University of Minnesota
Department of Pediatrics

Study of Epidermal Grafting Using the CelluTome® Epidermal Harvesting System for the Treatment of Individual Lesions in Persons with Epidermolysis Bullosa (EB)

CPRC #2015LS154
MT2015-36

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Confidential

Revision History

Revision Number	Version Date	Summary of Changes	Consent change?
	11/04/2015	original to CPRC	n/a
	12/01/2015	in response to CPRC review with additional clarifications; section 1.3 – replaced tables 1 and 2 with differently formatted tables section 8 - make separate x charts for each treatment arm	n/a
1	10/30/2016	Synopsis, section 4.1 and Appendix I – simplify transplant related inclusion criteria for Arm A as it does not affect safety nor interfere with the study endpoints Minor edits and clarifications: Study Overview, Treatment Flow and Section 2 – replace Tegraderm® with a non-adhering silicone mesh Clarify throughout that the pain and itching surveys are part of iScorEb Sections 8.1, 8.2, and 8.3 –clarifications and updates to the schedule of events, add footnote that Evaluations do not need to be repeated if recently done in association with a treatment study Section 9.1 - clarify that grade 3 and greater adverse events at least possibly related will be documented. Delete text regarding expected harvest site and treated wound healing times Section 10.1 – clarify where endpoint data will be stored Section 11 – add 1 year to outcome measures Section 13 – Conduct of the Study section missing from previous version. Other minor edits/updates throughout document	Yes
1.1	10/30/2016corrected	Study Overview: Correction of typographic error to remove the word “chronic” from the description of key eligibility criteria.	No
2	4/4/2017	Simplified study schema Throughout protocol clarified that follow up donor assessments, and 6 week follow up recipient assessments may be performed remotely if patient/donor is unable to return to UMN. Section 4.1 and eligibility checklist – clarified definition of chimerism.	Yes

		Section 4.2 and donor eligibility checklist – added donor infectious disease panel Section 8.1 clarified subject only needs to take pre-treatment iscoreEB assessment once Sections 8 and 10 – removed blister testing and skin biopsies	
3	11/29/2017	Sections 4, 8.1, and eligibility checklist. Clarified eligibility criteria with regard to donor chimerism; throughout protocol noted that the device may possibly cause infrequent, mild scarring and/or hypopigmentation	Yes
4	12/27/2018	Dr. Christen Ebens new PI of study. Background, Section 4 and eligibility checklist amended to remove the word “recessive” in order to expand eligibility to include all genotypic forms of dystrophic EB Synopsis, section 3 – added Arms C and D (analogous to Arms A and B but for subjects who were enrolled previously) Sections 4.1 and eligibility checklist. Removed detail of how somatic reversion is proven for Arm B subjects given variability in relevance of individual tests by patient and evolving methodologies in the field. Section 8: Added optional research biopsies (Arm A and B) Section 12:Added a note to describe analysis plan for re-enrolled subjects.	No
5	07/07/2021	Removed Drs. Weston Miller, Paul Orchard and John Wagner from study team; Added Dr Gupta Updated language on medical photography to all for updates in specialized equipment as needed and to allow for the specialty camera to be used per PI discretion at the specified time points Updated link to MCC DSMP	No

