Safety and Feasibility of Adipose Derived Regenerative Cells (ADRCs) in the Treatment of Deep Partial Thickness and Full Thickness Thermal Wounds (RELIEF)

RELIEF Trial

Version 04

14 May 2018

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Contents

PROTOCOL APPROVAL FORM

Study Title: Safety and Feasibility of Adipose Derived **R**egenerative C**el**ls (ADRCs) **i**n the Tr**e**atment of Deep Partial Thickness and **F**ull Thickness Thermal Wounds **(RELIEF)**

Short Title: **RELIEF Trial**

Protocol Date: 24 April, 2018

This study protocol was subjected to critical review. The information it contains is consistent with the sponsor's current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2008, and the Good Clinical Practice (GCP) Consolidated Guidance in guidelines of the International Conference on Harmonization (ICH) E2 & E6, and 21CFR 11, 50, 56, 812, 814 and 820. The study will also be carried out in keeping with all applicable laws, rules, and regulations.

Terrie Heidemann
Senior Manager, Regulatory & Quality Assurance

Date (dd/mmm/yyyy)

Marc H. Hedrick, MD
Chief Executive Officer and
Chief Medical Officer

Date (dd/mmm/yyyy)

INVESTIGATOR STATEMENT PAGE

Study Sponsor: **Cytori Therapeutics, Inc.**
Clinical Trial: **RELIEF Trial**
Date of Protocol: **24 April, 2018**

Study Title: Safety and Feasibility of Adipose Derived **R**egenerative C**el**ls (ADRCs) **i**n the Tr**e**atment of Deep Partial Thickness and **F**ull Thickness Thermal Wounds **(RELIEF)**

The information contained in this document and all information provided to you related to this protocol are the confidential and proprietary information of Cytori Therapeutics, Inc. (Sponsor) and except as may be required by federal, state or local laws or regulations, may not be disclosed to others without prior written permission of Sponsor. The Principal Investigator at each site may, however, disclose such information to supervised individuals working on the protocol, provided such individuals agree to be bound to maintain the confidentiality of such protocol information.

I agree to abide by the statement of confidentiality.

I agree to conduct the study according to this protocol. Any changes in procedure will only be made if necessary to protect the safety, rights, or welfare of subjects.

I agree to comply with the current ICH, GCP and CFR Guidelines.

I agree to conduct the study in person or to supervise the study.

I agree to ensure that all who assist me in the conduct of the study have access to the study protocol and any amendments and are aware of their obligations.

I agree to conduct the investigation in accordance with the agreement, the investigational plan, Part 812 and other applicable FDA regulations, and conditions of approval imposed by the reviewing IRB and FDA.

I agree to supervise all testing of the device involving human subjects.

I agree to ensure that the requirements for obtaining informed consent are met.

I agree to provide sufficient and accurate financial disclosure information and update information if any relevant changes occur during the investigation and for one year following the completion of the study.

_____

Principal Investigator (Print Name and Title)

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Principal Investigator (Signature) Date (dd/mmm/yyyy)

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RELIEF TRIAL PROTOCOL SUMMARY

Title	Safety and Feasibility of Adipose Derived **R**egenerative C**el**ls (ADRCs) **i**n the Tr**e**atment of Deep Partial Thickness and **F**ull Thickness Thermal Wounds **(RELIEF)**
Test Product	Celution® System used for processing of Autologous Adipose-Derived Regenerative Cells (ADRCs)
Objective	The primary objective of the protocol is to evaluate preliminary safety and feasibility of ADRCs via intravenous delivery in the treatment of deep partial and full thickness thermal wounds.
Design	A prospective, open-label, parallel group, usual care controlled, multi-center randomized (2:1, active: usual care alone) safety and feasibility study targeting thermal burns. Subjects will have at least one deep partial or full thickness burn wound of $\geq$ 250 cm^2 that is to be autografted with a meshed split thickness skin graft (STSG) Subjects randomized to ADRCs will undergo small volume fat harvest (100 to 150 mL) performed under general anesthesia during scheduled burn surgery followed by peripheral intravenous delivery of ADRCs within 4 hours of completion of Celution® processing. Harvested tissue will be processed in the Celution® System to isolate and concentrate ADRCs. Subjects randomized to usual care will not undergo a fat harvesting procedure.
Sample Size	Up to 15 subjects will be enrolled. Each subject will contribute up to three qualified wound areas for the analysis.
Enrollment	The 1st 5 subjects enrolled in active treatment will be observed for adverse events over a 7 day period. If no SAEs occur during that period, for that individual subject, the next subject enrolled in active treatment may be entered with the same 7 day observation period. The safety results of the first 5 subjects will be reviewed by the DMC and their evaluation will be used in conjunction with the clinical data collected to date, and if appropriate, to potentially remove the staggering approach to the study. Enrollment after the first 5 subjects enrolled in active treatment may continue only after the DMC has completed its safety review and recommends continuation.
Study Location	Up to 10 regional burn centers in the US
Indication	Celution® System is intended for the preparation of autologous adipose-derived regenerative cells (ADRCs) for intravenous use as an adjunct to autologous split thickness skin grafts used in the current standard treatment of full thickness and deep partial thickness thermal burn injuries
Post Procedure Evaluations	Evaluations at early dressing changes (time of first dressing change, day 10 (±2) days and weeks 2 (±3 days), 3 (±3 days), 4 (±3 days), 8 (±7 days), 12 (±14 days), 26 (±14 days) and 52 (±21 days) post-grafting
Treatment Arm	Adjunct to thermal burn skin wound coverage with autologous meshed split thickness skin graft (STSG) delivered intravenously on the same day as STSG. Dose: 20 x 10^6 ADRCs

Primary Endpoint	Safety and Feasibility • Safety will be evaluated based on the incidence, type and seriousness of adverse events related to the IV administration of test substance as well as the low volume lipoharvest procedure. • Feasibility will be determined as the ability to obtain > 100 mL adipose tissue, obtain ≥ 20 x 106 ADRCs, and deliver the prepared cell dose.
Key Secondary Endpoints	• Percent epithelialization of the graft at time of first dressing change, day 10 and weeks 2, 3 and 4 post grafting (assessed by surgeon visual evaluation and blinded independent review of standardized photographs) • Percent take of the graft at time of first dressing change and day 10 and weeks 2, 3 and 4 • Percent of group with complete wound healing at weeks 2, 3, 4, 8 and 12
Exploratory Endpoints	• Transepithelial water loss at weeks 2, 3 and 4 to be performed at 2 different sites within the wound (central and peripheral) • Wound pain VAS (visual analogue scale) at time of first dressing change and day 10 and weeks 2, 3 and 4 • Pruritis VAS at weeks 3, 4, 8, 12, 26 and 52 • Patient and Observer Scar Assessment Scale (v2.0) at weeks 3, 4, 8, 12, 26 and 52 • Skin elasticity measurement at weeks 12, 26 and 52 • Vancouver Scar Scale at weeks 12, 26 and 52 • Skin pigmentation and hardness at weeks 12, 26 and 52 • ADRC cell population subtypes (FACS) • Biomarkers related to burn injury (such as C-reactive protein (CRP), IL-6, IL8, IL1β, interleukin-1 receptor antagonist (IL-1ra); neutrophil gelatinase-associated lipocalin (NGAL); Tumor Necrosis Factor-α (TNFα), and tumor necrosis factor receptor 1a (TNFR-1a)) and related to scarring (such as amino-terminal propeptide of type III procollagen, (PIIINP), TIMP1, MMP2, MMP9 and VEGF-A) will be collected at baseline (prior to treatment), days 6, and 10 and weeks 2, 3, 4, 8, and 12 post ADRC administration. Serum sample will be collected from 4 to 5 ml of whole blood that is centrifuged, separated into 500-µL aliquots, and stored at -80°C at the clinical site serum bank or central lab until use. • Serum biomarkers will be measured using commercial Enzyme-Linked ImmunoSorbent Assay (ELISA).
Statistics	The intent-to-treat (ITT) population will include all randomized subjects. Randomization will occur on the STSG procedure day prior to liposuction. The per-treatment-evaluable (PTE) population will include all enrolled subjects who have day 6 follow-up information available. The groups compared will be the subjects receiving ADRCs and STSG and the usual care controlled subjects (both grouped and stratified by mesh size). Analysis will be performed by a one way ANOVA.

Inclusion & Exclusion Criteria	**Inclusion Criteria** 1. Males or females age ≥ 18 to ≤ 65 2. BMI > 20 kg/m^2 3. Burn TBSA 20% - 50% 4. At least one deep partial thickness and/or full thickness thermal burn ≥ 250 cm^2 on the arms, legs, back, abdomen or chest that is anticipated to be covered with a meshed autologous STSG and that has not been treated previously with a biologic dressing such as Alloderm® or Integra® 5. Ability to safely undergo tissue harvest that is anticipated to yield >150 mL of adipose tissue at a site that is free from infection 6. Donor site availability for skin graft harvest 7. Able to provide written informed consent signed by either the patient or their legally authorized representative 8. Women and men of child-bearing potential agreeing to use contraception during the study. Acceptable methods include surgical sterility, IUDs, hormonal contraception or double barrier methods. **Exclusion Criteria** 1. Subjects with burns > 3rd degree (i.e. involvement of deeper tissues, such as muscle, tendons, or bone) 2. Subjects with electrical or chemical burns 3. Subjects with significant inhalation injuries necessitating intubation and mechanical ventilation or requiring > 50% FI02 on a continuous basis to maintain oxygenation (0_2 sat > 90%) 4. In the opinion of treating physician, patient not expected to survive beyond 30 days 5. Pre-existing condition requiring current use of immunosuppressive medication or systemic steroids 6. Pregnant or lactating status. Pregnancy as determined by a positive pregnancy test at screening or baseline 7. Known history of HIV infection, or active Hepatitis B or active Hepatitis C infection 8. Cancer requiring chemotherapy or radiation within previous 6 months or resection within the last 5 years (other than basal cell carcinoma) 9. Known chronic renal failure (serum creatinine > 2 mg/dL) or chronic liver disease 10. Pre-existing medical conditions that would interfere with wound healing (i.e., diabetic patients with Hemoglobin A1c ≥8.0%, malignancy, autoimmune disease) 11. Subjects with psychiatric conditions that are anticipated to result in protocol noncompliance 12. Chronic illicit drug or alcohol abuse that is anticipated to interfere with patient compliance with the protocol 13. Participation in another clinical trial within 60 days of the screening visit 14. Any concurrent disease or condition that, in the opinion of the investigator, would make the patient unsuitable for participation in the study

ABBREVIATIONS

ADRC	Adipose-Derived Regenerative Cells
ADSC	Adipose-Derived Stem Cells
AE	Adverse Event
BARDA	Biomedical Advanced Research and Development Authority
CFU-F	Colony Forming Units Fibroblast
CRF	Case Report Form
DMC	Data Monitoring Committee
DNase	Deoxyribonuclease
ET	Early Termination
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
ID	Identification
IRB/EC	Institutional Review Board/Ethic Committee
ITT	Intent-to-Treat
LVL	Low Volume Lipoharvest
PBS	Phosphate Buffered Saline
PI	Principal Investigator
POSAS	Patient and Observer Scar Assessment Scale
PTE	Per-Treatment-Evaluable
SAE	Serious Adverse Event
STSG	Split Thickness Skin Graft
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	Standard of Care
SVF	Stromal Vascular Fraction
TBSA	Total Body Surface Area
TF	Tumescent Fluid
UADE	Unanticipated Adverse Device Effects
VAS	Visual Analog Scale
VSS	Vancouver Scar Scale
WHO	World Health Organization

1 OBJECTIVE

The objective of the protocol is to evaluate preliminary safety and feasibility of adipose-derived regenerative cells (ADRCs) delivered intravenously for the treatment of full thickness and deep partial thickness thermal wounds in addition to standard of care.

