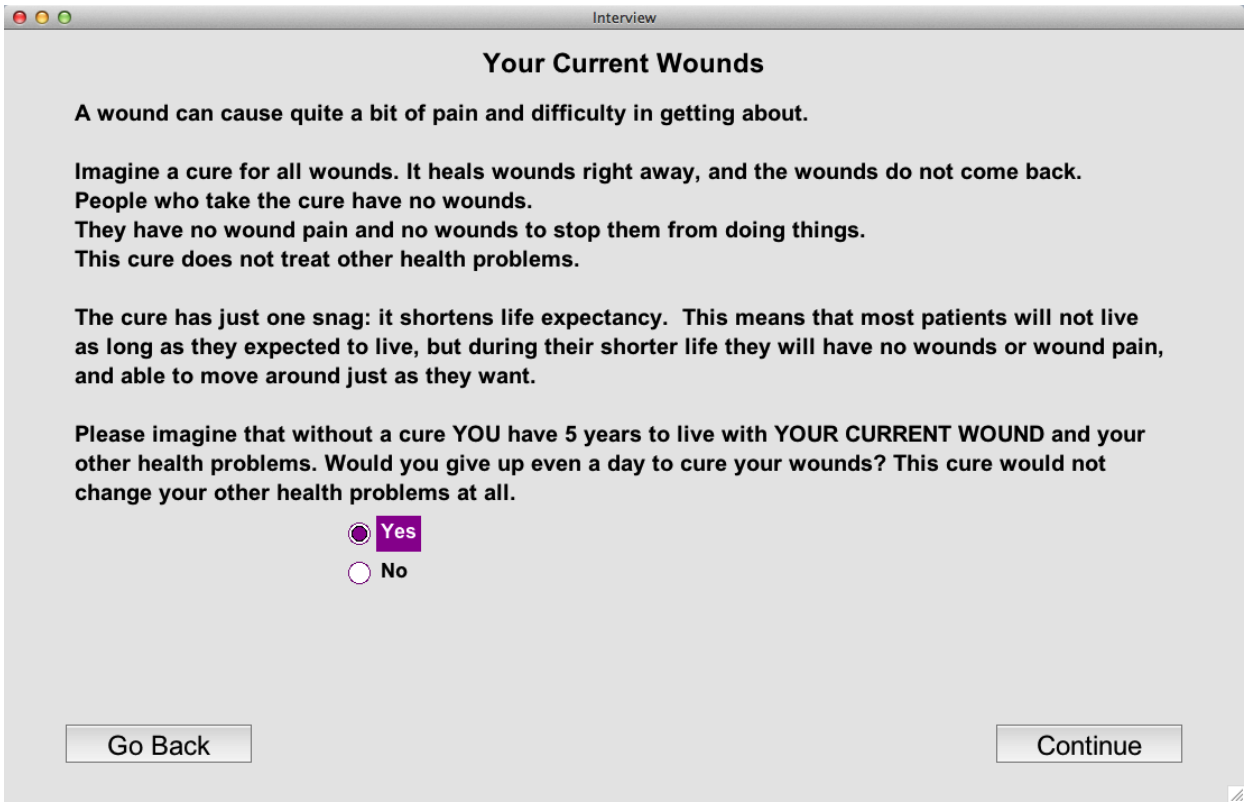


16. Patient will read the text;

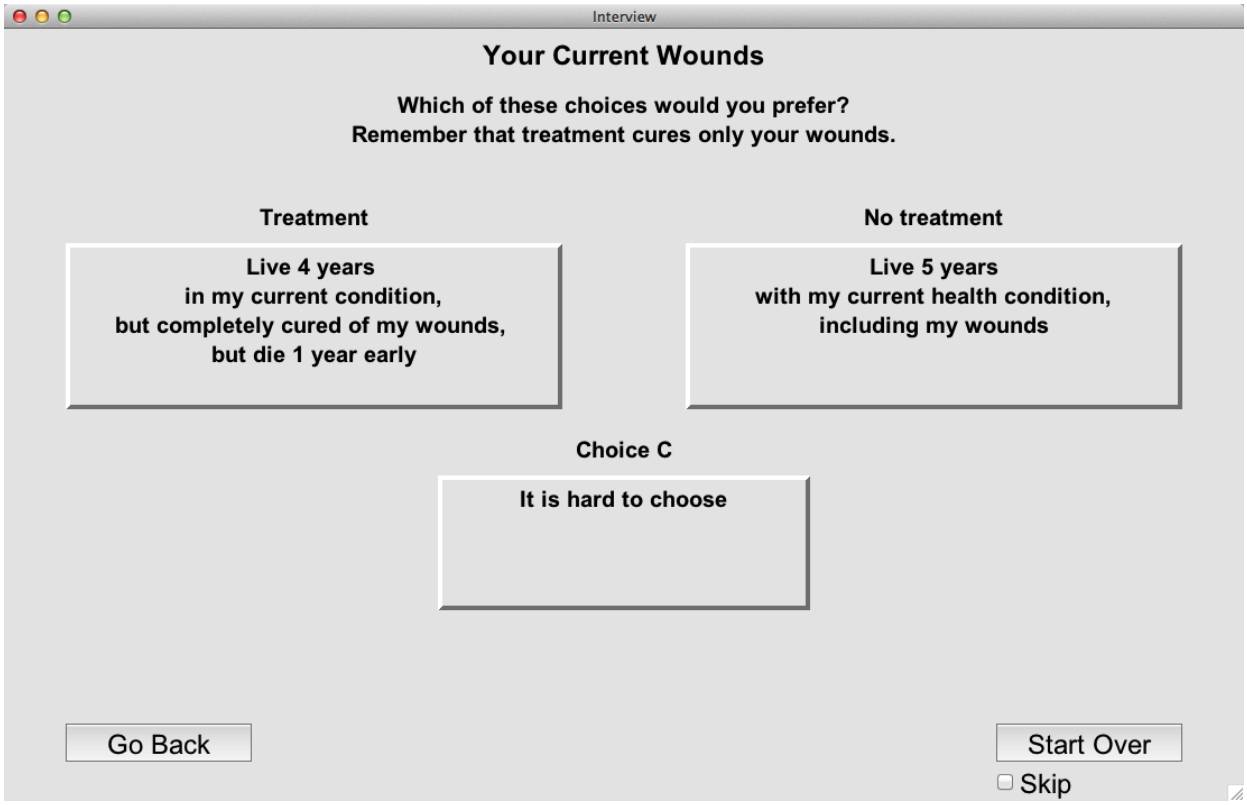
17. Hit continue;



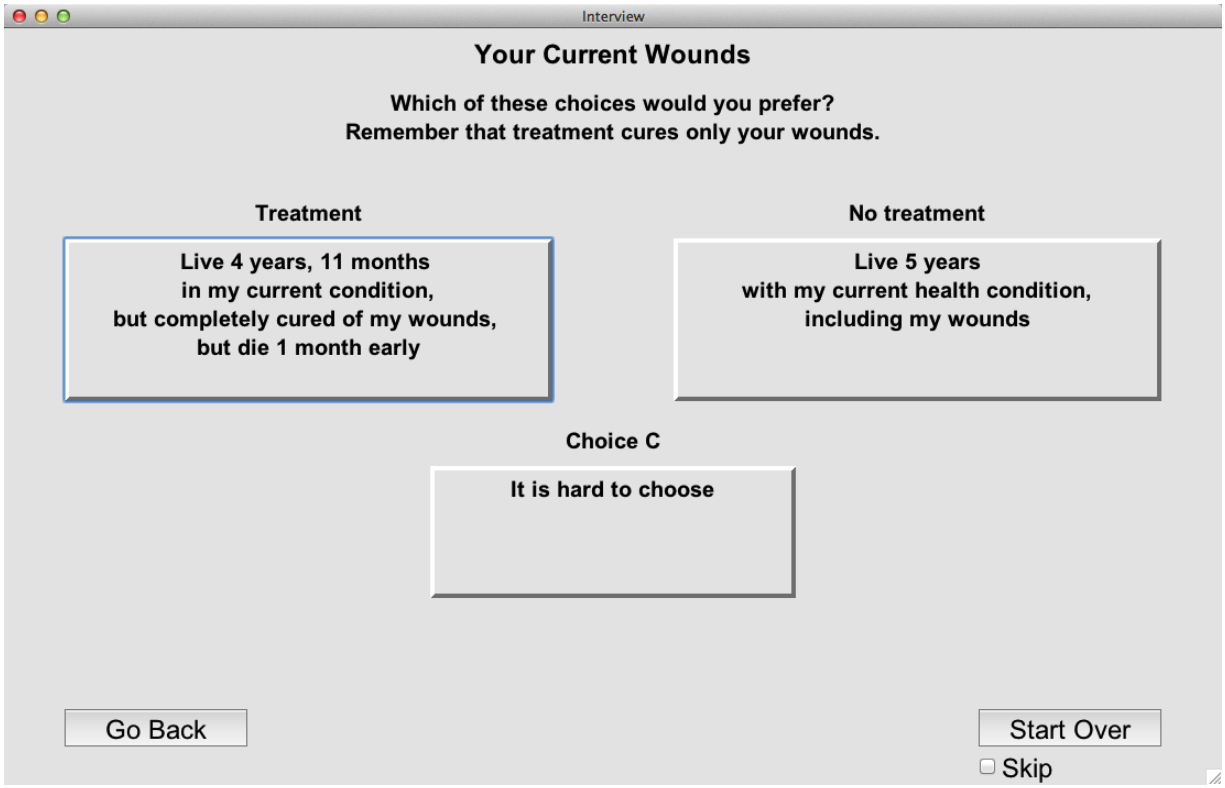
18. Patient will read and choose either yes or no. If no then this trade stops and goes into ricky treatment section;
19. Check yes it will go to trade section where patient will choose preferences again;
20. Hit continue either way;