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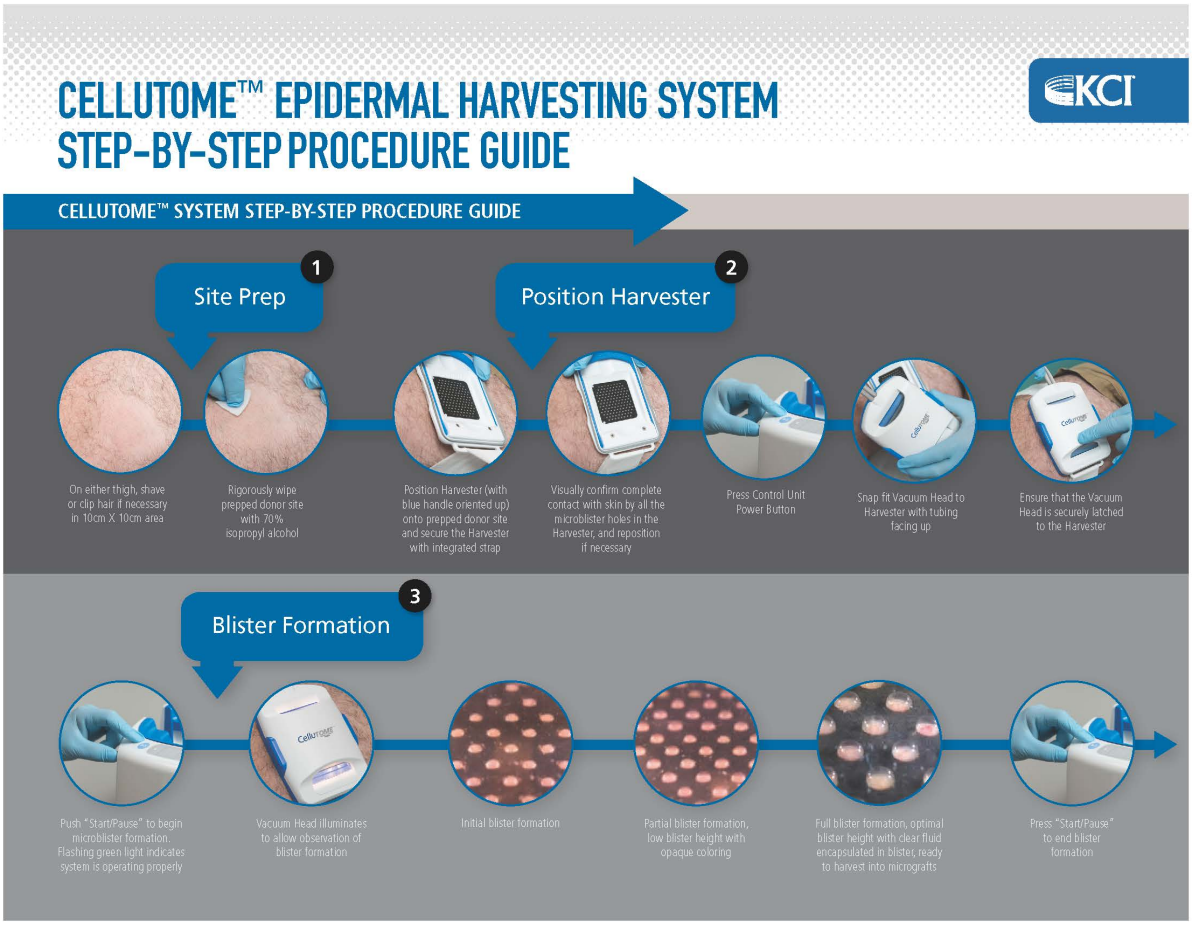
CELLUTOME™ EPIDERMAL HARVESTING SYSTEM
STEP-BY-STEP PROCEDURE GUIDE
KCI
CELLUTOME™ SYSTEM STEP-BY-STEP PROCEDURE GUIDE
1
Site Prep
2
Position Harvester
On either thigh, shave or clip hair if necessary in 10cm X 10cm area
Rigorously wipe prepped donor site with 70% isopropyl alcohol
Position Harvester (with blue handle oriented up) onto prepped donor site and secure the Harvester with integrated strap
Visually confirm complete contact with skin by all the microblister holes in the Harvester, and reposition if necessary
Press Control Unit Power Button
Snap fit Vacuum Head to Harvester with tubing facing up
Ensure that the Vacuum Head is securely latched to the Harvester
3
Blister Formation
Push "Start/Pause" to begin microblister formation. Flashing green light indicates system is operating properly
Vacuum Head illuminates to allow observation of blister formation
Initial blister formation
Partial blister formation, low blister height with opaque coloring
Full blister formation, optimal blister height with clear fluid encapsulated in blister, ready to harvest into micrografts
Press "Start/Pause" to end blister formation