2 BACKGROUND

2.1 Wounds

Wound healing is a complex multifactorial process involving the interaction of inflammation, granulation tissue formation, re-epithelialization, and angiogenesis. For deep partial thickness and full thickness burns, acute burn management involves multiple activities including protecting the wound from further injury, initial cleansing and debridement, and determination of dressings with or without a topical antimicrobial agent. Fluid resuscitation, prevention of hypothermia and pain management are also critical aspects of management. Surgical procedures involve escharectomy with further debridement and determination of skin grafting (often autologous split thickness skin graft (STSG) or a temporary graft (allograft or xenograft) or a tissue engineered substitute such as Integra®). The use of a tissue engineered substitute/matrix or temporary graft is generally followed by an autologous skin graft. Maturation of the graft may take several weeks and, depending on the severity and location of wound and the degree of meshing required for a STSG, the cosmetic outcome is often unsatisfactory. In addition, complications such as infection or contractures may occur, which can result in further treatments and procedures, as well as prolongation of healing. Furthermore, in the event of a catastrophic event, the current systems may be overwhelmed in the ability to handle substantial numbers of burn cases, which is compounded by the prolonged nature of healing with current techniques and the high level of expertise required to limit complications and increase the likelihood of a satisfactory functional and aesthetic result. Overall, there is a growing need to develop improved techniques in acute burn management[1].

Adipose Derived Regenerative Cells (ADRCs) are of emerging interest in the application of wound healing. Cells obtained from adipose tissue have been shown to inhibit inflammation[2-5] , promote angiogenesis[5-7, 7-13], stimulate recruitment of endogenous regenerative cells[14, 15] and promote salvage of damaged cells[2, 5, 16]. On the basis of these properties, several preclinical studies have been performed using freshly isolated and cultured adipose-derived cells in a number of animal models of normal and impaired wound healing. The data consistently show improvement of epithelialization and wound healing. ADRCs are an advantageous resource because they can be found in abundant quantities, they can be harvested with a minimally invasive procedure[17, 18], and they can be safely and effectively transplanted into an autologous host.

2.2 Pre-Clinical Data

A large number of studies in the literature have described the ability of freshly isolated adipose cells and cultured adipose stem cells to improve healing in several small and large animal preclinical models of cutaneous wound healing including normal animals, diabetic mice, irradiated skin, mitomycin-treated wounds, and ultraviolet-treated skin[14, 19, 20]. All investigators report improved wound healing parameters including increased wound closure rate, increased granulation tissue, and improved wound vascularity and perfusion.

Pre-clinical testing (animal and biocompatibility) of ADRCs and the Celution® System has demonstrated safety and biocompatibility across numerous animal models of human disease. Data with intravenous[12],

subcutaneous or topical (spray)[4] administration show efficacy of ADRCs over a control group treated with vehicle alone (lactated Ringer's solution). Intravenous administration has the advantage of administration not having to be available at the time of grafting (i.e. can be delivered following completion of the surgical procedure) and potentially may treat more wounds via systemic delivery relative to only treating selected wounds via topical delivery. Complete pre-clinical animal and biocompatibility data are provided in the Celution® System Investigator's Brochure.

2.3 Clinical Data

The Celution® System is approved for use as a medical device in the European Union. Hundreds of patients have been treated with autologous ADRCs prepared using the Celution® System. These patients range in clinical indication from cardiovascular disease to soft tissue reconstruction following breast cancer therapy.

The RESTORE-2 study was a Phase IV (post-market) study evaluating safety and efficacy of ADRC-enriched fat transplantation in the treatment of subjects with breast deformities post segmental breast resection (lumpectomy) with or without radiation therapy. This prospective, single-arm, open-label, multi-center study enrolled 71 subjects with defects ranging from 25-150 mL at 7 European clinical centers. A minimum of 270 mL of adipose tissue were harvested by tissue aspiration. The efficacy data demonstrated high degrees of patient (75%) and investigator (85%) satisfaction following ADRC-enriched fat grafting to treat breast defects post-breast conservation therapy. The procedure was also shown to be safe and well tolerated. RESTORE-2 results provide evidence of safety and efficacy following ADRC delivery for subcutaneous soft tissue indications.

PRECISE was a prospective, double-blind, randomized, parallel group, placebo-controlled, sequential dose escalation study that evaluated the safety and feasibility of intramyocardial administration of ADRCs in subjects with chronic myocardial ischemia not amenable to revascularization. Following baseline electromechanical mapping, subjects received ≤ 15 intramyocardial injections of ADRCs (n=21; median dose of ADRCs 42 x 10^6 per subject) or placebo (n=6: indistinguishable solution) in the area(s) of the heart with inducible ischemia. ADRCs were processed using the Cytori Celution® System. ADRC-treated subjects demonstrated improvement in maximum oxygen consumption relative to the control group. Adverse events and serious adverse events were equally distributed across all treatments groups.

The APOLLO Clinical Trial was a safety and feasibility study of ADRCs processed by the Celution® System and delivered via the intracoronary (IC) route in the treatment of subjects with ST-segment elevation myocardial infarction. This was a prospective, double-blind, 3:1 randomized (ADRC: Placebo), parallel group, placebo-controlled trial. A total of 14 subjects were enrolled and treated according to protocol, 10 subjects received ADRCs and four received placebo. The APOLLO study demonstrated that harvest of adipose for the purpose of processing and returning autologous ADRCs via the intracoronary infusion of 20 x 10^6 ADRCs is a safe and practical procedure. ADRC–treated subjects following acute myocardial infarction yielded several promising efficacy signals including a > 50% reduction in infarct size by MRI compared to placebo treatment.

The ADVANCE clinical study (NCT01216995) is a safety and efficacy randomized (2:1), 2-arm, placebo-controlled, double-blinded clinical study in patients with ST elevation acute myocardial infarction. The ADRCs were injected via the intracoronary route at a dose of 20 x 10^6± 10% or placebo. A total of 23 patients were enrolled, with 13 patients receiving ADRCs and 6 receiving placebo, with 4 subjects not analyzed. While the ADVANCE study did not demonstrate a therapeutic benefit for ADRCs

treatment in patients after STEMI other than a possible preservation on LV mass, the ADVANCE study did demonstrate intracoronary injection of ADRCs obtained via liposuction was well tolerated, with no test material- or injection procedure-related adverse events reported, and was not associated with an increased risk of Major Adverse Cardiac and Cerebrovascular Events (MACCEs).

The ATHENA trials are two ongoing prospective, double-blind, 2:1 randomized (ADRC: Placebo), placebo controlled trials in the United States investigating the safety and efficacy of ADRCs in subjects with heart failure due to ischemic heart disease. ADRCs (low or high dose) are administered directly into the myocardium during a left heart catheterization procedure via 15 injections. A total of 31 subjects were randomized prior to termination of enrollment. Tissue harvest in compromised cardiac subjects was demonstrated to be feasible and can be performed with an acceptable safety profile. One-year follow-up has indicated an improvement in health related quality of life with ADRCs relative to placebo; however, no physiologic changes through echocardiogram or SPECT testing were observed[21].

The ACT OA trial is an ongoing double-blind 2:1 randomized placebo controlled trial investigating the safety and feasibility of intra-articular injection of ADRCs in the treatment of osteoarthritis of the knee. The study completed enrollment of 94 subjects in June 2015. All subjects have completed the study. There have been no UADEs. Tissue harvest was demonstrated to be feasible and safe in all enrolled subjects.

The STAR trial is a phase III a double-blind placebo controlled study evaluating subcutaneous treatment with ADRCs for hand dysfunction resulting from scleroderma. The trial is enrolled with 88 subjects as of June 2016. No related unanticipated adverse events have been reported. The tissue harvest procedures and cell injections have been well tolerated without complications.

A summary of pre-clinical and clinical experience is provided in the Celution® System Investigator's Brochure.

3 RATIONALE

Patients with thermal wounds often require intensive management and have the risk of incomplete wound closure, prolonged period to wound closure, dysfunctional tissue healing (i.e. contractures, keloids) and infection. Such injuries may ultimately lead to chronic pain and disability. For digits and limbs, progressive injury may result in amputation. In addition, more rapid and complete wound healing following acute burns could reduce other complications and decrease the time spent in specialized hospital units. Cells present within the subject's own adipose tissue, ADRCs have the potential to improve healing following thermal injury by potentially reducing inflammation, improving restoration of blood flow, and stimulating local repair cells. Preclinical studies performed by Cytori in a full thickness thermal injury porcine model have demonstrated local and systemic delivery of autologous ADRCs without a scaffold increase wound re-epithelialization[4, 12]. Using a similar model but with Integra® skin substitute, local delivery of ADRCs is associated with improved wound tissue vascularity and maturation[11]. In previous clinical studies, these cells have a favorable safety profile in acute myocardial injury, heart muscle damage, breast deformity following lumpectomy, and OA of the knee.

4 STUDY DESIGN

4.1 Study Overview

The RELIEF Trial is a prospective, open-label, parallel group, usual care controlled, multi-center randomized (2:1, active: usual care alone) safety and feasibility study targeting thermal burns. Subjects will have at least one deep partial or full thickness burn wounds of $\geq$ 250 cm^2 that is to be autografted with a meshed split thickness skin graft (STSG). Subjects randomized to ADRCs will undergo small volume fat harvest (100 to 150 mL) performed at initiation of general anesthesia for scheduled burn surgery followed by intravenous delivery of ADRCs within 4 hours following completion of Celution processing. The lipoaspirate will be processed in the Celution® System to isolate and concentrate ADRCs.