21. Patient will select their preferences related to their wound. Screens will change based on their selection;



22. Another example of screen change. If they click on Choice C then this trade is done (Next screen);

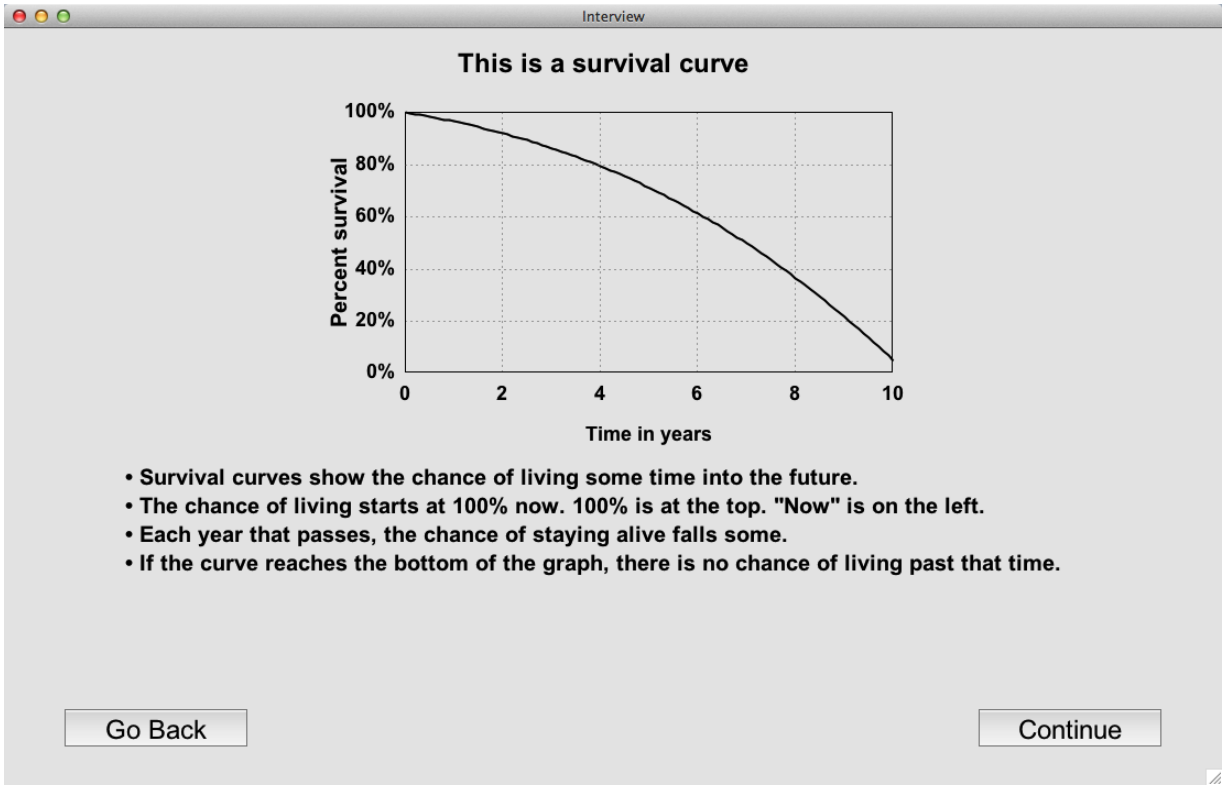


23. Status of interview;

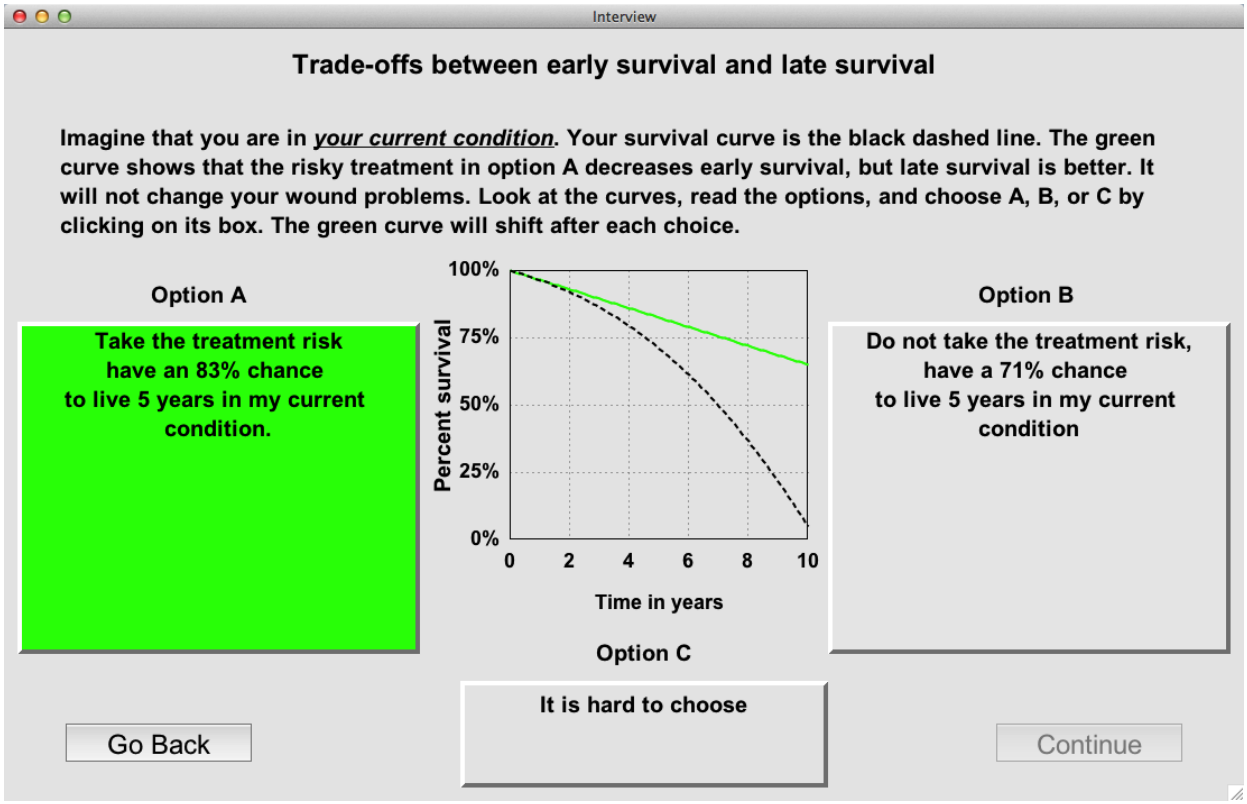
24. Hit continue;

25. The next set of trades require a basic understanding of survival curves. This one slide gives a general overview that is enough to complete next sections of trades. The research assistant can also guide the patient here;

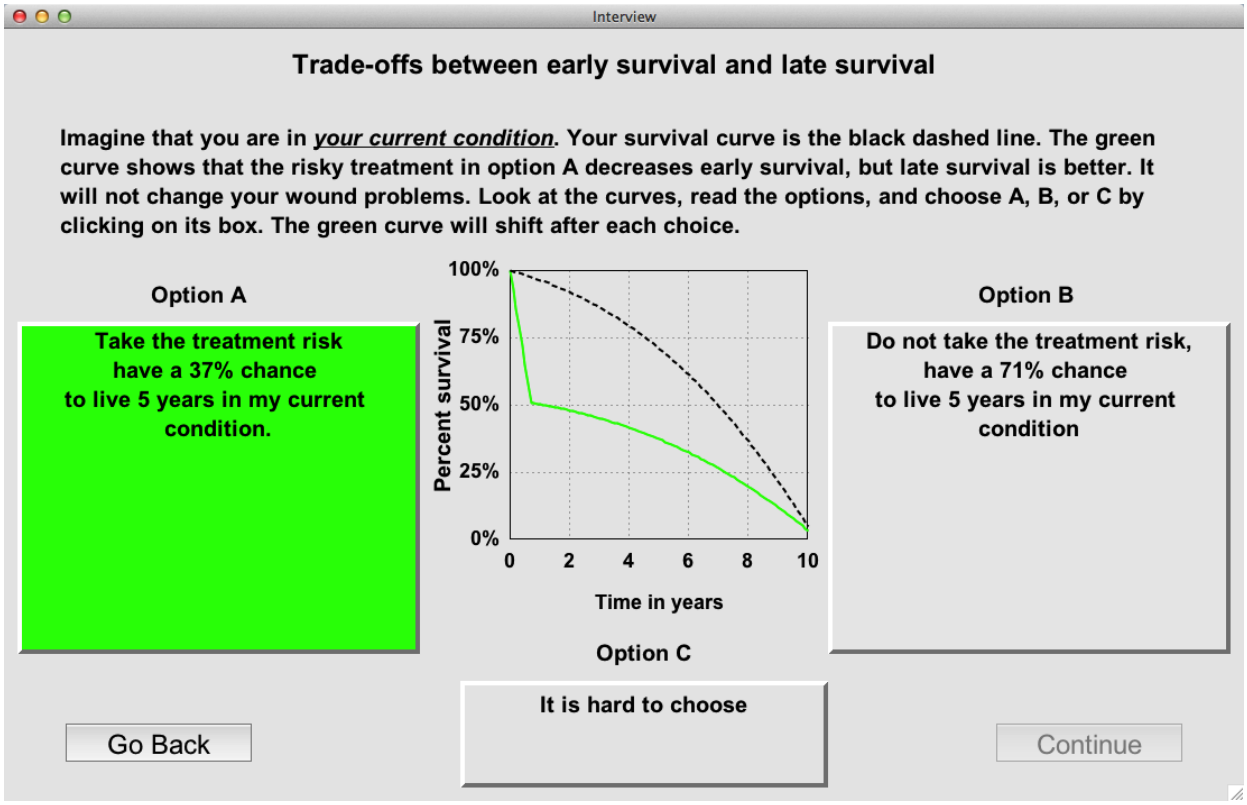
26. Hit continue after review;