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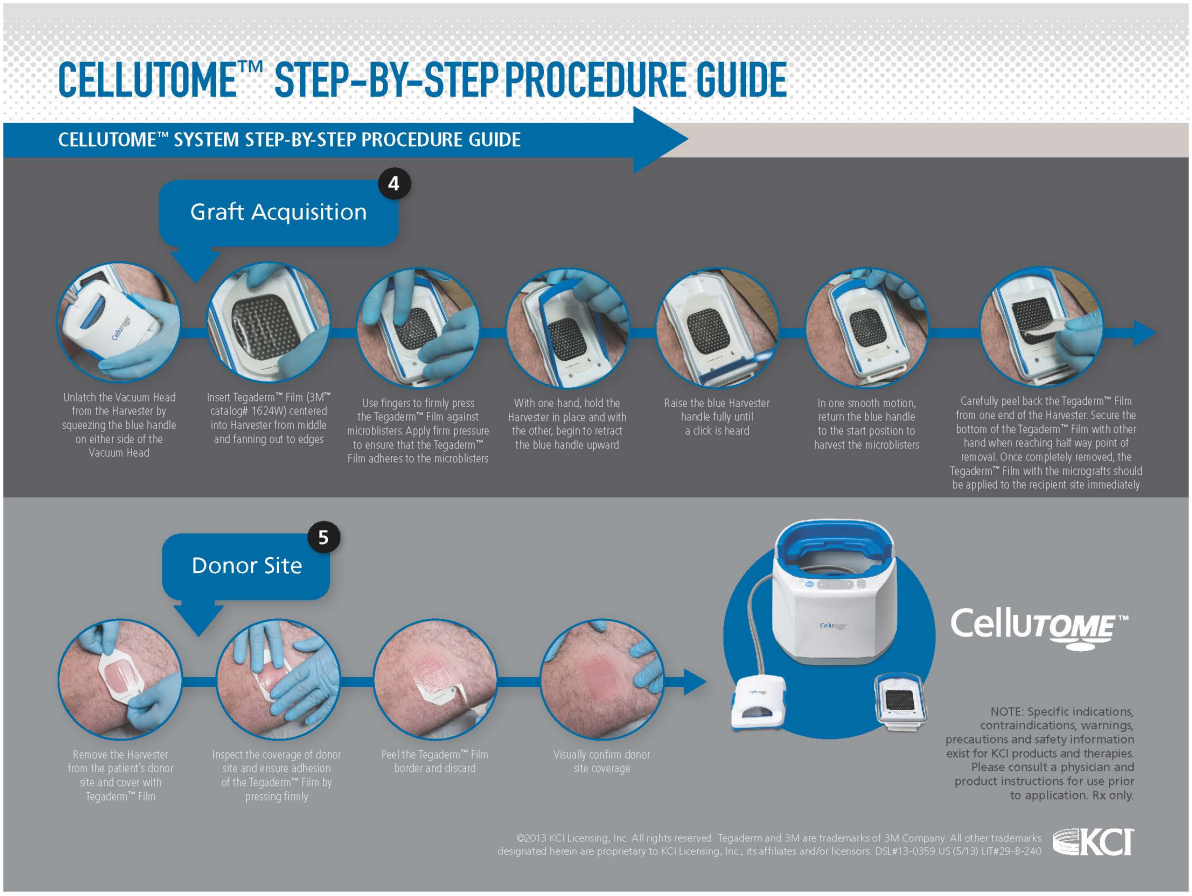
CELLUTOME™ STEP-BY-STEP PROCEDURE GUIDE
CELLUTOME™ SYSTEM STEP-BY-STEP PROCEDURE GUIDE
4
Graft Acquisition
Unlatch the Vacuum Head from the Harvester by squeezing the blue handle on either side of the Vacuum Head
Insert Tegaderm™ Film (3M™ catalog# 1624W) centered into Harvester from middle and fanning out to edges
Use fingers to firmly press the Tegaderm™ Film against microblisters. Apply firm pressure to ensure that the Tegaderm™ Film adheres to the microblisters
With one hand, hold the Harvester in place and with the other, begin to retract the blue handle upward
Raise the blue Harvester handle fully until a click is heard
In one smooth motion, return the blue handle to the start position to harvest the microblisters
Carefully peel back the Tegaderm™ Film from one end of the Harvester. Secure the bottom of the Tegaderm™ Film with other hand when reaching half way point of removal. Once completely removed, the Tegaderm™ Film with the micrografts should be applied to the recipient site immediately
5
Donor Site
Remove the Harvester from the patient's donor site and cover with Tegaderm™ Film
Inspect the coverage of donor site and ensure adhesion of the Tegaderm™ Film by pressing firmly
Peel the Tegaderm™ Film border and discard
Visually confirm donor site coverage
CelluTOME™
NOTE: Specific indications, contraindications, warnings, precautions and safety information exist for KCI products and therapies. Please consult a physician and product instructions for use prior to application. Rx only.
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Study Overview