Following informed consent and initial screening assessments, eligible subjects will undergo pre-operative testing. On the procedure day, subjects will be randomized to ADRCs (with usual care) or usual care alone. Low volume lipoharvest will only be performed on subjects randomized to ADRCs in order to obtain 100-150 mL lipoaspirate, which will then be transferred to the Celution® System for processing to isolate and concentrate ADRCs for same-day administration. All treatment will be delivered in a total volume of 10 mL which will be delivered by slow intravenous administration into a peripheral vein.

Following surgery, subjects will be evaluated at time of first dressing change, day 10 (±2) (only if wound dressing change planned) and weeks 2 (±3 days), 3 (±3 days), 4 (±3 days), 8 (±7 days), 12 (±14 days), 26 (±14 days), and 52(±21 days).

All subjects will undergo digital photography of the wounds, clinical evaluations, microbiology and laboratory testing prior to and after the procedure as outlined in *Section 9.0, Study Visit Requirements,* and in the *Schedule of Procedures (Appendix A).*

The 1st 5 subjects enrolled in active treatment will be observed for adverse events over a 7 day period. If no SAEs occur during that period, for that individual subject, the next subject enrolled in active treatment may be entered with the same 7 day observation period. The safety results of the first 5 subjects will be reviewed by the DMC and their evaluation will be used in conjunction with the clinical data collected to date, and if appropriate, to potentially remove the staggering approach to the study. Enrollment after the first 5 subjects may continue only after the DMC has completed its safety review and recommends continuation.

4.2 Study Population

The study population will include subjects with a 20% - 50% TBSA and at least one deep partial or full thickness burn wound with an area of $\geq$ 250 cm^2 who will undergo an escharectomy and a planned autologous meshed STSG to the identified area(s).

The rationale for including subjects with TBSA of 20-50% is that these subjects often have meshed STSG, increasing their risk of wound contraction and scarring. Thus, they could benefit from ADRCs as an adjunctive therapy with the potential for enhancing the engraftment and healing process. These subjects, those between 20% - 50%, also tend to be more hemodynamically stable in that their fluid requirements are less than those with greater wound size area. This relative clinical stability earlier in their initial treatment phase may allow them to better tolerate the lipoaspiration procedure which serves as the source for the ADRCs.

4.3 Sample Size

Up to a total of 15 subjects will be enrolled. Each patient may contribute up to three qualified wound

areas for the analysis. If more than one wound is identified, each must be noncontiguous with the other(s). Therefore, between 15 and 45 wounds will be evaluated. Wound areas that have been treated with a biologic dressing such as Alloderm® or Integra® are not qualified for analysis. However, a wound area that receives excision and grafting as described in this protocol without the application of a biologic dressing may be qualified even if other wound areas in the subject are not qualified because they have been treated with a biologic dressing.

4.4 Clinical Sites

Considering the patient population is expected to have an overall large %TBSA, based on the general prevalence of the this type of burn injuries in the US population, up to ten (10) regional burn centers within the United States will be selected to participate in the RELIEF Trial.

4.5 Study Duration

Enrollment is anticipated to be completed over 18 months. For each patient, the study duration will include a 12 month follow-up period. The total duration of the RELIEF Trial is estimated to be 30 months.

4.6 Study Endpoints

4.6.1 Primary Endpoints

- Safety and Feasibility

Safety will be evaluated based on the incidence, type and seriousness of adverse events related to the IV administration of test substance as well as the low volume lipoharvest procedure. Adverse events will be assessed according to the NIH Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for scale grading adverse events.

Microbiological testing (culture and sensitivity) will be performed on the ADRC treatment dose produced and administered on site.

Feasibility will be determined as the ability to obtain > 100 mL adipose tissue, obtain $\geq$ 20 x 10^6 ADRCs, and deliver the prepared cell dose.

4.6.2 Key Secondary Endpoints

- Percent epithelialization of the graft at time of first dressing change and day 10 (only if wound dressing change planned) and weeks 2, 3 and 4
- Percent take of the graft at time of first dressing change and day 10 (only if wound dressing change planned) and weeks 2, 3 and 4

Digital photography will be performed at surgery and at each follow-up visit for blinded independent review.

Graft take will be assessed as the area over which vascularization is evident and sufficient anchorage to the underlying tissue exists to support epidermal growth. Take will be expressed as a percentage of the area originally covered by the graft. Percent take should be estimated separately for each graft-treated site.

Both of the above endpoints will be evaluated by (a) visual inspection by the treating surgeon, and (b) standardized digital photography and scoring centrally by a blinded trained assessor.

- Percent of group with complete wound closure at each assessment through to 12 weeks

4.6.3 Exploratory Endpoints

- Transepithelial water loss at weeks 2, 3 and 4
- Wound pain VAS at time of first dressing change and day 10 and weeks 2, 3 and 4
- Pruritis VAS at weeks 3, 4, 8, 12, 26 and 52
- Patient and Observer Scar Assessment Scale (v2.0) weeks 3, 4, 8, 12, 26 and 52
- Skin elasticity (Delfin Elastimeter) measurement at 12, 26 and 52 weeks
- Vancouver Scar Scale at 12, 26 and 52 weeks
- Skin pigmentation (Delfin SkinColorCatch) measurement at weeks 12, 26, and 52
- Skin hardness (Delfin Skin Fibrometer measurement) at weeks 12, 26, and 52
- Biomarkers related to burn injury[22] (such as C-reactive protein (CRP), IL-6, IL8, IL1β, interleukin-1 receptor antagonist (IL-1ra); neutrophil gelatinase-associated lipocalin (NGAL); Tumor Necrosis Factor-α (TNFα), and tumor necrosis factor receptor 1a (TNFR-1a)) and related to scarring[23, 24] (such as amino-terminal propeptide of type III procollagen, (PIIINP), TIMP1, MMP2, MMP9 and VEGF-A) will be collected at baseline (prior to treatment), days 6, and 10 and weeks 2, 3, 4, 8 and 12 post ADRC administration. Serum sample will be collected from 4 to 5 ml of whole blood that is centrifuged, separated into 500-µL aliquots, and stored at -80°C at the clinical site serum bank or central lab until use.
- Serum biomarkers will be measured using commercial Enzyme-Linked ImmunoSorbent Assay (ELISA).

4.7 Inclusion and Exclusion Criteria

4.7.1 Inclusion Criteria

Subjects will be enrolled into this study only if they meet ALL of the following criteria:

1. Males or females, ≥ 18 to ≤ 65 years of age
2. BMI > 20 kg/m^2
3. Burn TBSA 20% - 50%
4. At least one deep partial thickness and/or full thickness thermal burn ≥ 250 cm^2 on the arms, legs, back, abdomen or chest that is anticipated to be covered with a meshed autologous STSG and that has not been treated previously with a biologic dressing such as Alloderm® or Integra®
5. Ability to safely undergo adipose tissue harvest procedure that is anticipated to yield ≥ 150 mL of adipose tissue at a site that is free from infection
6. Donor site availability for skin graft harvest

7. Able to provide written informed consent, signed by either the patient or their legally authorized representative
8. Women and men of child-bearing potential agreeing to use contraception during the study. Acceptable methods include surgical sterility, IUDs, hormonal contraception or double barrier methods.

4.7.2 Exclusion Criteria

Subjects will not be enrolled into this study if they meet ANY of the following criteria:

1. Burn > third degree (i.e. involvement of deeper tissues, such as muscle, tendon, or bone)
2. Subjects with electrical or chemical burns
3. Subjects with significant inhalation injuries necessitating intubation and mechanical ventilation or requiring > 50% $FI0_2$ on a continuous basis to maintain oxygenation (0_2 sat > 90%)
4. In the opinion of treating physician, patient not to expected to survive beyond 30 days
5. Pre-existing condition requiring current use of immunosuppressive medication or systemic steroids
6. Pregnant or lactating. Pregnancy as determined by a positive pregnancy test at screening or baseline
7. Known history of HIV infection, or has active Hepatitis B or active Hepatitis C infection.
8. Cancer requiring chemotherapy or radiation within previous 6 months or resection within the last 5 years (other than basal cell carcinoma)
9. Known chronic renal failure (serum creatinine > 2 mg/dL) or chronic liver disease
10. Pre-existing medical conditions that would interfere with wound healing (i.e., diabetic patients with Hemoglobin A1c ≥8.0%, malignancy, autoimmune disease)
11. Subjects with psychiatric conditions that are anticipated to result in protocol noncompliance
12. Chronic illicit drug or alcohol use that is anticipated to interfere with patient compliance to the protocol
13. Participation in another clinical trial within 60 days of screening visit
14. Any concurrent disease or condition that, in the opinion of the investigator, would make the patient unsuitable for participation in the study

5 ENROLLMENT

5.1 Subject Enrollment

Subjects are considered enrolled in this study when all of the following have occurred:

1. Written informed consent is obtained
2. All inclusion criteria and no exclusion criteria have been met
3. All screening evaluations are complete
4. Commencement of fat harvest (except for usual care control subjects who will not undergo a lipoaspirate procedure)

5.2 Randomization and Subject Identification

Randomization to ADRC and usual care will occur within 2 hours prior to the scheduled surgery for STSG.

Randomization will be done via an interactive web response system (IWRS). Subjects will be randomized in a 2:1 ratio to receive intravenous delivery of either Celution® System processed ADRCs with usual care or to usual care alone.

A total of up to 15 subjects will be enrolled. Each patient will contribute up to three qualified wound areas for the analysis. If more than one wound is identified, each must be noncontiguous with the other(s). Therefore, between 15 and 45 wounds will be evaluated.

The 1st 5 subjects enrolled in active treatment will be observed for adverse events over a 7 day period. If no SAEs occur during that period, for that individual subject, the next subject enrolled in active treatment may be entered with the same 7 day observation period. The safety results of the first 5 subjects will be reviewed by the DMC and their evaluation will be used in conjunction with the clinical data collected to date, and if appropriate, to potentially remove the staggering approach to the study. Enrollment after the first 5 subjects enrolled in active treatment may continue only after the DMC has completed its safety review and recommends continuation.

5.3 Stopping Rules

The study will be stopped and patient enrollment will be suspended if any of the following events occur:

1. Acute cardiovascular adverse event occurring within seventy-two (72) hours of the ADRC infusion.
2. Acute allergic or anaphylactic response within seventy-two (72) hours of the ADRC infusion.
3. Acute pulmonary adverse events such as acute respiratory distress syndrome (ARDS) and renal adverse events such as acute renal failure (ARF) within seventy-two (72) hours of the ADRC infusion.

The Sponsor will notify the DMC and FDA immediately for their review and recommendation on study continuation.

5.4 Blinding Procedures and Safeguards

The “blinded party” (photographic assessor(s) with no knowledge of the subjects treatment assignment) are those involved in the independent evaluation of the wound digital photographs.

All others involved in the study are considered the “unblinded parties”, meaning those that will or may have knowledge of a patient’s treatment assignment.