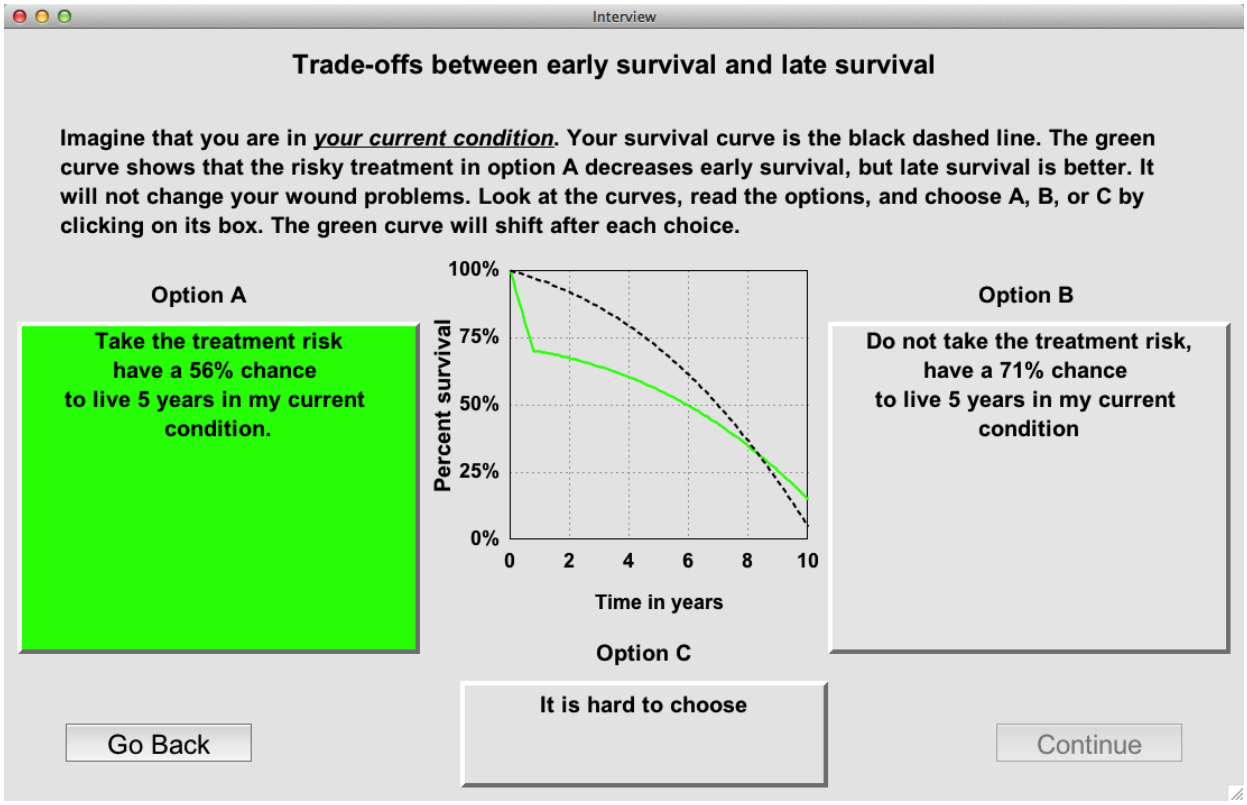
27. This section focuses on risky treatment that may decrease early survival but if they survive their prognosis for longer survival becomes better. In the example below, the patient will most likely choose the green box as it gives better probability of survival over 5 years. Once they select their preference the trades begin – go to next page;



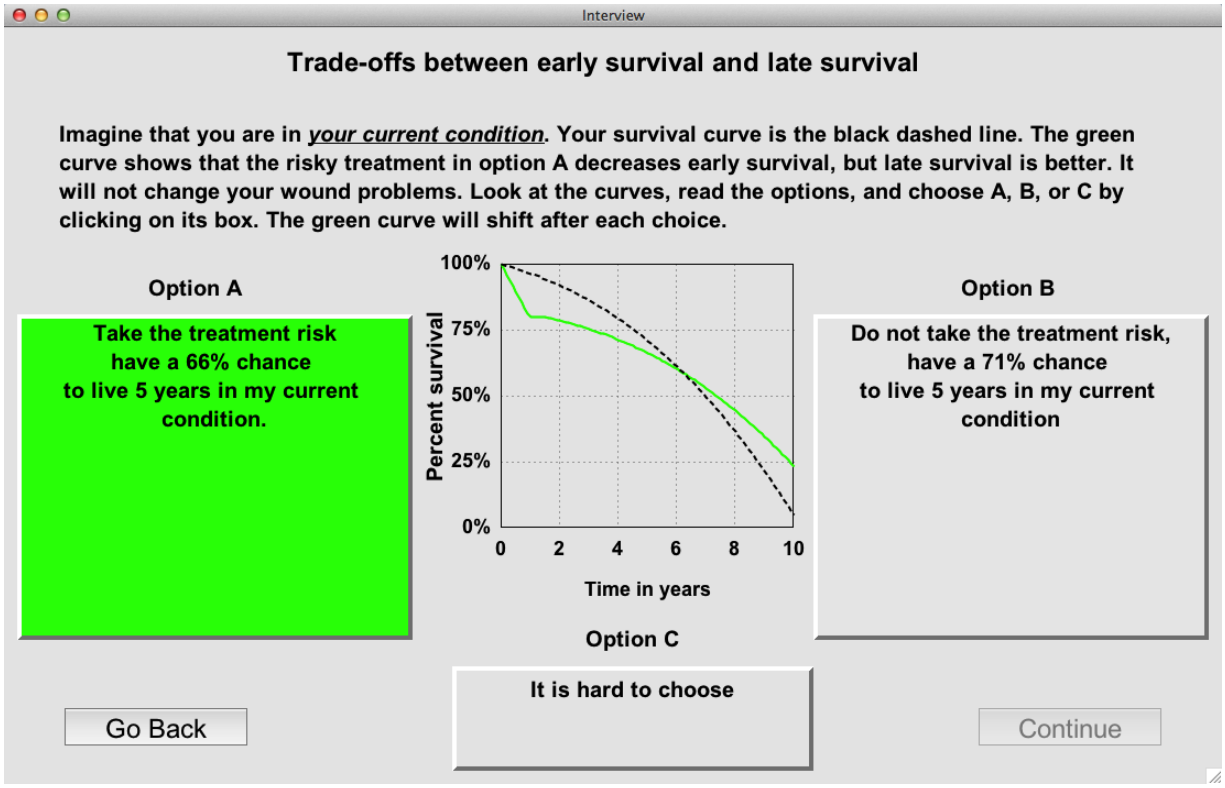
28. Notice that the survival curve changes with their selection in previous section. Patient now will most likely click on option B. Page transitions to next preference trade – go to next page;