Study of Epidermal Grafting Using the CelluTome® Epidermal Harvesting System for the Treatment of Individual Lesions in Persons with Epidermolysis Bullosa

CPRC #2015LS154

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Few but persistent wounds often remain even after successful hematopoietic cell transplantation (HCT) for systemic genodermatosis epidermolysis bullosa. We propose local wound therapy using epidermal skin grafting from the same donor that provided the hematopoietic graft, or from the same EB individual with a mosaic (naturally gene corrected) skin. In both cases permissive immune system and skin chimerism is expected to enable long-term epidermal engraftment and wound healing. We will use FDA approved vacuum device (CelluTome®, Regulation number 878.4820) that enables generally scar-free harvesting of epidermis with negligible bleeding and minimal pain (commonly described as a sensation of warmth and mild pressure) and its transfer on a non-adhering silicone mesh dressing (i.e. Adaptic Touch™) to the recipient's wound.

The **primary** study objective is to achieve > 50% wound closure within 12 weeks of skin grafting.

Secondary objectives are to [1] assess safety, longevity and functionality of grafted skin in the recipient over the period of 1 year, [2] measure changes in quality of life (QOL) through pain, itching and general QOL questionnaire via iscoreEB assessment scores and [3] safety and seamless, scar-free healing of the body sites of the donor from which epidermis has been harvested over the period of 1 year.

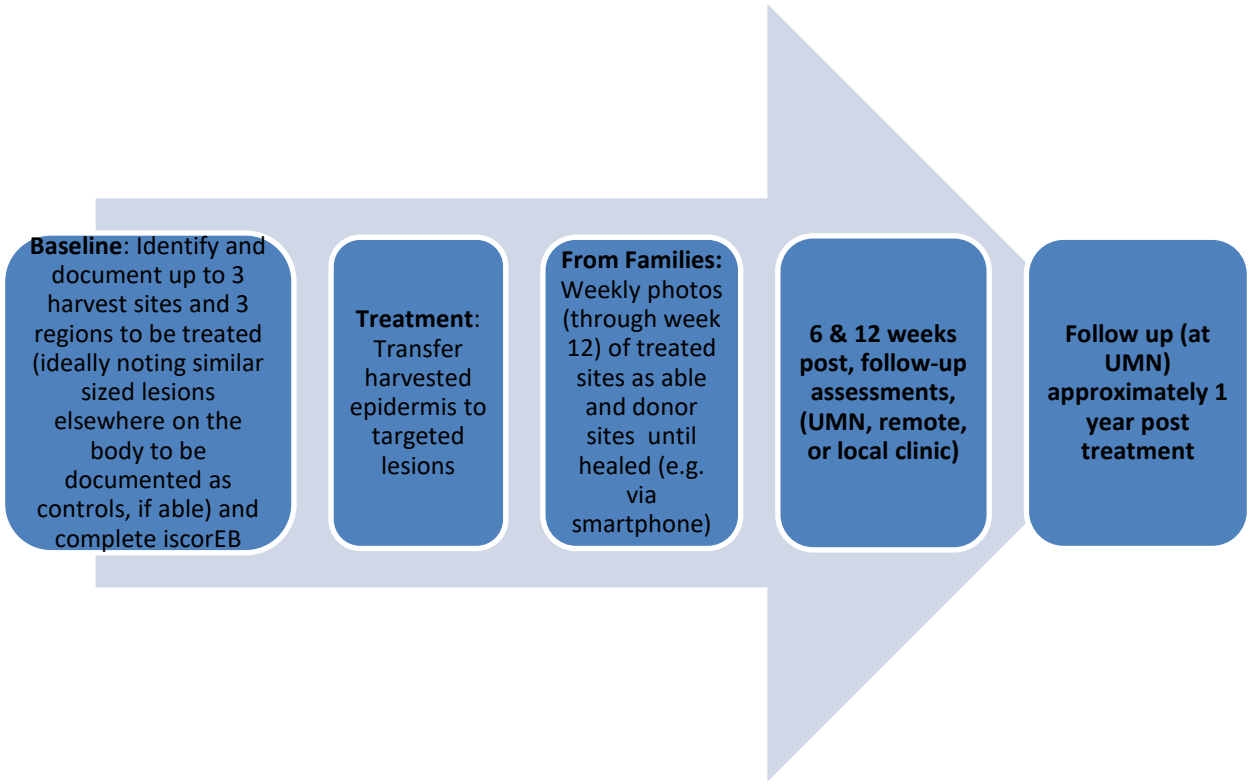
Key Eligibility:

Diagnosis of Dystrophic Epidermolysis Bullosa (DEB) or Junctional Epidermolysis Bullosa (JEB) with at least one wound and fitting into one of the following study arms:

- More than 6 months after HCT with stable (mixed or full) donor hematopoietic engraftment and original transplant donor is available and agrees to be the epidermal donor for this study (arm A).
- Participant has a patch of healthy unaffected skin available for autologous harvest (arm B)

Target enrollment is 25 patients on arm A (graft from hematopoietic stem cell donor) and 10 subjects on arm B (self-donor) over 3 years. Up to an additional 25 patients may re-enroll in the study. Re-enrolled allogenic graft recipients will be considered Arm C (treatment and follow up identical to Arm A) and re-enrolled self-donor recipients will be considered Arm D (treatment and follow-up identical to Arm B).

Schema



Treatment Flow

Identify Donor Skin Availability:
Arm A: graft from HCT donor
Arm B: self- donor – intact skin patch

↓

Identify and document up to 3 regions for treatment and approximately equal control* (untreated) wounds

↓

Harvest donor cells from up to three sites using Cellutome; transfer via a non-adhering, open-mesh silicone dressing to 3 regions / wound sites (refer to appendix III for procedure overview)

↓

Parent submits weekly digital photos of donor harvest site and wounds*

↓

Patient and donor (if applicable) follow-up at 6 weeks +/1 week at the U of MN, their local clinic, or via remote assessment if photographs suggest not clinically indicated

↓

Patient and donor (if applicable) for follow-up at 12 weeks +/- 1 week at the U of MN, their local clinic, or via remote assessment
If at 12 weeks wound healing is good with no significant toxicity, the controls and/or other wounds may be treated (up to 3 wounds per time, no closer than 12 weeks apart) – refer to section 6.4

↓

Patient and donor (if applicable) return to the UMN for follow-up 1 year +/- 3 months (final study visit for primary and secondary endpoints). After each year of treatments, patients may re-enroll on study (Arms C & D).

*If possible

1 Background and Study Objectives

Skin—like bone marrow—is a complex immune tissue that can be harvested and transplanted. This unique capacity has been used with significant success in the field of hematopoietic cell transplantation and in practice of skin grafting. This study aims to merge these concepts for the benefit of patients with systemic blistering disease, epidermolysis bullosa (EB).

1.1 Phenotype and Genotype

EB is a group of heritable mechanobullous skin diseases characterized by skin fragility, blister formation, and scarring. The most severe forms of EB are characterized by mutilating scarring, blisters over large proportions of the body surface and later on, mitten deformities, joint contractures, esophageal strictures, corneal erosions, chronic cutaneous infections and aggressive squamous cell carcinoma (SCC)[1-3]. Children and adults with dystrophic EB (RDEB) and junctional EB (JEB) are often faced with a life of pain, often dying of SCC. SCC can appear as early as 13 years of age with 50% having SCC if they survive to age 40 years. The prognosis of a EB patient with SCC is very poor with nearly all dying of metastatic disease. Patients with the severe DEB have profound physical disabilities. Daily activities (e.g., toileting, feeding, bathing, walking) are major challenges. Quality of life also tends to decline with age. Caretakers are consumed by the day to day care of the child with EB.

DEB is caused by loss of function mutations in *COL7A1* gene and JEB by loss of function mutations in *LAMA3*, *LAMB3*, *LAMC2* or *COL17A1* genes. Type VII collagen (C7) is synthesized by human keratinocytes and fibroblasts alpha 3, beta 3 or gamma 2 chains of laminin 332 (L332), and type XVII collagen (C17) are synthesized in keratinocytes. These proteins are secreted around the basement membrane zone (BMZ) lying between the epidermis and dermis of skin. C7 is the major component of anchoring fibrils (AF), which are necessary for normal epidermal-dermal adherence, L332 and C17 are operational in structures that attach keratinocytes to the skin basement membrane or keratinocytes to each other. DEB and JEB are typically inherited as autosomal disease with blisters and scarring often present at birth or shortly thereafter. The blisters in the neonate often cover the whole body, including oral and esophageal mucosa, and it continues throughout life.