5.5 Subject Withdrawal and Replacement

Subjects may withdraw from the study at any time and for any reason without penalty or prejudice to his or her future medical care. If a subject is prematurely withdrawn from the study for any reason, the reason(s) for withdrawal must be recorded in the Subject’s source documentation and on the eCRF.

The Investigator must make every effort to perform the Early Termination evaluations. This visit shall take place as soon as possible after it is determined that the Subject will be withdrawn from the study and will comprise the observations and procedures scheduled at the next visit.

6 CELUTION® SYSTEM

6.1 Celution® System Description

The Celution® System consists of a stand-alone re-useable hardware unit called the Celution® 800/IV Device with v5.1 firmware with Heater Attachment accessory, the Celution® 805/IV Consumable Set, Celase® and Intravase®. The Celution® System prepares an ADRC output from adipose tissue collected by standard syringe aspiration techniques. The tumescent fluid is composed of: 500 mL of lactated Ringers solution, 20 mL of 1% lidocaine, and 1mg of epinephrine.

Following the collection and preparation of tissue via fat harvest, the adipose tissue is introduced into the device's collection canister in order to wash the adipose tissue and remove deleterious substances (such as cellular debris) (Detailed instructions for use are found in the *Celution® User Manual*).

6.2 Device Characterization and Intended Use

The Celution® 800/IV Device is intended to be used in conjunction with the Celution® 805/IV Consumable Set, Celase and Intravase to digest human adipose tissue in order to further extract, wash, and concentrate adipose derived regenerative cells intended for autologous reinfusion (intravenous) for burn wounds for investigational use in the RELIEF clinical trial.

6.3 Contraindications

1. Non-autologous use
2. Use of any tissues other than adipose

6.4 Intended Clinical Performance

The Celution® System is designed to process lipoaspirated tissue to separate ADRCs from adipose tissue by enzymatic disassociation of the tissue and using buoyancy differences and centrifugation to separate and then concentrate the ADRCs from lipid containing adipocytes. When ADRCs are prepared using the Celution® System, the resultant cell suspension contains all of the components naturally occurring in the native tissue. Preparation of autologous ADRCs for delivery using the Celution® System involves no cell culture. The prepared ADRC dose can be administered to the same patient within four hours.

A percentage viability of $\geq$ 70% of the ADRC output will be used as the acceptance criteria.

The total viable cell count will be performed using a validated system that counts both viable and non-viable nucleated cells (NucleoCounter NC-100® Automated Cell Counting System). This count is used to calculate the total number of live nucleated cells from the entire preparation, to arrive at a quantity of viable cells for treatment of the patient.

6.5 Celution® System Output: Physical, Chemical, and Mechanism of Action

The product of the Celution® System is an ADRC output obtained from autologous lipoaspirate. The suspension of viable ADRCs has been characterized with a average viability of >70% and the average

yield is 3.22 x 10^5 ± 1.48 cells per gram of adipose tissue processed in the Celution® System. An index of regenerative cell potential, the CFU-F assay, shows that 1.51% of those ADRCs form colonies (mean range of 0.13 to 4.08% colony forming units).

The product is a suspension of cells in a diluent. The cellular components of the suspension are made up of nucleated and non-nucleated cells. The non-nucleated cells are red blood cells (RBC) that comprise > 95% of the total cell suspension. The nucleated cells make up approximately 0.1 – 5% of the total cell suspension.

The combination of these four phenotypes will account for approximately 90% of nucleated cells in the product. Table 1 summarizes the ADRC cellular suspension processed by the Celution® System.

Table 1. ADRC cellular suspension components and concentrations

Cell Type	Phenotype	Relative Identity of Nucleated Cell Population
Non-nucleated Cells	Red Blood Cells	
Endothelial Cells	$CD45^-$, $CD31^+$, $CD34^+$	> 2%
Stromal Cells	$CD34^+$, $CD31^-$, $CD45^-$	> 5%
Leukocytes	$CD45^+$, $CD31^-$, $CD34^-$	> 5%
Mural Cells	$CD45^-$, $CD34^-$, $CD\ 31^-$,$CD146^+$	> 2%

6.6 Proposed Mechanism of Action of ADRCs

The proposed mechanism of action of the ADRCs prepared with the Celution® System is postulated to be modulation of inflammation, promotion of angiogenesis and prevention of cell apoptosis. Studies suggest that ADRC therapy changes the inflammatory response and augments the regenerative response starting early in the injury process[2-4, 8, 11, 12]. Studies have detected significant amounts of paracrine growth factors, which have been shown to facilitate vascularization, and factors associated with anti-inflammatory effects, thereby ameliorating injury by pro-inflammatory cytokines[8]. This has been demonstrated to repair healing impaired, deep, and chronic wounds[8, 9, 25-27]. Viability characterization of ADRCs as described above, in addition to pre-clinical animal data from Cytori and other published reports, support this hypothesis.

7 STUDY TREATMENTS

Subjects randomized to ADRCs will receive active treatment (intravenous infusion of 20 × 10^6 ADRCs into a peripheral vein). Subjects randomized to the control group will not undergo fat harvest and not receive any study treatment. All subjects will continue their usual care treatment.

7.1 Product Description - ADRCs

Lipoaspirate will be obtained through a low volume lipoharvest (100-150 mL) which will be performed at the start of general anesthesia for the scheduled burn surgery. The lipoaspirate will be processed using the

Celution® System to produce ADRCs. The ADRCs will be delivered via intravenous approximately within 4 hours of Celution processing.

The adipose tissue is introduced into the device's collection container in order to wash and process the tissue. Based on a fat harvest of 100-150 mL, the expected cell output would be approximately 20 to 40 million cells. The target dose for the study is 20 million cells.

The excipients in the cell suspension are composed of residual Celution® System reagents and diluent. Specifically, the residual reagents are collagenase (<0.005 Wünch Units/mL) and DNase (<100 Kunitz Units/mL). The diluent is Lactated Ringers solution (USP).

Adipose tissue processing is detailed in the *Celution® System Users Manual*. Preparation of the active study treatment is provided in *the Study Reference Manual*.

7.1.1 Treatment Dose Control

ADRC count and viability will be determined using a validated system that counts both viable and non-viable nucleated cells (NC-100 NucleoCounter® Automated Cell Counting System, Enfield, Connecticut, USA) using the following criteria:

- Nominal cell count: 20×10^6 ADRCs
- A minimum cell count within 15% of the nominal cell count is required (i.e., a dose as low as 17×10^6 ADRCs is acceptable if 20×10^6 ADRCs is not available)
- Cell viability: ≥ 70%
- If cell viability is < 70%, as determined by the NucleoCounter® Automated Cell Counting System, the subject will not be treated but will be followed per study protocol for safety.
- Negative result in Gram stain testing (see *Section 7.2*)
- If the Gram stain testing result is positive, the subject will not be treated but will be followed per study protocol for safety.

7.2 Study Treatment Sterility Testing

Following cell processing in the Celution System sterility testing will be performed as described below and more fully in the *Study Reference Manual* and the *Cell Preparation Manual*.

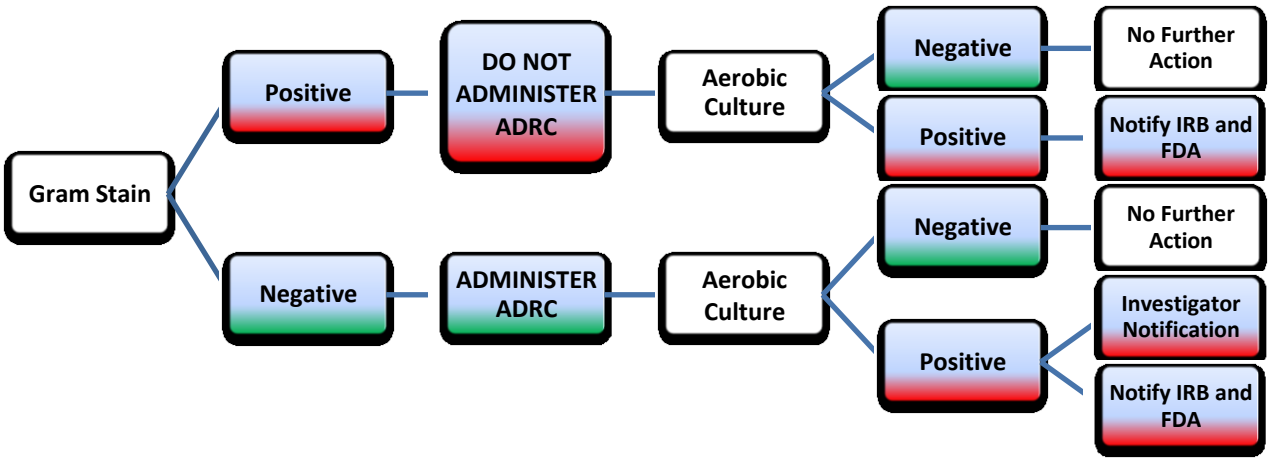
A negative Gram stain test result is required prior to injections of ADRCs. If the sample is determined to be positive for bacteria then the subject should not receive any study treatment, but will be followed per study protocol for safety.

Further testing (aerobic bacteria culture (e.g. BacT Alert or BacTec)) will be performed in order to test for the presence of any low level microbial contamination that might not be apparent in the gram stain test. Should a culture be deemed positive for microbial activity, the investigator will be immediately notified. The study patient will be evaluated and a course of treatment and/or antibiotics may be administered, as determined by the study investigator. The source of the contamination will be evaluated as appropriate. If the culture test indicates potential bacterial contamination of the study treatment and the clinical follow up

indicates potential or actual infection of the subject, the FDA and IRB will be notified within 15 days of the Sponsor being made aware of any positive test results.

The following flow chart describes the activities associated with positive or negative outcomes in these two tests of sample sterility.

Figure 1: Sterility Testing of ADRC Samples



7.3 ADRC Characterization

Additional ADRC phenotypic characterization beyond the cell counting and bacterial testing required for dose preparation may be performed for subjects for whom ADRCs in excess of the number needed for dosing, cell counting, and bacterial testing are available. Such testing should be performed according to methods developed by Cytori. Flow cytometry methods will be used to determine ADRC cellular composition[28]. Particularly, detailed characterization will be performed on left over cells once the cell dose preparation has been prepared for the injection procedure. Within the ADRC population, cells will be identified phenotypically by combination of the following markers: CD45, CD34, CD31 and CD146.

Once the cell dose is prepared according to criteria described in *Section 7.1.1*, any excess ADRCs may be stained and fixed at the clinical site using antibodies directed to CD31, CD34, CD146 and CD45 proteins according to instructions in the *Study Reference Manual (Cytori Work Instruction)*. Samples will be then shipped to the testing facility within 92 hours.