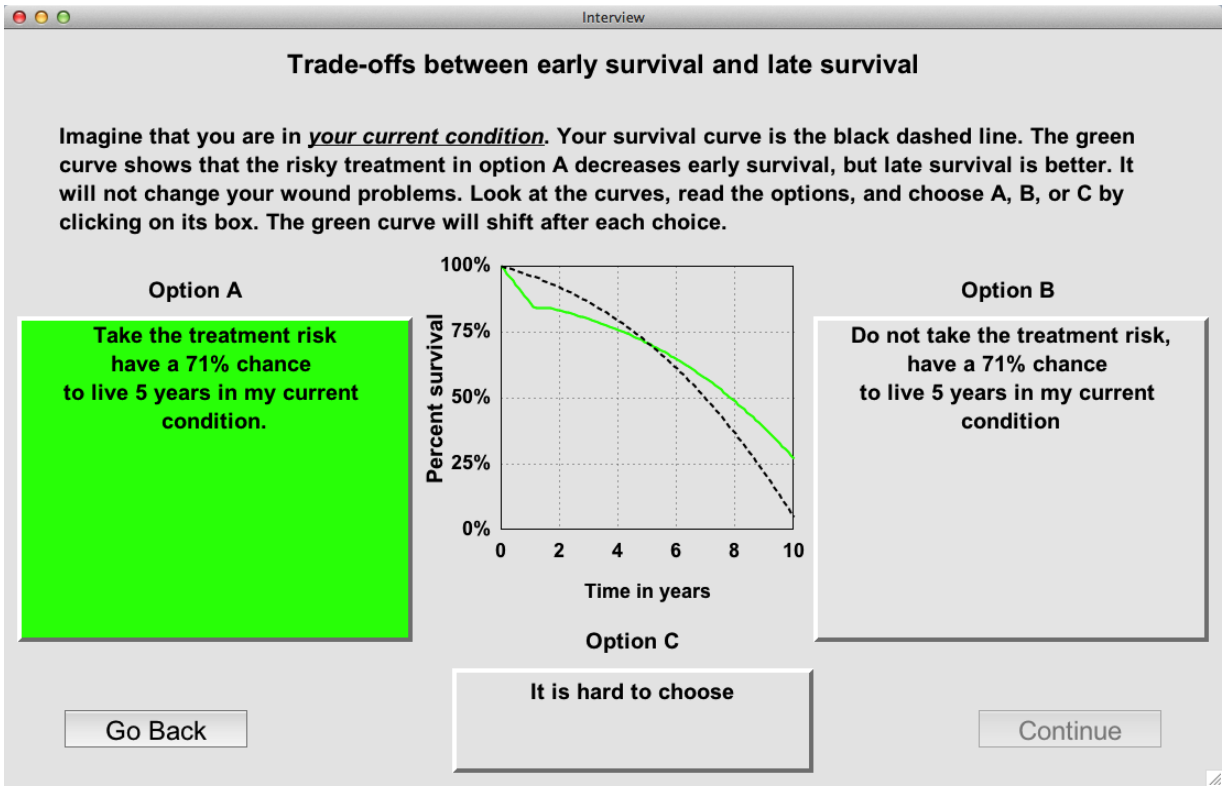
29. The scenario changes in this section with new trade preference options. Also notice the survival curve changes based on choices. Patient selects Option B and a new trade appears on next page;