1.2 Hematopoietic Cell Transplantation for Severe EB

To begin to address the lack of effective therapies for DEB, in 2008 we started a clinical trial of hematopoietic cell transplantation (HCT) in children with generalized

severe DEB. The rationale for this intervention was based on murine data showing that HCT could lead to restoration of c7 in the skin basement membrane of *col7a1*-/- mice as well as to their improved survival.[5,6] The first human study of HCT for DEB was reported in 2010.[7] Analysis of the skin of six children in this trial showed that, in most subjects, donor cells engrafted in recipient skin. Moreover, in five of six individuals this was paralleled by increase of C7 expression at the dermal-epidermal junction (DEJ), healing of mucocutaneous lesions, and improved quality of life.[7]

Nevertheless, none of the HCT recipients has become entirely free of wounds, and several children experienced severe complications, including death, as a result of the HCT-related toxicities of the high-dose chemotherapy used for myeloablative conditioning (MAC), of DEB disease progression, or both. Therefore, with the ultimate aim of improving the efficacy and safety of HCT for the systemic and durable therapy of generalized severe DEB, we sequentially modified the HCT treatment protocol with the addition of supplemental cells and reduced intensity conditioning (RIC). The addition of mesenchymal stem/stromal cells (MSCs) was based on identification of non-hematopoietic donor cells in the skin after HCT,[7] on the capacity of mesenchymal stromal/stem cells (MSCs) to secrete C7, and on the immune-modulatory potential of these cells to mitigate graft-versus-host disease. The change of conditioning to RIC was adopted to assess whether less-than-complete hematopoietic chimerism could mediate adequate clinical responses while being expected to result in less morbidity and mortality after allogeneic HCT.[8]

In a cohort of patients with JEB, two children were transplanted using the MAC and four using the RIC. Four of six patients died between days 42 and 146 after HCT, predominantly from progression of their primary genodermatosis. As severe JEB is genetically heterogeneous, it is relevant to note that the two surviving individuals with severe JEB have loss-of-function mutations in *LAMA3* gene, while those who died had genetic inactivation of *LAMB3* and, in one case *ITGB4* gene. As only six JEB patients have been treated since the study was opened there are still too few to accurately assess outcome/efficacy for this subgroup. Thus the review below has been confined to the 26 DEB patients.

Of the 26 individuals who underwent HCT, 13 were treated with MAC: busulfan (0.8 mg/kg of body weight per dose for children who weighed 12 kg or more, and 1.1 mg/kg for those who weighed less than 12 kg, delivered intravenously every 6 hours on days -9 to -6 before transplantation, with dose modifications based on pharmacokinetics, targeting 1,000 µmol per minute for the area under the curve), fludarabine (25 mg/m^2 of body surface area per day, given intravenously on days -5 to -3 before transplantation), and cyclophosphamide (50 mg/kg per day, given

intravenously on days -5 to -2 before transplantation). An additional 13 patients were treated with RIC: cyclophosphamide (50 mg/kg per day, given intravenously on day -6 before transplantation), fludarabine (40 mg/m^2 of body-surface area per day, given intravenously on days -5 to -2 before transplantation), horse anti-thymocyte globulin (30 mg/kg per day, given intravenously on days -4 to -2 before transplantation), and total body irradiation (200 cGy for the first seven patients in the RIC cohort or 300 cGy for the following six patients, delivered on day -1 before transplantation). On the day of transplantation, patients received unfiltered marrow stem cells or umbilical cord blood cells from HLA-matched or partially HLA-matched related or unrelated donors; after 4 hours they also received third-party HLA-unmatched MSCs (2 million cells/kg of body weight). Immunoprophylaxis against graft-versus-host disease (GvHD) consisted of cyclosporine (targeting trough levels of 200 to 400 µg per liter from day -3 before transplantation to day +100 after transplantation, with the dose tapered by 10% each week thereafter) and mycophenolate mofetil [15 mg/kg from day -3 before transplantation to day +30 (RIC) or day +45 (MAC) after transplantation].