Based on the expression of these four markers, the major populations will be defined as follow:

Leukocytes	CD45+
Endothelial Cells	CD34+/CD31+/CD45-
Stromal Cells	CD34+/CD31-/CD45-
Mural cells	CD34-/CD31-/CD45-/CD146+

Other markers (such as CD206, CD14, CD90, CD73, CD105 and CD44 or other related markers) may be used to further characterize ADRCs subpopulation.

8 STUDY ASSESSMENTS

All parameters will be conducted at specific time points as outlined in this section. As a reference point during the study, a Schedule of Procedures is provided in *Appendix A*.

8.1 Medical History, Physical Examination & Vital Signs

A complete medical history will be collected at Screening and will include demographic information, current and past medical conditions and a summary of the burn event. Any adverse event that occurs after the ICF is signed and prior to the fat harvest is to be recorded as an update to the Medical History.

Physical examinations should be based on the following body systems: general appearance, head (ear, eyes, nose and throat), respiratory, abdomen, musculoskeletal, neurological, and skin, including total burn surface area (TBSA) on admission, percent of third degree burns, and other trauma related to the burn event. Vital signs to be obtained include the following:

- Blood pressure (systolic and diastolic; mm/Hg)
- Heart rate (beats per minute)
- Body temperature (°C)
- Respiration rate (per minute)

8.2 Adverse Events

Adverse events will be assessed throughout the study according to the NIH Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for scale grading adverse events.

8.3 Burn Evaluations

The physical characteristics of the selected burn areas and the graft (up to 3 per subject) as well as the subjective evaluation of symptoms will be evaluated using several assessment tools. The assessment tools are listed below and detailed information and instructions provided in the *Study Reference Manual*. Planned study days are listed below but an acceptable range of days (±) is in the schedule of events.

- Percent epithelialization at early dressing changes (estimated time of first dressing change and day 10 after graft application and at weeks 2, 3 and 4 (by surgeon evaluation and blinded independent review of standardized photographs)
- Percent take of the graft at early dressing changes (estimated time of first dressing change and day 10 after graft application (if wound dressing must be changed) and at weeks 2, 3 and 4
- Occurrence of complete wound healing at each assessment weeks 4 to 12
- Transepithelial water loss at weeks 2, 3 and 4
- Wound pain VAS at time of first dressing change and day 10 and weeks 2, 3 and 4
- Pruritis VAS at 3, 4, 8, 12, 26 and 52 weeks
- Patient and Observer Scar Assessment Scale (POSAS) at 3, 4, 8, 12, 26 and 52 weeks
- Vancouver Scar Scale at 12, 26, and 52 weeks
- Skin elasticity measurement at 12, 26 and 52 weeks (Delfin SkinElastimeter)
- Skin pigmentation (Delfin SkinColorCatch) measurement at weeks 12, 26, and 52
- Skin hardness (Delfin SkinFibrometer) measurement at weeks 12, 26, and 52

- Percent of wounds with complete wound closure at weeks 2, 4, 8 and 12
- Digital photography of treated wounds on day 1 and each follow up visit
- Biomarkers related to burn injury will be assessed from 4-5mL blood (serum) samples drawn at baseline (prior to treatment), time of first dressing change and day 10 and weeks 2, 3, 4, 8 and 12 post-ADRC administration.

8.4 Prior and Concomitant Medications

Any medication a subject receives is considered a concomitant medication, and must be recorded in the subject's source documentation throughout the study. All concomitant medications will be recorded in the eCRF starting at screening through the 52 week visit. Information to be recorded on the eCRF will include: generic medication name, route of administration, dosage, indication, start date, and stop date.

Medications used as part of the tissue harvest procedure and the escharectomy/grafting surgeries do not need to be recorded in the eCRF but should be recorded as part of the operative records.

8.4.1 Prohibited Medications

Subjects should not receive the following medications for the duration of the study:

- Any other investigational drug or agent
- Any chemotherapy or immunosuppressive medication
- Systemic steroids exceeding prednisone 10 mg daily (or equivalent) for periods exceeding 4 weeks
- Any anticoagulant administered for systemic anticoagulation that would contraindicate a lipoaspiration procedure. Subcutaneous heparin or low molecular weight heparin for deep vein thrombosis prophylaxis is acceptable
- A substantial anticoagulant such as a Glycoprotein IIb/IIIa inhibitor/anti-platelet class drug was administered within 2 weeks prior to fat harvest (such as ReoPro, Integrilin or Aggrastat)

If treatment with any prohibited medication becomes necessary during study participation, the subject will be followed; however, the subject would not be included in the PTE population.

8.5 Laboratory Procedures

All laboratory assessments will be performed locally at each center's laboratory by means of their established methods (using site specific laboratory normal values). Blood samples should be taken using standard venipuncture techniques according to the laboratory guidelines. The approximate total blood volume that will be taken for the laboratory and biomarker assessments will be 50 to 60 mL.

8.6 STUDY VISIT REQUIREMENTS

The Schedule of Procedures is outlined in Appendix A.

8.6.1 Day -2 to Day -1: Screening

Within 48 hours prior to scheduled excision and STSG surgery, subjects will undergo the following screening assessments:

- Written informed consent from the patient or their legally authorized representative

- Age, gender, height and weight
- Inclusion and exclusion criteria evaluation
- Medical history
- Concomitant medications
- Physical examination
- Vital signs
- Biomarkers
- Laboratory tests
    - CBC with platelets
    - INR, PTT
    - Electrolytes, alkaline phosphatase, AST, ALT, bilirubin, total protein, albumin, BUN, creatinine, and, for patients with diabetes only, evaluation of HbA1c levels
    - Blood draw (4-5mL) to obtain serum for subsequent testing for burn biomarkers
- Pregnancy test for female subjects
- Evaluation of suitability and probable body location for fat harvest by the surgical specialist who will perform the lipoaspirate procedure

Enrollment will occur by a designated staff following confirmation by the treating surgeon that the patient is to proceed with a skin graft from the patient's donor site. Enrollment will occur within 2 hours of the scheduled procedure.

8.7 Procedure Day (Day 1)

The following assessments are to be conducted:

- For All Subjects
    - Randomization
    - Vital signs
    - Assessment for adverse events
    - Digital photography of wounds
    - Laboratory tests
        - CBC with platelets
        - INR, PTT
        - Electrolytes, alkaline phosphatase, AST, ALT, bilirubin, total protein, albumin, BUN, creatinine

- For Subjects Randomized to Treatment Arm only
    - Low volume fat harvest
    - Microbiology testing of dose samples (ADRCs) at baseline (prior to ADRCs administration)
    - ADRC dose preparation and delivery

8.7.1 Fat Harvest

8.7.1.1 General Requirements

- Fat harvest will be performed at the start of general anesthesia for the planned excision and STSG

- In order to ensure that surgical resources for standard of care escharectomy are not diverted to the fat harvesting procedure, fat harvest will be performed by a qualified surgeon not otherwise involved in the burn surgery at that time.
- Standard tissue calipers or expert surgical specialist examination should be used to assess possible body sites for subcutaneous fat tissue. Any subject with an active skin infection at the site of lipoharvest will be excluded from the study
- Fat harvest should NOT be performed if an anticoagulant has been administered within 1 hour prior, a substantial anticoagulant such as a Glycoprotein IIb/IIIa inhibitor/anti-platelet class drug was administered within 2 weeks prior (such as ReoPro, Integrilin or Aggrastat), or if there is an abnormally high INR or PTT

Operative details such as start and stop time, weight of lipoaspirate, dose preparation time will be recorded on the appropriate eCRF.

8.7.1.2 Fat Harvest Procedure:

A specific set of requirements has been established for the collection of fat that can then be used for the successful preparation of ADRCs using the Celution® technology. Therefore, the following conditions and procedures are to be used. Additional information will be provided in the Celution® Instructions for Use:

- Syringe-based fat harvest is required
- A total volume of 100-150 mL of adipose tissue must be harvested
- Symmetric fat removal is required, keeping the overall area of aspiration as small as possible
- The abdominal region is the preferred site for fat harvest, however multiple sites should be evaluated and prepared and non-abdomen regions (e.g., inner thigh) used if abdomen not available
- Make an approximately 0.5 cm stab skin incision
- Infiltrate tumescent solution using a 14 gauge, up to 30 cm, blunt LAMIS™ infiltrator for tumescent fluid administration ; tumescent fluid should be infiltrated at a ratio of approximately 1:1 to 3:1 of tumescent solution to fat to be harvested
- The tumescent fluid formula to be used is:
  - 500 mL of Lactated Ringers solution
  - 20-50 mL of 1% Lidocaine
  - 1 mg of epinephrine
- To sufficiently induce vasoconstriction/hemostasis during the tissue harvest procedure a minimum of 100 mL of tumescent fluid must be used. Estimated volume should generally be limited to 500 mL
- Approximately 10 minutes after the tumescent fluid injection, the adipose tissue is harvested through the same incision, using an up to 32 cm long, 3-5 mm inner diameter Toomey cannula with a Mercedes standard tip attached to a sterile 60 mL Toomey syringe
- After the first syringe is filled (complete filling is not required) it is detached from the cannula, capped with a sterile cap and set aside; additional syringes are filled until the desired amount of fat is harvested
- Fat harvest will be limited to areas where tumescent fluid has been infiltrated
- Vital signs are monitored during tissue harvest procedure
- Close surgical incision as per the surgeon's standard procedure. Apply pressure bandage immediately upon conclusion of tissue harvest

8.7.1.3 Fat Harvest Stopping Rules

The physician performing the fat harvest procedure should continuously monitor the patient for signs of significant bleeding from the puncture site(s) and overall hemodynamic stability. The tissue harvest procedure should be terminated if any of the following occur:

- Drop in systolic blood pressure < 90 mm/Hg requiring pressor support
- Profuse or uncontrollable bleeding from the puncture site(s)
- Rapid expansion of subcutaneous space at the fat cell tissue harvest site(s) related to bleeding into the subcutaneous space
- Hemoperitoneum or pneumopertineum
- Hemothorax or pneumothorax
- Evidence of perforation of a vascular structure that requires ligature or surgical control
- Evidence of perforation into abdominal cavity, peritoneum or gastrointestinal organ

Should any of the above occur, the fat harvest procedure should be terminated and the patient treated according to standard of care. DO NOT PROCEED WITH ADRCs ADMINISTRATION. Complete the study specific SAE form and notify the sponsor within 24 hours.

8.7.2 Digital Photography

Digital photographs of the target wounds must be taken shortly after application of the STSG. Images of wounds will be taken by using the Silhouette Star System in accordance with the detailed work instruction provided in the Study Manual. Digital photographs will be taken at a fixed distance of approximately 30 - 50 cm from the wound. A reference image to identify the location of the target wound(s) with reference to anatomical landmarks should also be captured at the time of grafting. Prior to imaging, a label (with date, Subject number, and wound identification) and ruler (to indicate the longest length and width) will be placed near each wound site (within the camera's field of view) to track and identify each image. Images will be captured from directly above the wound (approximately perpendicular to the center of the wound surface) and at an oblique angle (approximately parallel to the center of the wound surface) to show scar height above adjacent skin. For the oblique images, a scale will be placed within the frame and immediately adjacent to the wound and perpendicular to the scar to allow assessment of scar height.