30. Choose Option B. New section comes open on next page. Survival curve changes with selections;

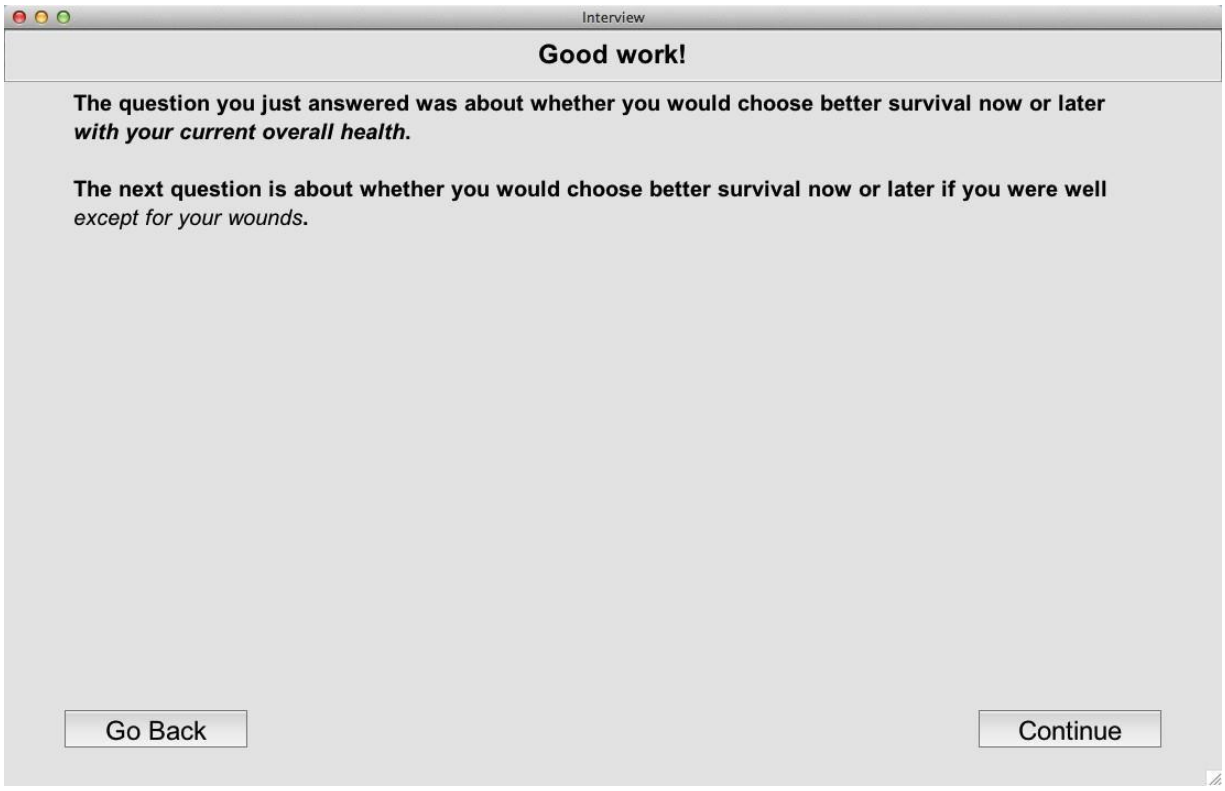


31. Patient chooses Option C and the trade stops. Next section automatically appears;

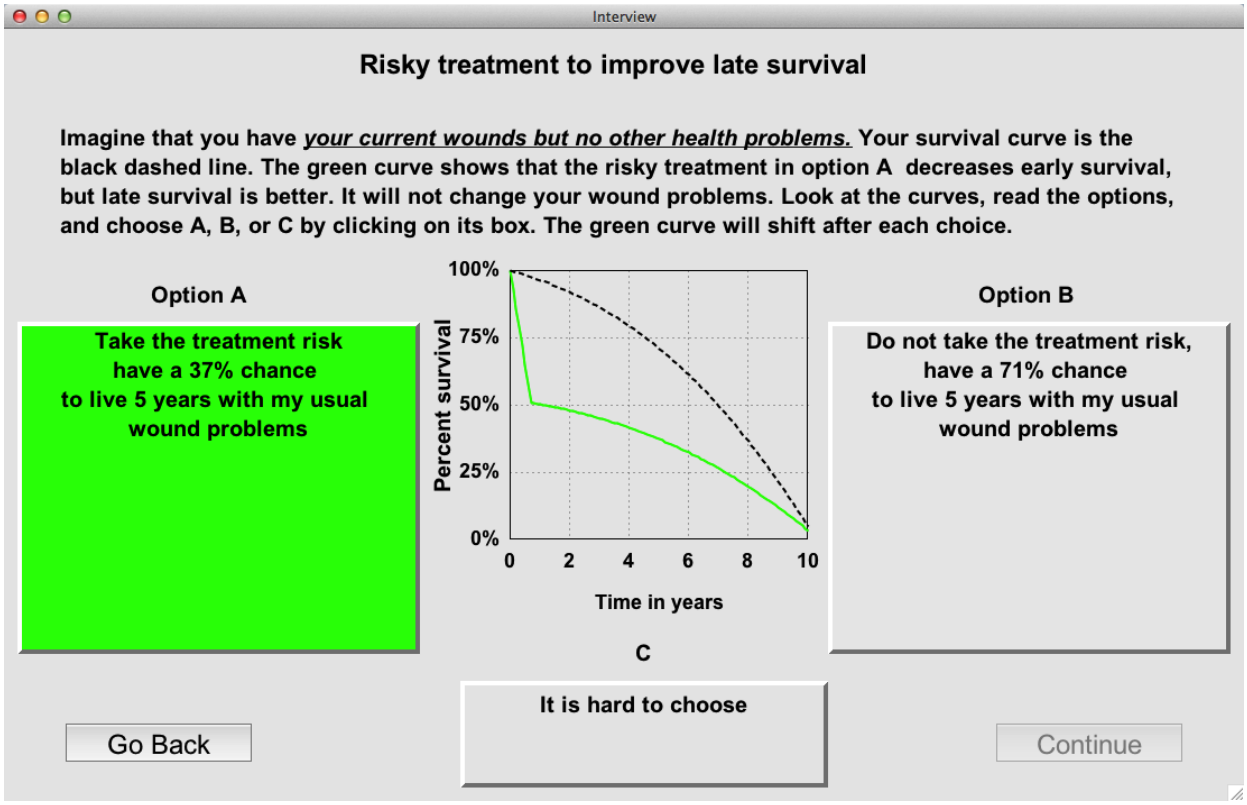


32. Status page;

33. Hit continue;

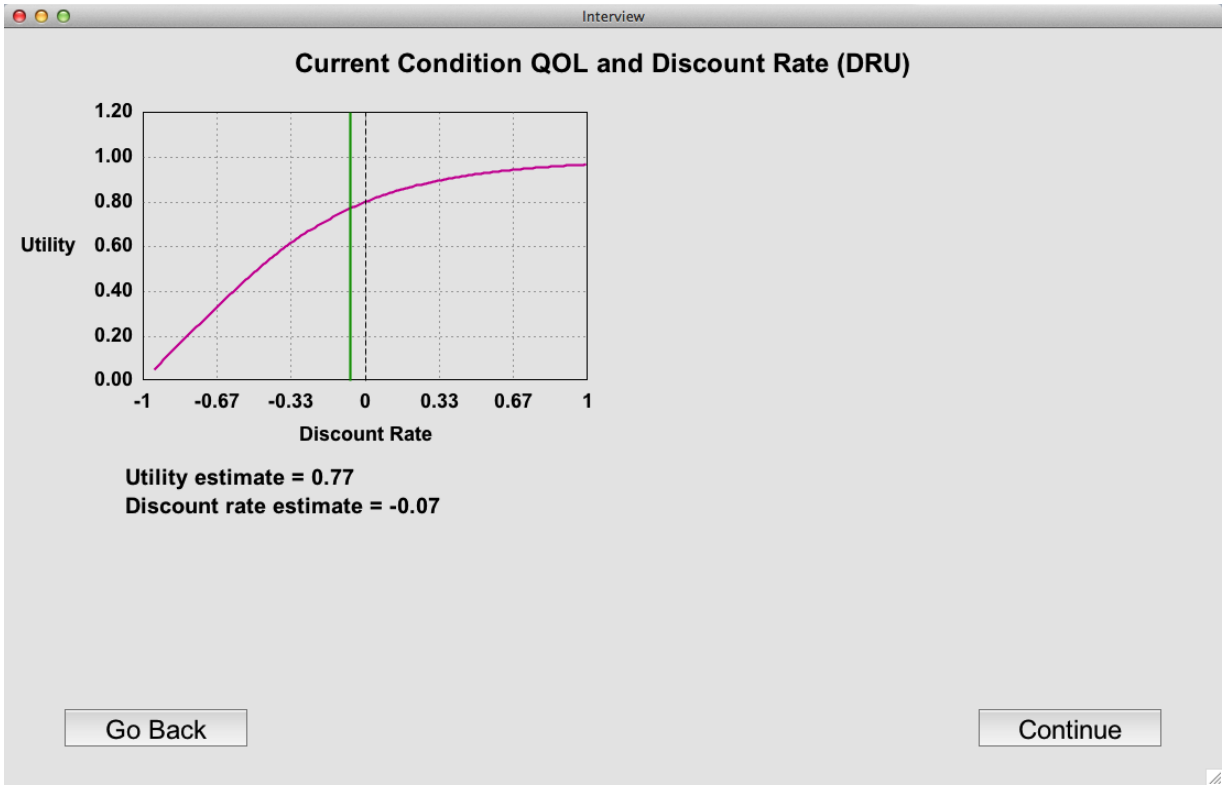


34. The next section is similar to the previous risky treatment scenario except it focuses on wounds only. Patient will select accordingly until they hit Option C when the survey is over. The next section gives quality of life utility measurements with discounted numbers;



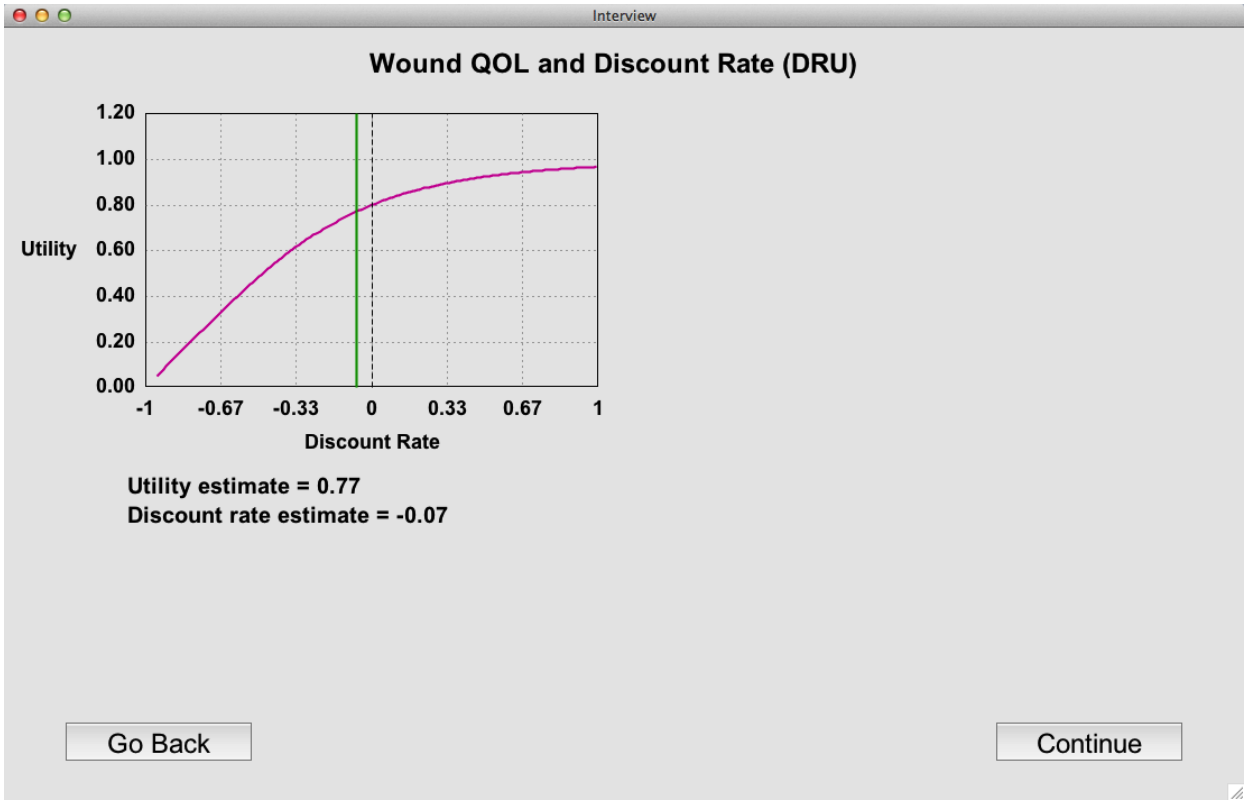
35. This screen shows current health condition utility measure between 0 and 1 (0.77). A discounted rate of 0.07 was subtracted from the utility measure giving the above answer 0.77.

36. Hit continue;



37. Wound quality of life utility measurement that is discounted;

38. Hit continue;



39. The interview is complete;

40. The researcher can save and quit or do another interview.

19.3 MODIFIED ACTIVITY-BASED COSTING METHODOLOGY

Introduction
Modified activity-based costing methodology will be used in this study to document direct costs (direct reimbursable medical cost, direct non-reimbursable medical costs, direct non-medical costs) and indirect costs related to each treatment group. Modified activity-based costing (ABC) is an accounting technique that allows healthcare organizations to determine actual costs associated with their services based on the resources they consume.

Direct medical costs related to care of venous leg ulcers include 1) labor for office visits, diagnostics, laboratory tests, debridement, application of treatment to the wound, applying compression bandages etc. This may be accomplished through a physician, nurse or other qualified healthcare professional; 2) materials such as wound dressings, compression bandages and medical supplies (gloves, saline, gauze etc.); 3) medications for pain, infection etc. and 4) the treatment under investigation. Indirect costs will be estimated by results from the Patient Benefit Index questionnaire (Appendix 19.6).

Sampling Institutions for Direct Human Clinical Resource, Medical Supplies and Diagnostics Costing Analysis
Wound clinics participating in the study will be randomly selected and given 5 costing worksheets (5 patients) for CelluTome™ group (see figures on next 3 pages) and 5 costing worksheets (5 patients) for the control group in each institution to document the weekly direct human clinical resource time for each group. This involves clinical contact within the wound clinic and during the week with non-wound clinic staff (e.g., primary care physicians, home care nurse, RN etc.). An assigned clinician or research assistant will collect the information contained within the costing worksheet for each patient randomly selected within each wound clinic. This data will be used to estimate direct human clinical resource consumption and average costs for each group.

CelluTome Group

Research Clinic:_____

Visit Date:_____ **Patient Id:**_____

Activity-Based Costing Worksheet

Clinic Labor

Pre-CelluTome Application: Includes compression bandage removal, dressing(s) removal, ulcer cleansing, patient assessment etc.

Please place a check (☑) in boxes indicating staff credentials administering care to this patient. Enter number of staff for each credential and then total minutes allotted for all respective clinicians:

☐ RN	Number of Registered Nurses:_____	Total Minutes for all RN's at this pre-CelluTome application phase:_____
☐ LPN	Number of Licensed Practical Nurses:_____	Total Minutes for all LPN's at this pre-CelluTome application phase:_____
☐ Nurse Other*	Number of Other Nurses:_____	Total Minutes for all other nursing at this pre-CelluTome application phase:_____
☐ Physician	Number of Physicians:_____	Total Minutes for all Physician's at this pre-CelluTome application phase:_____

*Please describe nurse other in CelluTome application phase:_____

CelluTome Application: Includes 1) site preparation; 2) position harvester; 3) blister formation; 4) graft acquisition & application to recipient site; 5) donor site care

Please place a check (☑) in boxes indicating staff credentials administering CelluTome to this patient. Enter number of staff for each credential and then total minutes allotted for all respective clinicians:

☐ RN	Number of Registered Nurses:_____	Total Minutes for all RN's at this CelluTome application phase:_____
☐ LPN	Number of Licensed Practical Nurses:_____	Total Minutes for all LPN's at this CelluTome application phase:_____
☐ Nurse Other**	Number of Other Nurses:_____	Total Minutes for all other nursing at this CelluTome application phase:_____
☐ Physician	Number of Physicians:_____	Total Minutes for all Physician's at this CelluTome application phase:_____

**Please describe nurse other in pre-CelluTome phase:_____

CelluTome Study ABC Worksheet, Confidential KCI 2015

Page 1 of 2

CelluTome *Clinic Labor (continued)*

Post CelluTome Application: Includes compression bandage application and other activity before patient leaves the research clinic