1.3 Transplant Outcomes

The overall probability of 2-year survival in the MAC cohort (n=13) was 69% (95% CI, 37-87%) versus 90% (95% CI, 47-99%) for the RIC cohort (n=13; p=0.3). Of the 20 recent DEB patients, three individuals died before one year after HCT (MAC: RII-4 of veno-occlusive disease, RII-7 of fungal sepsis; RIC: RIII-6 of fungal sepsis); one patient died after one year (MAC: RII-3 of extensive chronic GvHD; **Tables 1** and **2**). Three individuals did not engraft with donor cells (MAC: RII-2, RIC: RIII-4, RIII-8; **Tables 1** and **2**). There were no statistically significant survival differences between related versus unrelated grafts, bone marrow versus umbilical cord blood grafts, and those who received additional MSCs versus those who did not. One individual in each cohort (MAC: RII-7, RIC: RIII-9) developed acute GvHD (grade 2, with stage 3 skin involvement - erythroderma); one individual developed extensive chronic GvHD (MAC: RII-3). Neutrophil and platelet engraftment were faster in the RIC group compared to the MAC group, although this improvement was not statistically significant when those with autologous recovery were excluded from analysis. Probably related to differences in quality and pace of immune reconstitution, we observed many fewer infections for RIC compared to MAC. While the incidence of blood bacterial infections was similar, and the incidence of viral infections was not statistically different. Remarkably, there was only one systemic fungal infection among the RIC-treated patients (versus 6 of 13 in the MAC-treated patients).

Table 1. Patient characteristics, grafts, and outcomes after hematopoietic cell transplantation for dystrophic epidermolysis bullosa.

Pt ID	Gender	Age in years at HCT	Conditioning	Graft	Clinical features[1]	Clinical outcome	Mucocutaneous chimerism %	Type VII collagen expression	Anchoring fibrils (AFs)	BSA change %
RII-1	F	2	MAC	UCB, MSC	Bloody emesis, corneal abrasions, anal fissure	Stable	11-30	Increased	Rare thick AFs; no C7 immune label	75 → <25
RII-2	F	9	MAC	UCB, MSC	Esophageal strictures	2nd HCT, no engraftment	0	Increased[2]	Rare thick AFs with C7 immune label	No change
RII-3	M	13	MAC	UCB, MSC	Pseudo-syndactyly, IDA, esophageal strictures, wheelchair bound	Death	7-33	None	Rare thick AFs with C7 immune label	No change
RII-4	F	1	MAC	MSD, MSC	Neonatal seizure	Death	5-6	None	Thin AFs; no C7 immune label	75 → <5
RII-5	M	5	MAC	MSD, MSC	Esophageal strictures, aspiration pneumonia	Improvement	6-48	None	Thick AFs with moderate C7 immune label	75 → <5
RII-6	M	4	MAC	URD, MSC	Neonatal sepsis, pneumonia, colitis, esophageal strictures, constipation	2nd HCT, improvement	3-22	None	Thick AFs; no C7 immune label	75 → <5
RII-7	M	10	MAC	MSD, MSC	eonatal intubation, esophageal strictures, Pseudo-syndactyly, corneal erosions, contractures, IDA	Death	11-97	Increased	Thin AFs with C7 immune label	No change

[1] All patients had: >55% skin surface covered with erosions and blisters; history of skin colonization and infections; FTT; pruritus, and pain. [2]total nucleated cells/kilogram of recipient's body weight. Four hours after hematopoietic cells, all patients received single intravenous dose of 2 x 10^6 HLA-unmatched (3rd party) mesenchymal stromal cells. [2]The C7 increase occurred early after HCT (day 28) and later comparisons were not possible, or indicated.

Legend: HCT, hematopoietic cell transplantation; BSA, body surface area; MAC, myeloablative conditioning; UCB, umbilical cord blood; MSC, mesenchymal stromal cells; C7, collagen type VII; IDA, iron-deficient anemia.