8.7.3 ADRCs Delivery

If subjects become hypotensive and require vasopressors to maintain organ perfusion between the collection of lipoaspirate and the intravenous introduction of ADRCs DO NOT PROCEED WITH ADRCs ADMINISTRATION.

Sterile technique should be maintained throughout the procedure. The intravenous ADRCs infusion will be by administered by peripheral intravenous administration. An intravenous catheter (20 or 22 gauge) will be used to administer the ADRCs. The 5 mL output from the Celution System will be diluted to achieve 10 mL lactated Ringer's solution containing 20 x 10^6 ADRCs in a sterile syringe. The syringe will be attached to the sterile macro (part # or designation) syringe filter and the contents of the syringe will be injected into the catheter slowly. The catheter will be flushed with 5 mL of lactated Ringer's solution from a separate sterile syringe.

8.8 Post-ADRC Administration time of first dressing change and Day 10 (±2 days)*

The following assessments are to be conducted:

- Physical examination
- Vital signs
- Assessment for adverse events
- Concomitant medications
- Biomarkers
- Laboratory tests (time of first dressing change only):
  - CBC with Platelets
  - Electrolytes, alkaline phosphatase, AST, ALT, bilirubin, total protein, albumin, BUN, creatinine

The following assessments, tests and questionnaires should be conducted in the order listed below (as applicable to each visit) and prior to the other listed tests:

- Percent epithelialization of the graft (surgeon evaluation)
- Percent take of the graft**
- Digital photographs of wound with ruler to indicate the longest length and width
- Wound pain VAS

*Day 10 assessments performed only if wound dressing is being changed between days 8 and 12.

**Graft take will be assessed as the area over which vascularization is evident and sufficient anchorage to the underlying tissue exists to support epidermal growth. Take will be expressed as a percentage of the area originally covered by the graft. Percent take is estimated separately for each of the grafted study sites.

8.9 Post-ADRC Administration Follow-up visits: Weeks 2 (±3 days), 3 (±3 days) and 4 (±3 days)

The following assessments are to be conducted:

- Physical examination
- Vital signs
- Assessment for adverse events
- Concomitant medications
- Biomarkers

The following assessments, tests and questionnaires should be conducted in the order listed below (as applicable to each visit):

- Percent epithelialization of the graft
- Percent take of the graft
- Transepithelial water loss
- Digital photographs of wound with ruler to indicate the longest length and width
- Occurrence of complete wound closure

- Wound pain VAS
- Pruritis VAS at Weeks 3 and 4
- Patient and Observer Scar Assessment Scale (POSAs) (Weeks 3 and 4 only)

8.10 Post-ADRC Administration Follow-up visits: Weeks 8 (±7 days), 12 (±7 days), 26 (±14 days), and 52 (±21 days)

The following assessments are to be conducted:

- Physical examination
- Vital signs
- Assessment for adverse events
- Concomitant medications
- Biomarkers (Weeks 8 and 12 only)

The following assessments, tests and questionnaires should be conducted in the order listed below (as applicable to each visit):

- Digital photographs of wound with ruler to indicate the longest length and width
- Occurrence of complete wound closure (Weeks 8 and 12)
- Pruritis VAS
- Patient and Observer Scar Assessment Scale (POSAS)
- Vancouver Scar Scale (Weeks 12, 26 and 52 only)
- Skin elasticity (Delfin SkinElastimeter) measurement (Weeks 12, 26 and 52 only)
- Skin pigmentation (Delfin SkinColorCatch measurement) at Weeks 12, 26, and 52
- Skin hardness (Delfin SkinFibrometer measurement) at Weeks 12, 26, and 52

A summary table of all follow up evaluations is outlined in *Appendix A*: Schedule of Procedures.

8.11 Early Termination Evaluation

Subjects that discontinue early from the study, unless consent is withdrawn, shall have an Early Termination (ET) evaluation. This visit shall take place as soon as possible after it is determined that the Subject will be withdrawn from the study.

The observations and procedures for the ET evaluation are the same as those required at the Week 52 visit.

The reason for early withdrawal must always be clearly documented in the Subject's source documentation. Contact with the Subject must be attempted to determine overall health status, and contact details must be documented in the Subject's source documentation. If lost to follow-up, details of three contact attempts followed by a certified letter must be documented in the Subject source documentation.

9 RISK/BENEFIT ANALYSIS

9.1 Context of the proposed investigation:

Wound healing is a complex multifactorial process involving the interaction of inflammation, granulation tissue formation, re-epithelialization, and angiogenesis. For deep partial thickness and full thickness burns, acute burn management involves multiple activities including protecting the wound from further injury, initial cleansing and debridement and determination of dressings with or without a topical antimicrobial agent. Fluid resuscitation, prevention of hypothermia and pain management are also critical aspects of management. Surgical procedures involve escharectomy with further debridement and determination of skin grafting (often autologous split thickness skin graft (STSG) or a temporary graft (allograft or xenograft) or a tissue engineered substitute such as Integra®). The use of a tissue engineered substitute/matrix or temporary graft is generally followed by an autologous skin graft. Maturation of the graft may take several weeks and, depending on the severity and location of wound and the degree of meshing required for a STSG, the cosmetic outcome is often unsatisfactory. In addition, complications such as infection or contractures may occur, which can result in further treatments and procedures, as well as prolongation of healing. There is a need to develop improved techniques in acute burn management[1]

Adipose Derived Regenerative Cells (ADRCs) are of emerging interest in the application of wound healing. Cells obtained from adipose tissue have been shown to inhibit inflammation[2-5] , promote angiogenesis[5-7, 7-13], stimulate recruitment of endogenous regenerative cells[14, 15] and promote salvage of damaged cells[2, 5, 16]. On the basis of these properties, several preclinical studies have been performed using freshly isolated and cultured adipose-derived cells in a number of animal models of normal and impaired wound healing. The data consistently show improvement of epithelialization and wound healing. ADRCs are an advantageous resource because they can be found in abundant quantities, they can be harvested with a minimally invasive procedure[17, 18], and they can be safely and effectively transplanted into an autologous host.

The Celution® System is designed to process lipoaspirated tissue to separate ADRCs from adipose tissue by enzymatic disassociation of the tissue and using buoyancy differences and centrifugation to separate and then concentrate the ADRCs from lipid containing adipocytes. When ADRCs are prepared using the Celution® System, the resultant cell suspension contains all of the components naturally occurring in the native tissue. Preparation of autologous ADRCs for delivery using the Celution® System involves no cell culture. The prepared ADRC dose can be administered to the same patient within four hours.

This study is a prospective, open-label, parallel group, usual care controlled, multi-center randomized (2:1, active: usual care alone) safety and feasibility study targeting thermal burns. Subjects will have at least one deep partial or full thickness burn wounds of $\geq$ 250 cm^2 that is to be autografted with a split thickness skin graft (STSG). Subjects randomized to ADRCs will undergo small volume fat harvest (100 to 150 mL) performed at initiation of general anesthesia for scheduled burn surgery followed by intravenous delivery of ADRCs within 4 hours following completion of Celution processing. The lipoaspirate will be processed in the Celution® System to isolate and concentrate ADRCs.

Following informed consent and initial screening assessments, eligible subjects will undergo pre-operative testing. On the procedure day, subjects will be randomized to ADRCs (with usual care) or usual care alone. Low volume lipoharvest will only be performed on subjects randomized to ADRCs in order to obtain 100-150 mL lipoaspirate, which will then be transferred to the Celution® System for processing to

isolate and concentrate ADRCs for same-day administration. All treatment will be delivered in a total volume of 10 mL which will be delivered by slow intravenous administration.

Following surgery, subjects will be evaluated at several intervals up to 52 weeks to assess safety and efficacy as measured by direct measurement of wound healing.

9.2 Assessment of risks of the proposed Investigation

The areas of risk include those of adipose tissue harvest and ADRCs delivery.

9.3 Risks

The primary risks associated with adipose tissue harvest are bleeding and infection associated with the removal of the adipose tissue (fat harvesting procedure) along with the potential risk of infection associated with intravenous delivery of ADRCs.

Tissue harvest by syringe-based manual aspiration has been used as a source for ADRCs in the 344 subjects in 7 clinical trials with the Celution system. Adverse events were those commonly associated with liposuction such as discomfort and ecchymosis. The only related serious adverse event reported was a single event of rectus sheath hematoma which was associated with the use of a glycoprotein IIb/IIIa inhibitor. The patient had unremarkable recovery. Fat harvest has been demonstrated to be feasible and is associated with an acceptable safety profile. The volume of adipose tissue removed in these studies generally varied between 150 mL and 450 mL. In the proposed feasibility and safety trial in burn patients a target volume between 100 to 150 mL of adipose tissue is to be removed and processed for ADRCs.

9.4 Intravenous Delivery Risk Analysis and Management

Risks involved with intravenous delivery of ADRCs cells include:

- Embolism
- Infection

The preclinical studies described herewith demonstrate no evidence of serious adverse events associated with ADRCs delivered intravenously, topically, subcutaneously, or intraarticularly. The clinical trial studies also have not reported significant safety issues when ADRCs are delivered subcutaneously, intraarticularly or intra-arterially. As in other trials applying intravascular administration of ADRCs, the ADRC product will be passed through a 47µm filter as part of the infusion process in order to further minimize risk of embolism. However, the possibility of infection exists with any percutaneous procedure. However, current experience in multiple clinical studies has not revealed any patient infections association with ADRCs delivery.

To mitigate potential risk of infection a gram stain of the ADRCs doses will be performed and must be negative in order to proceed with intravenous administration. A sample will be sent to the hospital laboratory for BacT (or equivalent) culture including aerobic microorganisms and sensitivity. All procedures related the Celution® System and cell counting are performed in aseptic conditions. If a culture result is positive for microbial activity, the investigator will be immediately notified. The study patient will be evaluated and standard clinical treatments of infection for patients with burn wound injury should be followed including antibiotics as deemed appropriate by the study investigator.

9.5 Celution® System and Delivery Device Risk Analysis and Management

The investigators and all study personnel will be trained in the details of all aspects of the study procedures relevant to their particular area of responsibility.

- Train investigators and all study personnel in the details of the operation of the Celution System and Cytori Work Instructions for ancillary procedures and devices such as cell counting and intravenous delivery.
- Inspect the package integrity and sterility indicators on all disposables used with the devices and maintain sterility of the fluid paths.
- Follow Instructions for Use for the Celution® System, including all warnings, precautions and contraindications.