Please place a check (☑) in boxes indicating staff credentials administering compression bandage to this patient and other care before patient leaves clinic. Enter number of staff for each credential and then total minutes allotted for all respective clinicians:

☐ RN	Number of Registered Nurses:_____	Total Minutes for all RN's at this post-CelluTome application phase:_____
☐ LPN	Number of Licensed Practical Nurses:_____	Total Minutes for all LPN's at this post-CelluTome application phase:_____
☐ Nurse Other***	Number of Other Nurses:_____	Total Minutes for all other nursing at this post-CelluTome application phase:_____
☐ Physician	Number of Physicians:_____	Total Minutes for all Physician's at this post-CelluTome application phase:_____

***Please describe nurse other in post-CelluTome phase:_____

Non-Clinic Care in Addition to Clinic Care

How many nursing visits to patients home has patient received in the last 7 days to treat their ulcer? ○ None ○ 1 ○ 2 ○ 3 ○ > 3

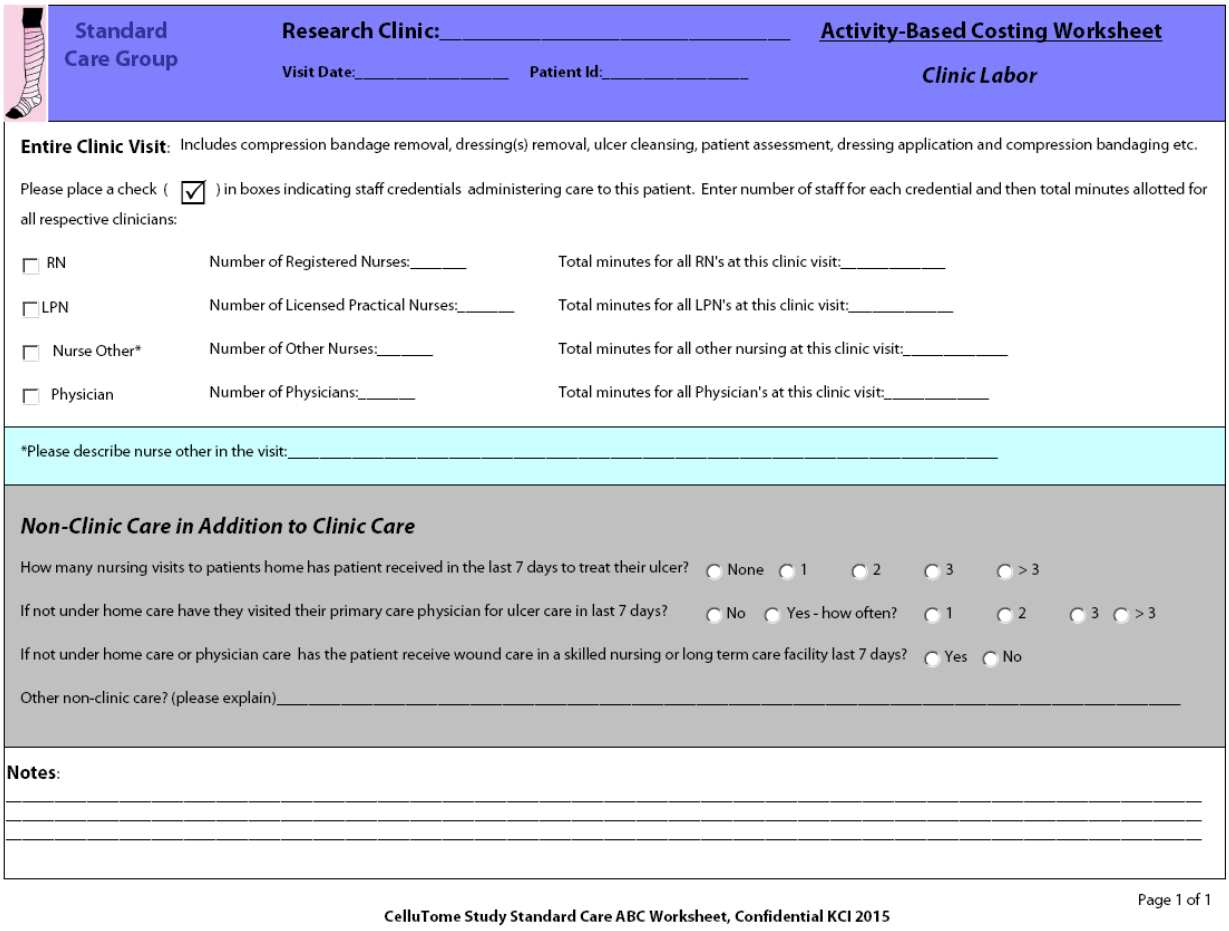
If not under home care have they visited their primary care physician for ulcer care in last 7 days? ○ No ○ Yes - how often? ○ 1 ○ 2 ○ 3 ○ > 3

If not under home care or physician care has the patient receive wound care in a skilled nursing or long term care facility last 7 days? ○ Yes ○ No

Other non-clinic care? (please explain)_____

Notes:
_____
_____
_____

Patient Id:_____ **Visit Date**:_____ **CelluTome Study ABC Worksheet, Confidential KCI 2015** Page 2 of 2



Standard Care Group

Research Clinic:_____ **Activity-Based Costing Worksheet**

Visit Date:_____ **Patient Id:**_____ ***Clinic Labor***

Entire Clinic Visit: Includes compression bandage removal, dressing(s) removal, ulcer cleansing, patient assessment, dressing application and compression bandaging etc.

Please place a check (☑) in boxes indicating staff credentials administering care to this patient. Enter number of staff for each credential and then total minutes allotted for all respective clinicians:

☐ RN	Number of Registered Nurses:_____	Total minutes for all RN's at this clinic visit:_____
☐ LPN	Number of Licensed Practical Nurses:_____	Total minutes for all LPN's at this clinic visit:_____
☐ Nurse Other*	Number of Other Nurses:_____	Total minutes for all other nursing at this clinic visit:_____
☐ Physician	Number of Physicians:_____	Total minutes for all Physician's at this clinic visit:_____

*Please describe nurse other in the visit:_____

Non-Clinic Care in Addition to Clinic Care

How many nursing visits to patients home has patient received in the last 7 days to treat their ulcer? ○ None ○ 1 ○ 2 ○ 3 ○ > 3

If not under home care have they visited their primary care physician for ulcer care in last 7 days? ○ No ○ Yes - how often? ○ 1 ○ 2 ○ 3 ○ > 3

If not under home care or physician care has the patient receive wound care in a skilled nursing or long term care facility last 7 days? ○ Yes ○ No

Other non-clinic care? (please explain)_____

Notes:
_____
_____
_____

Page 1 of 1

CelluTome Study Standard Care ABC Worksheet, Confidential KCI 2015

Patient Consumption of Drugs, Skin Care, Medical Supplies and CelluTome™

The patients randomly selected at the eight wound clinics will have their consumption of medical supplies (e.g., dressings, compression bandages, debridement, tape, gloves, saline and, gauze etc.), drugs (e.g., antibiotics, pain meds, antimicrobials etc.) and CelluTome™ documented in the patient record (CRF) over the course of their assigned therapy. A research assistant or assigned nurse will pull the records for each patient and document the consumption and costs of medical supplies and CelluTome™ for health economic analysis.

Laboratory and Miscellaneous Diagnostics

A record of diagnostics used within the treatment process is contained in the randomly assigned patient medical record and case report form. A research assistant or assigned nurse will pull the records for each patient and document the consumption and costs of diagnostics for health economic analysis.

Cost Analysis

Multiple costing perspectives are considered in the health economic analysis:

Hospital/Outpatient Perspective: Direct institutional medical costs for the treatment and control group will be collected through modified activity-based costing methodology. Hourly payment rates will be collected directly from the institutions where care is provided for each care provider involved in the treatment for each cohort. An average hourly payment rate per resource will be determined across the

institutions participating in the study. For resource consumption outside of the wound clinic national hourly wage rates will be determined for home care visits, nursing home staff, family caregiver etc. as detected in modified activity-based costing data collection forms. All direct medical costs from the hospital/outpatient sector will be compiled for each cohort and compared between groups as the patients flow through the study/system. This allows a pragmatic view of costs and cost savings from the hospital/outpatient perspective in relation to their charges and reimbursement.

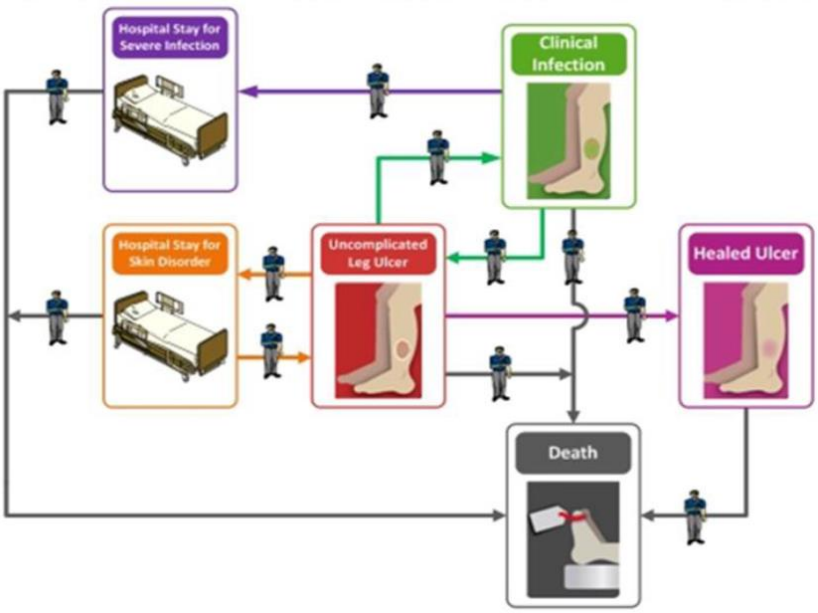
Payer Perspective: Reimbursement from Medicare, Medicaid and Private payers during the course of the study for each group are compiled from each institution participating in the study. Average reimbursement for each product/procedure/stay for each cohort and patient will be determined across the institutions. This information will come from data sources within each institution as compiled by an internal research assistant. Cost effectiveness analysis from the payer perspective is expected to show reduced reimbursement costs for CelluTome group in respect to expedited healing and reduction in complications.