9.6 Fat Harvest Risk Analysis

The risks of standard small volume liposuction in healthy patients are known and are minimal and recovery is usually fast. Following standard liposuction the following effects can be expected:

Common

- Edema and redness of the liposuction areas for a few days
- Moderate contusion of the liposuction areas for a few months

Uncommon

- Persistent edema
- Persistent bleeding
- Hematoma
- Visible puncture sites

Rare

- Infection
- Deep Vein thrombosis (DVT)
- Persistent bleeding requiring transfusion

9.6.1 Fat Harvest Risk Management

The Investigators and all study personnel will be trained in the details of all aspects of the study procedures. Standardization of fat harvest techniques is critical to a good clinical outcome. To minimize risks and increase the chances of a favorable clinical outcome the following measures are required for the liposuction procedure:

- Performed by an appropriately licensed and credentialed physician
- Monitoring of vital signs throughout the procedure
- Sterile technique is followed throughout
- The adipose tissue is handled gently at all times
- HGB, HCT prior to and 1-2 hours post-procedure

- To sufficiently induce local vasoconstriction/hemostasis during the fat harvest procedure, a minimum of 500 mL and a recommended maximum of 2000 mL of tumescent fluid is used containing:
  - 500 mL of Lactated Ringers solution
  - 20-50 mL of 1% Lidocaine
  - 1 mg of epinephrine
- A blunt 14 to 16 gauge LAMIS infiltrator (or equivalent) will be used for infiltration with tumescent solution
- After approximately 10 minutes, the adipose tissue may be harvested through the same incision, using the aspiration cannula attached to a syringe
- A blunt cannula with a minimum inner diameter of 3 mm is preferred for lipoaspiration.
- Lipoaspiration is done manually using a syringe (60 mL Toomey syringe is preferred); no machine aspiration is permitted.
- Monitoring for prevention or early detection of bleeding:
- Enrollment of only patients who qualify per the inclusion / exclusion criteria defined in the protocol) including screening blood work
- Strict adherence to the study protocol
- Standard of care for the monitoring and treatment of subjects who experience any adverse event for liposuction
- Target volume of adipose tissue is limited to approximately 100-150 mL
- Close surgical incision with appropriate suture
- Apply pressure bandage immediately upon conclusion of tissue harvest

Fat harvest should NOT be performed on patients who have received any anticoagulant within 1 hour of the procedure, substantial anticoagulation (eg: GIIb/IIIa inhibitor class drugs) within two weeks prior to fat harvest, or who have an abnormal PTT.

9.7 Overall Study Risk Management

The following measures implemented by Cytori will minimize the risks associated with patients' participation in clinical studies:

- All examinations, treatment procedures, and interpretation of clinical data generated during the study is directed, overseen and analyzed by an appropriately licensed and credentialed physician who has been trained to the clinical study protocol.
- Protocol and training programs to ensure that the study personnel at Cytori and at the clinical sites have a strong knowledge and understanding of the clinical protocol, including patient selection criteria and procedure requirements.
- Investigators are trained to the procedure requirements in the protocol, and the Instructions for Use for the Celution® System.
- Clearly defined inclusion and exclusion criteria to ensure that only properly selected patients will be enrolled in the clinical study.
- The protocol is designed so that patient treatment and follow-up procedures will be consistent with those of the clinically established standard of care.

9.8 Assessment of the Benefits of the Proposed Investigation

This clinical investigation is designed as a safety and feasibility study. Therefore, it is not known at this time whether any direct subject benefit can be expected.

Patients with significant thermal burn injury typically require, on average, approximately 1 to 1.5 days of in-hospital care for each 1% of burned TBSA. Burn treatment involves labor-intensive procedures over a long period of time. For the patient, this includes substantial pain and morbidity from repeated excisions and harvest of skin autografts. Currently available preclinical data indicate that ADRC treatment may improve the quality of granulation tissue formed following escharectomy, promote more rapid re-epithelialization of the burn wound and reduce hypertrophic scar formation. These effects have the potential to improve the efficiency of integration of an autologous STSG into the wound site (commonly referred to as 'graft take'). They also have the potential to accelerate the rate at which the meshed component of the skin graft fills in with new epithelium. Earlier wound closure and improved graft take will likely lead to reduced risk of infection, shorter duration of stay, reduced rate of hypertrophic scarring formation and contracture, and reduced need for repeat procedures such as re-grafting and subsequent procedures to address hypertrophic scarring.

9.9 Consideration of Patient Preference information

None can be provided at this time.

9.10 Assessment of Uncertainty

The key source of the uncertainty in the available evidence is the safety of the procedure in burn wound subjects and the level of potential effect on wound healing and the improvement in the clinical course of the subject. The safety profile of liposuction in subjects with burn wounds has not been established. Liposuction, in order to obtain ADRCs via the Celution system, has been used in seriously ill subjects in several studies that evaluated the effects of ADRCs on congestive heart failure and in subjects with an acute myocardial infarction (MI) within a 72 hour window post MI. No adverse events occurred secondary to liposuction other than the one subject with a rectus sheath hematoma whose had received a glycoprotein IIb/IIIa inhibitor. The procedure of low volume liposuction appears to be tolerated in subjects with significant myocardial decomposition. The safety of the subjects in this trial will be evaluated within a staggered enrollment with safety evaluations as well as by a Data Monitoring Committee (DMC).

The extent of benefit to subjects is unknown as no other clinical trial using intravenously administered ADRCs in the treatment of burn wounds has been done. Preclinical data generated in animal models of burn wounds suggests that wound healing should be accelerated.

9.11 Conclusions

The overall risk benefit profile with the proposed safety assessment measures makes the balance of risk to benefit sufficient to proceed with this clinical trial.

10 STATISTICAL METHODS AND DATA ANALYSIS

The RELIEF Trial is a multi-center, open-label, randomized, usual care controlled study to evaluate the feasibility and safety of ADRCs with usual care compared to usual care alone in subjects with thermal burns. The primary endpoint will be safety and feasibility.

The ITT population will be used for the primary analysis; the analysis will also be presented in the PTE population.

10.1 General Considerations

All statistical tests will be two-sided and statistical significance will be assessed with respect to a nominal p-value <0.05. Prior to the data base lock, a separate statistical analysis plan (SAP) will be finalized. Any deviations from the planned analyses will be documented and justified in the final clinical/statistical study report. The SAP will detail all planned analyses and will serve as a statistical programming requirements document.

10.2 Determination of Sample Size

The objective of the pilot study is to evaluate the safety and feasibility of autologous Adipose-Derived Regenerative Cells (ADRCs) for the treatment of thermal wounds. The sample size of up to 15 subjects (each subject contributing up to 3 wounds to the dataset) is not powered to test specific hypotheses. The sample size is proposed to provide preliminary safety and feasibility data in order to design a larger study with sufficient power to test a hypothesis regarding benefits of ADRCs.

10.3 Data Analyses

Safety and efficacy endpoints will be summarized by treatment group using descriptive statistics (n, mean, SD, median, minimum and maximum), for quantitative variables and frequencies and percentages for categorical variables.

For quantitative efficacy variables, the change from baseline to each post-baseline time point will be analyzed using Student's t-test. For each endpoint, the treatment groups will be compared using two-sided tests at the alpha=0.05 level of significance.

The final dataset includes grafts with different mesh size (for example; 2:1 and 3:1). While the study is not powered for analysis of efficacy, data analysis for comparison between subjects receiving usual care and those receiving usual care plus ADRCs with respect to exploratory endpoints associated with healing (e.g.: percent epithelialization, pigmentation, hardness, Vancouver Scar Scale) will be stratified by mesh size (for example: 2:1, 3:1, 4:1, and all grouped).

Subjects randomized to the ADRC-treatment arm who undergo liposuction but who are not treated with ADRCs because the ADRCs do not meet release criteria may be followed up for the full duration of the study. Such subjects will be (a) included in the full data analysis for feasibility, (b) analyzed separately for safety, (c) analyzed in the ITT group for efficacy, and (d) excluded from the PTE group for efficacy.

10.3.1 Primary Endpoint Analysis

The primary endpoints for the study are safety and feasibility.

Safety endpoint will be summarized by treatment group using quantitative and descriptive statistics of adverse events. Adverse events will be assessed according to the NIH Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for scale grading adverse events. Related adverse events will be analyzed separately for those related to ADRCs and to the delivery of ADRCs (i.e. intravenous delivery), and for those related to tissue harvest.

Feasibility will address the following:

- Ability to perform tissue harvest and obtain 100-150 mL of adipose tissue
- Completion of dose preparation with the target dose
- Ability to deliver ADRCs via intravenous administration
- Successful secondary endpoint evaluations

10.3.2 Key Secondary and Exploratory Endpoint Analysis

The key secondary endpoints are

- Percent epithelialization
- Graft take
- Proportion of population with complete wound closer

Epithelialization will be assessed by surgeon visual evaluation and blinded independent review of standardized digital photographs. Graft take will be assessed as the area over which vascularization is evident, and sufficient anchorage to the underlying tissue exists to support epidermal growth. Take will be expressed as a percentage of the area originally covered by the graft. Percent take is estimated separately for each of the grafted study sites. The proportion of subjects with complete wound healing will be determined and is defined as skin re-epithelialization without drainage or dressing requirements.

All other secondary endpoints will be treated as exploratory. P-values for each analysis will be computed separately, without any corrections for multiple comparisons. These analyses will be considered as part of the totality of the evidence concerning the safety and effectiveness of the product.

10.4 Background, Demographic and Concomitant Medication Analyses

Demographic data, medical history, concomitant disease and concomitant medication will be summarized by means of descriptive statistics (n, mean, SD, median, minimum and maximum) or frequency tables.

10.5 Withdrawal, ITT, PTE

Reasons for withdrawal pre- and post-randomization will be summarized. The treatment analyses will be performed on intent-to-treat basis as well as on a per-treatment-evaluable basis, as defined below:

Intent-to-Treat (ITT): All randomized subjects; randomization will occur on procedure day prior to the tissue harvest procedure. Subjects will be included in the analysis according to the treatment assigned if population is different than PTE.

Per-Treatment-Evaluable (PTE): All enrolled subjects who have day 5 follow-up information available.

10.6 Handling of Missing Data

Given the relatively small sample, missing data will not be imputed.

10.7 Protocol Deviations

Deviations from the protocol, including violations of inclusion/exclusion criteria, will be assessed as "minor" or "major" by the Sponsor. Deviations will be defined prior to un-blinding.

10.8 Data Monitoring Committee

The independent Data Monitoring Committee (DMC) will be comprised of burn specialists and a biostatistician who are not participants in the study. The DMC will be provided with all safety data as well as efficacy data for the primary endpoint in order to fully evaluate the benefit to risk profile of the study procedures. The DMC will be responsible for the review of the data and generation of recommendations on the conduct of the trial.

The DMC will be asked to review data and generate recommendations after 5, 10, and 15 subjects have at least 1 week of follow-up. Enrollment after the first 5 subjects enrolled in active treatment may continue only after the DMC has completed its safety review and recommends continuation.

11 ADVERSE EVENTS / SERIOUS ADVERSE EVENTS

11.1 Definitions

An **Adverse Event (AE)** is defined as any undesirable experience occurring to a Subject during the course of the study, whether or not it is related to the test material or the study procedure. Expected adverse events are those that are listed as known risks of the tissue harvest or ADRC application procedure including those described in this protocol or the informed consent.

A **Serious Adverse Event (SAE)** is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires or prolongs Subject hospitalization
- Results in persistent or significant disability or incapacity
- Is an important medical event(s) that may not be immediately life-threatening or result in death or hospitalization but that may jeopardize the Subject or require intervention to prevent one of the above outcomes
- Is a congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in deciding whether a case is serious and whether expedited reporting is appropriate.

Pain and ecchymosis/hemorrhage around the fat harvest site are expected and should not be recorded as an adverse event unless meeting one or more of the following criteria: (a) serious adverse event (SAE), (b) greater severity than expected according to Investigator clinical judgment, or (c) hemorrhage requiring evacuation.

An **Unanticipated Adverse Device Effect (UADE)** is defined as any serious adverse effect on the health and safety or any life-threatening problem or death caused by, or associated with the Celution® system

device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application or any other unanticipated serious problem with the Celution® system device that relates to the rights, safety or welfare of subjects.

11.2 Procedures for AE Reporting

Adverse events will be assessed according to the NIH Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for scale grading adverse events.

All AEs (including SAEs and UADEs) will be recorded by the investigator or designee on the appropriate electronic case report form (eCRF). Events that occur prior to the tissue harvest, including those that are considered serious, are to be recorded in the medical history. Events, including SAEs, which occur after tissue harvest procedure day, are to be recorded on the AE eCRF. The investigator will evaluate the relationship of the adverse event to both the test material and study procedure as unrelated, unlikely, possibly, probably, or definitely related and will record the findings, including all pertinent details of the event on the eCRF.

The investigator shall submit to the sponsor and to the reviewing IRB a report of any UADE occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect.

In addition, the Investigator or designee will report any serious adverse events occurring during this study to the Cytori Medical Monitor as soon as possible, but no later than 24 hours after the Investigator first identifies the adverse event.

The Investigator will take appropriate measures to ensure the subject's well-being and document these measures on the appropriate eCRF.

All serious adverse events experienced will be monitored until the event has resolved, any abnormal laboratory values have returned to baseline or stabilized at a level acceptable to the Investigator and Medical Monitor, or until there is a satisfactory explanation for the changes observed.

12 EMERGENCY DEVIATION FROM THE PROTOCOL

In case of an emergency threatening the continued well being of the Subject, the Investigator may deviate from the protocol. The circumstances necessitating the deviation and the intervention(s) required will be recorded on the subject's source documentation. The Sponsor must be notified as soon as possible, but no later than 5 days after event(s) occurred. The site's Institutional Review Board (IRB) should be notified as per the IRB regulations.

13 REGULATORY OBLIGATIONS AND STUDY REQUIREMENTS

13.1 FDA and Institutional Review Board Approval

Study initiation will not take place until the following regulatory requirements are met:

- Food and Drug Administration (FDA) approval with accompanying approval letter.
- Local Institutional Review Board (IRB) full approval with accompanying approval letter.

13.2 Informed Consent

Written informed consent will be obtained from all subjects, or their legally authorized representative, prior to the initiation of any study specific procedures. No tests or procedures required in this protocol that are outside the standard practice for treating thermal wounds will be initiated prior to obtaining written informed consent. The original IRB approved informed consent form will be filed in the Investigator Site Files according to site procedure.

13.3 Monitoring Procedures and Data Management

13.3.1 Site Qualification

A site qualification visit will be performed to evaluate site facilities, Investigator qualifications, adequacy of staffing, and understanding of clinical and regulatory requirements.

13.3.2 Site Training

All Investigators and appropriate study staff will be required to participate in a site training (or initiation if appropriate) to provide orientation and training to the Celution System, the RELIEF Protocol, eCRFs and other study related documents and procedures.

13.3.3 Monitoring

During the course of the study, a representative of the Sponsor will make site visits to review protocol compliance, compare eCRFs and individual Subject's medical records, assess test article accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that Subject confidentiality is maintained.

Regulatory authorities (United States Food and Drug Administration) and/or the Sponsor's Clinical Quality Assurance Group may wish to carry out source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality.

13.3.4 Reporting Requirements

The following reports are required of the Investigator:

- eCRFs for each Subject entered into the study (including all screen failures)
- Reports of any SAEs or UADEs within 24 hours of becoming aware of events
- Final report at the conclusion of the study
- Progress reports to the Sponsor and IRB at regular intervals, annually at a minimum
- Report to the Sponsor, within 5 working days, of approval withdrawal by the reviewing IRB
- Report to the Sponsor and IRB of any emergency changes made to the protocol to protect the life or physical well being of a Subject no later than 5 working days after the emergency occurred

13.3.5 Data Management

Data management will be conducted by the Sponsor or designee, and will include a data management plan and data verification guide developed according to protocol requirements.

13.4 Study Center and Investigator Requirements

To protect the rights and welfare of Subjects, the study will be conducted in conformance with the Declaration of Helsinki, the guidelines on GCPs, Code of Federal Regulations (21 CFR 11, 50, 56, 812, 814 and 820) and applicable local laws and regulations pertaining to the conduct of the study and the protection to human Subjects.

All Investigators, sub-investigators and study personnel are required to read and follow the protocol, as well as any literature that accompanies the products, prior to conducting the study procedure for the first time. In addition, all Investigators will sign and date the Investigator Statement Page of this protocol. All Investigators participating in this study will be trained in the proper use of the study procedure and in the components of the RELIEF protocol.

The Principal Investigator is ultimately responsible for the conduct of this study; however, he/she may designate a member of his/her staff to assist with the collection of data and completion of eCRFs. The designee(s) will be documented on an authorization form that is signed by the PI and kept in the Regulatory Binder, to be updated as necessary. Current copies of the authorization form will be forwarded to the Sponsor.

Obtaining informed consent in accordance with national policy is mandatory for Subject participation. All Subject data is kept confidential and procedures will be implemented to ensure that Subject confidentiality is not compromised.

If additional materials are used for screening and recruitment or provided to study Subjects (i.e. advertisements, recruitment materials) they must be approved by Cytori Therapeutics and the Institutional Review Board at the clinical site prior to use. Documentation of the approved informed consent must be provided to Cytori prior to study commencement at the clinical site.

After a Subject (or legal next of kin) has signed the Informed Consent Form, an eCRF will be initiated. After this point, the reason(s) must be documented on the report form for any Subject who has dropped, withdraws, or for any reason cannot complete this study.

13.4.1 Study Center Requirements

Due to the nature of the RELIEF Protocol, centers will be considered for participation only if they meet the following minimum facility requirements:

- Familiarity with FDA authorized IDE and/or IND Clinical Trials within the United States
- Pharmacy or laboratory (or qualified designee) capable of performing clinical trial randomization and cell dose preparation
- Ability to adhere to strict compliance with the clinical protocol for all study related procedure and observations

14 PUBLICATIONS

Following completion of the study, Cytori will present the study data in a clinical study report that will be delivered to the FDA according to US regulations.

When investigators external to Cytori conduct analyses of Company-sponsored study data or develop other works that use our data, we maintain the right to be informed of any plans for publication and to review any resulting works, including abstracts, presentations, or manuscripts, before they are submitted. We will return our comments to the authors in a timely manner so that they may submit the work for publication. In the event the investigator wishes to present or publish any study data (partial or complete), Cytori requests that he/she must submit the presentation / publication draft to Cytori for review prior to 30 days of the planned submission or presentation.

15 APPENDIX A: SCHEDULE OF PROCEDURES

	Day -2 to Day -1	Procedure Day 1	At First Dressing Change	Day 10 (±2d)[1]	Week 2 (±3d)	Week 3 (±3d)	Week 4 (±3d)	Week 8 (±7d)	Week 12 (±7d)	Week 26 (±14d)	Week 52 (±21d)
Informed consent	X										
Medical history (including demographics)	X										
Physical examination (including height and weight at screening)	X		X	X	X	X	X	X	X	X	X
Vital signs (BP, HR, Temp, RR)	X	X[2]	X	X	X	X	X	X	X	X	X
Concomitant medications	X		X	X	X	X	X	X	X	X	X
Laboratory Testing[5]	X	X	X								
Pregnancy test (urine)	X										
Inclusion and exclusion criteria	X										
Adverse events		X	X	X	X	X	X	X	X	X	X
Digital photography of wounds		X	X	X	X	X	X	X	X	X	X
Low Volume Lipoharvest		X									
Microbiology[3]		X									
ADRC dose prep and delivery		X									
% Epithelialization			X	X	X	X	X				
Biomarkers[4]	X		X	X	X	X	X	X	X		
% Graft Take			X	X	X	X	X				
Transepithelial water loss					X	X	X				
Wound Pain VAS			X	X	X	X	X				
Pruritis VAS						X	X	X	X	X	X
POSAS questionnaires						X	X	X	X	X	X
Vancouver Scar Scale									X	X	X
Skin elasticity measurement									X	X	X

	Day -2 to Day -1	Procedure Day 1	At First Dressing Change	Day 10 (±2d)[1]	Week 2 (±3d)	Week 3 (±3d)	Week 4 (±3d)	Week 8 (±7d)	Week 12 (±7d)	Week 26 (±14d)	Week 52 (±21d)
Skin pigmentation									X	X	X
Skin hardness									X	X	X
Complete Wound Closure					X	X	X	X	X		
Randomization		X									

[1]Day 10 assessments performed only if wound dressing is being changed between days 8 and 12. [2] Vital signs will be monitored according to institutional procedures. [3]Microbiological testing of dose samples (ADRCs) at baseline (prior to ADRCs administration). [4]Biomarkers will be measured in serum from 4 to 5 ml of whole blood that is collected, centrifuged, separated into 500-µL aliquots, and stored at -80°C will be collected at baseline (prior to ADRC administration), days 5 and 10 and weeks 2, 3, 4, 8 and 12 post ADRC administration. [5] Laboratory testing will include CBC with Platelets, INR, PTT,Electrolytes, alkaline phosphatise, AST, ALT, bilirubin, total protein, albumin, BUN and creatinine and, for patients with diabetes only, evaluation of HbA1c levels (see Exclusion Criterion 10).

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