Patient Perspective: Patient costs will be determined from the patient or proxy related to the direct care (medicine, hospital, therapy etc.) that are out-of-pocket, indirect work loss, worker replacement, and reduced productivity from the ulcer.

A comparative cost-benefit, cost-utility and cost-effectiveness analysis will be derived from a discrete-event simulation model with a time-frame conducive to each perspective above (Appendix 19.4). For the hospital/outpatient clinic a return-on-investment (cost-benefit) is determined over 6 to 12 treatment weeks which is standard length-of-time patient is engaged with that sector. From a payer perspective, the model will highlight costs from 1 to 3 years as VLU patients that do not heal within 6 months continue with treatment expenses. Fortunately, patients that do not heal within 6 months are few (~10 percent) but still costly. Reduction in patient out-of-pocket expenses will be compared over the entire span of the model (3 years).

19.4 DISCRETE EVENT SIMULATION MODEL TO MEASURE COST-EFFECTIVENESS AND COST-BENEFIT

A discrete event simulation model (see figure this page) is used to measure the costs (e.g., clinical human resources, medical supplies, drugs, diagnostics, investigations and CelluTome) and consequences (e.g., healing, clinical infection, hospitalization and death) over the course of the study time period from the perspective of the hospital/outpatient, payer and patient. The model will contain health states (e.g., healed ulcer, infected ulcer, severely infected ulcer, hospitalization (e.g., cellulitis, amputation), uncomplicated ulcer and death. The patient and respective ulcer will travel over time in the model for three years accumulating costs and consequences. Cost analysis for differing perspectives and time-horizons are completed using the advantages of the model framework.



In particular, average quality of life utility score values will be assigned to

each health state from the use of Wound-Trade©, time trade-off assessment (Appendix 19.2) matched to the health states in a separate matching analysis between scores and health state of the ulcer/patient measured in the case report form. As the patient/ulcer traverses through the model over time weekly assigned utility values in each health state will be compiled and totaled for each patient and respective group at years end. This will facilitate the determination of cost per quality adjusted life year comparison between groups.

A cost-benefit (return-on-investment) measurement will also be completed from the hospital/outpatient perspective to show value to the institution. Average direct medical costs attributed to resource utilization, medical supplies, drugs, diagnostics and CelluTome™ will be estimated during the trial for each group. Financial benefits include averted costs of complications from rapid healing that may be consequential to the hospital/outpatient clinic.

Cost-effectiveness is used to demonstrate the value of CelluTome™ for rapid healing, reduced complications and therefore reduced financial impact for the payer.

Return-on Investment Metrics:

Metric	Definition	Formula
Costs	Total amount of money spent on new improvement process	$= \sum_{i=1}^{n} \text{Cost}_i$
Benefits	Total amount of money gained from a new and improved process	$= \sum_{i=1}^{n} \text{Benefit}_i$
Net present value	Discounted benefits based on inflation	$= \frac{\text{Benefits}}{(1+\text{Inflation\_Rate})^{\text{Years}}}$

19.5 QUALITY OF LIFE QUESTIONNAIRE (w-QOL)

Quality of life with chronic wounds – "W-QoL" questionnaire

With the following questions, we aim to find out how your chronic wound(s) affect(s) your quality of life.

In every line, please tick what has applied to you in the last 7 days.

	In the last seven days…	not at all	a little	moder-ately	quite a lot	very much
1	…my wound **hurt**	O	O	O	O	O
2	…my wound had a bad **smell**	O	O	O	O	O
3	…there was a disturbing **discharge** from the wound	O	O	O	O	O
4	…the wound has affected my **sleep**	O	O	O	O	O
5	…the **treatment** of the wound has been a burden	O	O	O	O	O
6	…the wound has made me **unhappy**	O	O	O	O	O
7	…I have felt frustrated because the wound is taking so **long to heal**	O	O	O	O	O
8	…I have **worried** about my wound	O	O	O	O	O
9	…I have been afraid of the wound **getting worse** or of new wounds appearing	O	O	O	O	O
10	…I have been afraid of **knocking** the wound	O	O	O	O	O
11	…I have had trouble **moving about** because of the wound	O	O	O	O	O
12	…climbing **stairs** has been difficult because of the wound	O	O	O	O	O
13	…I have had trouble with **day-to-day activities** because of the wound	O	O	O	O	O
14	…the wound has limited my **leisure activities**	O	O	O	O	O
15	…the wound has forced me to limit my **activities with others**	O	O	O	O	O
16	…I have felt **dependent** on help from others be-cause of the wound	O	O	O	O	O
17	…the wound has been a **financial** burden to me	O	O	O	O	O

"W-QoL" questionnaire, German Center for Health Services Research in Dermatology (CVderm), University Medical Center Hamburg
Items adapted from Freiburg Quality of Life Assessment (FLQA-w), Würzburg Wund Score (WWS), Cardiff Wound Impact Schedule (CWIS)

19.6 PBI-P – PATIENT BENEFIT INDEX, WOUND VERSION

At the beginning of treatment for each group a patient benefit index questionnaire is given to the patient for completion. It is intended to give the patient a chance to give their goals of treatment:

PBI-P – Patient Benefit Index, wound version

Importance of Treatment Goals

With the help of the following questions, we'd like to know how important the below mentioned goals are to you personally in the **current treatment.**

For each of the following statements, please mark **how important** this treatment goal is to you. If a statement does not apply to you, e.g. because you do not have pain, please mark "*does not apply to me*".

	As a result of therapy, how important is it for you to ...	**not at all**	**somewhat**	**moderately**	**quite**	**very**	*does not apply to me*
1	...be free of pain	O	O	O	O	O	O
2	...have no discharge from the lesion	O	O	O	O	O	O
3	...not have an unpleasant smell from the lesion	O	O	O	O	O	O
4	...be healed from the lesion(s)	O	O	O	O	O	O
5	...be able to sleep better	O	O	O	O	O	O
6	...feel less depressed	O	O	O	O	O	O
7	...experience a greater enjoyment of life	O	O	O	O	O	O
8	...have no fear that the disease will become worse	O	O	O	O	O	O
9	...be able to lead a normal everyday life	O	O	O	O	O	O
10	...be more productive in everyday life	O	O	O	O	O	O
11	...be less of a burden to relatives and friends	O	O	O	O	O	O
12	...be able to engage in normal leisure activities	O	O	O	O	O	O
13	...be able to lead a normal working life	O	O	O	O	O	O
14	...be able to have more contact with other people	O	O	O	O	O	O
15	...be comfortable showing yourself more	O	O	O	O	O	O
16	...be less burdened in your partnership	O	O	O	O	O	O
17	...be less dependent on doctor and clinic visits	O	O	O	O	O	O
18	...need less time for daily treatment	O	O	O	O	O	O
19	...have fewer out-of-pocket treatment expenses	O	O	O	O	O	O
20	...have fewer side effects	O	O	O	O	O	O
21	...find a clear diagnosis and therapy	O	O	O	O	O	O
22	...have confidence in the therapy	O	O	O	O	O	O

Please check once more if you have exactly marked each statement with an 'x'.

Our sincerest thanks for your cooperation!

PBI-Patient Benefit Index (index of benefits and needs in therapy), Augustin 2006

At the end of treatment, the patient will complete the form below indicating if the respective treatment met their goals:

PBI-P – Patient Benefit Index, wound version

Treatment benefits

At the start of the treatment, you indicated in a questionnaire how important various goals were in the treatment of your skin disease.

Please mark each of the following statements according to the extent that these treatment goals **were achieved**, thereby indicating if the treatment has benefitted you. If a statement did not apply to you, e.g. because you had no pain, please mark "*did not apply to me*".

	The current treatment has helped me to...	**not at all**	**somewhat**	**moderately**	**quite**	**very**	*did not apply to me*
1	...be free of pain	O	O	O	O	O	O
2	...have no discharge from the lesion	O	O	O	O	O	O
3	...not have an unpleasant smell from the lesion	O	O	O	O	O	O
4	...be healed from the lesion(s)	O	O	O	O	O	O
5	...be able to sleep better	O	O	O	O	O	O
6	...feel less depressed	O	O	O	O	O	O
7	...experience a greater enjoyment of life	O	O	O	O	O	O
8	...have no fear that the disease will become worse	O	O	O	O	O	O
9	...be able to lead a normal everyday life	O	O	O	O	O	O
10	...be more productive in everyday life	O	O	O	O	O	O
11	...be less of a burden to relatives and friends	O	O	O	O	O	O
12	...be able to engage in normal leisure activities	O	O	O	O	O	O
13	...be able to lead a normal working life	O	O	O	O	O	O
14	...be able to have more contact with other people	O	O	O	O	O	O
15	...be comfortable showing yourself more	O	O	O	O	O	O
16	...be less burdened in your my partnership	O	O	O	O	O	O
17	...be less dependent on doctor and clinic visits	O	O	O	O	O	O
18	...need less time for daily treatment	O	O	O	O	O	O
19	...have fewer out-of-pocket treatment expenses	O	O	O	O	O	O
20	...have fewer side effects	O	O	O	O	O	O
21	...find a clear diagnosis and therapy	O	O	O	O	O	O
22	...have confidence in the therapy	O	O	O	O	O	O

Please check once more if you have exactly marked each statement with an 'x'.

Our sincerest thanks for your cooperation!

PBI-Patient Benefit Index (index of benefits and needs in therapy), Augustin 2006

19.7 SWAB COLLECTION PROCEDURE

1. Ensure that complete haemostasis has been achieved before obtaining the specimen.

2. Moisten wound area to be swabbed with saline. Care should be taken not to flood the wound with excessive saline.

3. Use the swabs provided by the Sponsor(s).

4. Avoid swabbing areas that contain blood, necrotic material or thick slough.

5. Press the head of the swab flat against the base of the wound and gently rotate it back and forth several times while applying pressure. Continue rotating the swab head until it is fully coated with wound fluid. The head of the swab should turn a tan color.

6. Collect additional swabs using the same procedure described in step 5 above. Apply additional saline drops to remoisten the wound between swabs.

7. Label the swabs with Subject ID#, date, and study visit number.

19.8 ANTIMICROBIAL DRESSING

In order to reduce the risk of infection, any of the following dressing may be used during the 2 week screening period and during TV4, TV8, and TV10 (for patients crossing over):

Prisma
Silver Alginate
Silver Hydrogel
Honey
Hydrofera blue

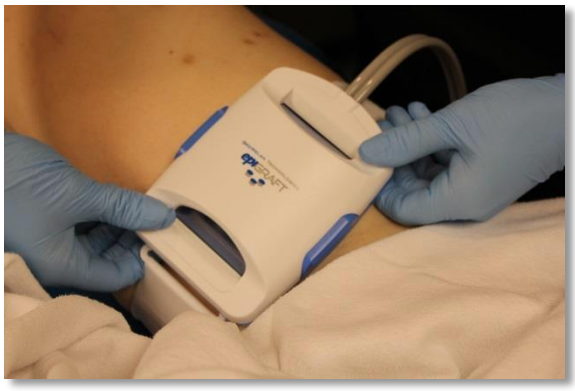
19.9 APPLICATION OF PRISMA™

1. Clean wound with saline; dry peri-wound skin
2. Cut PrismTM to cover the wound bed
3. Apply Prisma™ directly on the wound if it is moist. Normal saline may be used for dry wounds before Prisma™ is applied.
4. Apply appropriate cover dressing to maintain a moisture balanced wound environment. Prisma™ will be absorbed over 3 days. Prisma™ may need replacing more frequently if wound is exudating heavily.

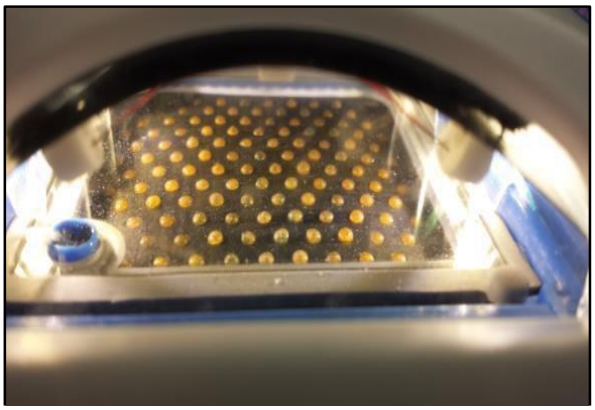
19.10 EPIDERMAL HARVESTING PROCEDURE

- Select donor site (inner thigh).
- Using clippers to remove any hair from the skin. (Do not use a razor to shave the patient)
- Prep the site with isopropyl alcohol.
- Anesthesia is not required.
- Apply the harvester and affix in place using the Velcro strap.

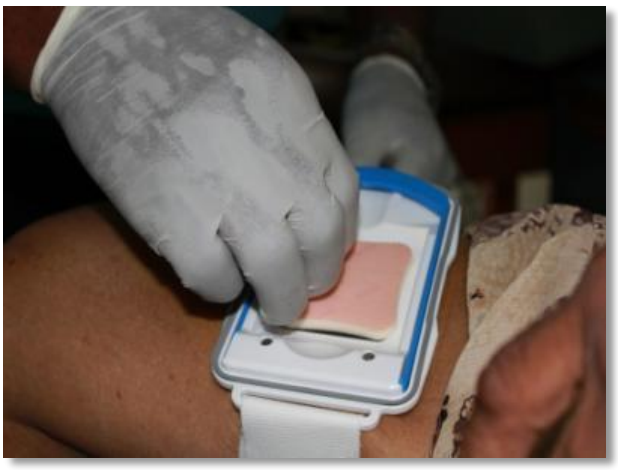
- Attach the vacuum hood. Press on the opposite sides of the hood until you here a click. This ensures that you have a seal.



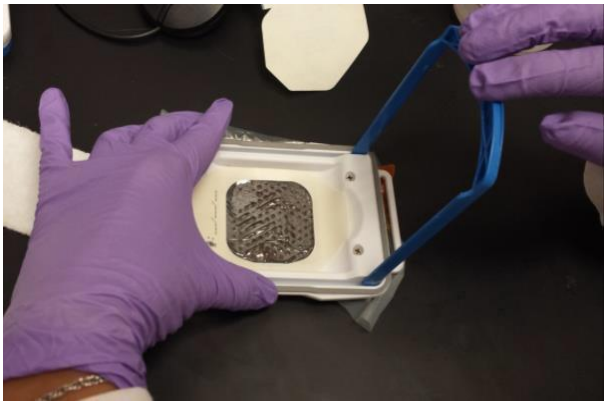
- Turn the machine on. The vacuum head will apply 200 mmHg negative pressure and heat the skin to 40 C. The patient may experience a warm feeling.
- The epidermal microdomes are ready for harvesting when the epidermis has formed blisters as shown.



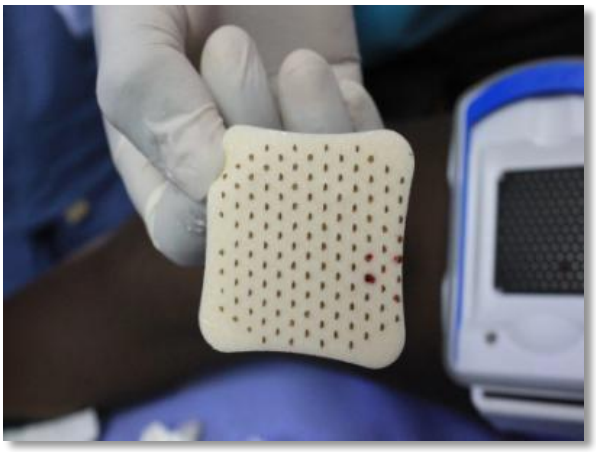
- Turn off the power.
- Remove the vacuum hood.
- Place the foam dressing provided in the harvester.



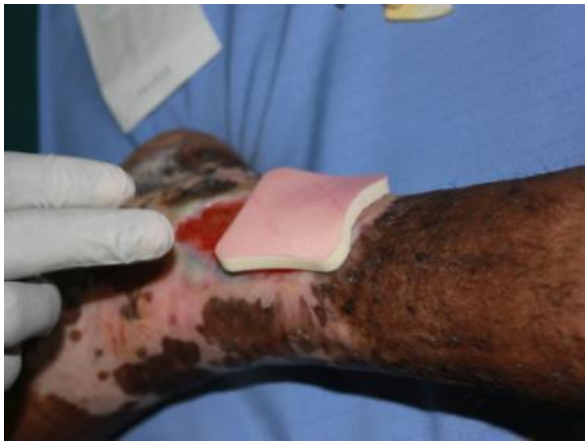
- Pull the blue handle back until you hear a “click.”
- Place the foam dressing provided in the harvester. Activate the blades cutting the epidermal microdomes by advancing the blue handle back onto the harvester.

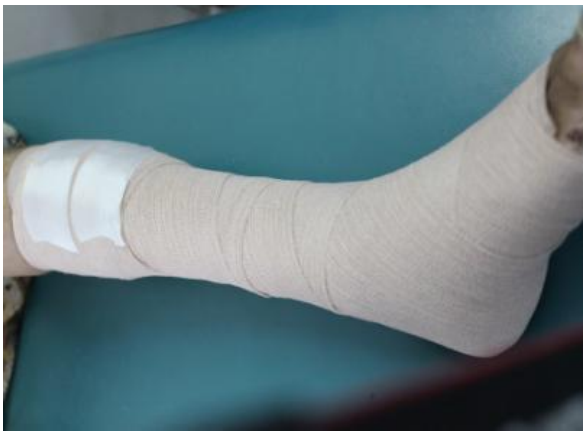


- Examine the foam and record the percentage of microdomes harvested.



- Transfer the foam to the wound and secure using the 3M Coban-2 compression wrap.





- Cover the donor site with Tegaderm® provided.