Table 2A. Patient characteristics, grafts and outcomes for DEB with reduced intensity conditioning (RIC, 200cGy TBI).										
Pt ID	**Gender**	**Age in years at HCT**	**Conditioning**	**Graft**	**Clinical features[1]**	**Clinical outcome**	**Mucocutaneous chimerism %**	**Type VII collagen expression**	**Anchoring fibrils (AFs)**	**BSA change %**
RIII-1	M	20	RIC 200 TBI	URD, MSC	Severe contractures, wheelchair bound, pseudo-syndactyly, IDA, dental carries, depression	Improvement	9-23	Increased	Common thin AFs with moderate C7 immune label	90 → <10
RIII-2	F	2	RIC 200 TBI	MSD, MSC	Bloody emesis, corneal abrasions	Improvement	0-2	Increased	Arching AFs; no C7 immune label	75 → <15
RIII-3	F	6	RIC 200 TBI	MSD, MSC	Esophageal strictures, corneal abrasions	Improvement	3-7	Continuous	Thick AFs with moderate C7 immune label	75 → <5
RIII-4	F	7	RIC 200 TBI	UCB, MSC	Esophageal strictures, corneal abrasions	No engraftment	0	None	Thin AFs; no C7 immune label	No change
RIII-5	M	0.75	RIC 200 TBI	URD, MSC	Hygroma, IDA, FTT, corneal abrasions	Improvement	0-15	Increased	Arched AFs with moderate C7 immune label	75 → <5
RIII-6	M	3	RIC 200 TBI	URD, MSC	Esophageal strictures, corneal abrasions	Death	8-11	Increased	Thick AFs; no C7 immune label	No change
RIII-7	F	0.9	RIC 200 TBI	URD, MSC	Bloody emesis	Improvement	0-3	None	Thin AFs; no C7 immune label	75 → <5

[1] All patients had: >55% skin surface covered with erosions and blisters; history of pruritus and pain.
Four hours after hematopoietic cells all patients received single intravenous dose of 2 x 10^6 HLA unmatched (3rd party) mesenchymal stromal cells
Legend: DEB, dystrophic epidermolysis bullosa; cGy, centigray; TBI, total body irradiation; HCT, hematopoietic cell transplantation; BSA, body surface area; URD, unrelated donor; MSC, mesenchymal stromal cells; IDA, iron-deficient anemia; C7, collagen type VII; FTT, failure to thrive.

Table 2B. Patient characteristics, grafts and outcomes for DEB with reduced intensity conditioning (RIC, 300cGy TBI).										
Pt ID	**Gender**	**Age in years at HCT**	**Conditioning**	**Graft**	**Clinical features[1]**	**Clinical outcome**	**Mucocutaneous chimerism %**	**Type VII collagen expression**	**Anchoring fibrils (AFs)**	**BSA change %**
RIII-8	M	3	RIC 300 TBI	MSD, MSC	Esophageal strictures	2nd HCT, improvement	0-16	None	Arching AFs with C7 immune label	75 → <5
RIII-9	F	1	RIC 300 TBI	MSD, MSC	Severe oral mucosa blistering	Improvement	0-22	Continuous	Numerous thin AFs with light C7 immune label	75 → <5
RIII-10	M	0.9	RIC 300 TBI	MRD, MSC	Corneal abrasions	Improvement	0-47	Continuous	High density AFs with heavy C7 immune label	75 → <10
RIII-11	F	4	RIC 300 TBI	MSD, MSC	Esophageal strictures, corneal abrasions	Improvement	0	None	Rare thick AFs with light C7 immune label	75 → <10
RIII-12	F	0.3	RIC 300 TBI	URD, MSC	Mild oral mucosa blistering	Improvement	3-8	None	Numerous thin AFs with moderate C7 immune label	55 → <5
RIII-13	M	0.5	RIC 300 TBI	MSD, MSC	Moderate oral mucosa blistering	Improvement	13	Continuous	Thin AFs; no C7 immune label	55 → <5

[1] All patients had: >55% skin surface covered with erosions and blisters; history of pruritus and pain. Four hours after hematopoietic cells all patients received single intravenous dose of 2 x 10^6 HLA unmatched (3rd party) mesenchymal stromal cells..
Legend: DEB, dystrophic epidermolysis bullosa; cGy, centigray; TBI, total body irradiation; HCT, hematopoietic cell transplantation; BSA, body surface area; MSD, matched sibling donor; MSC, mesenchymal stromal cells; C7, collagen type VII.

1.4 EB Outcomes

The underlying hypothesis was that infusion of bone marrow or umbilical cord blood from a healthy unaffected donor will correct C7 deficiency and reduce skin fragility in generalized severe DEB. A secondary hypothesis was that changes in conditioning intensity (from MAC to RIC regimen), and composition of the graft (addition of MSCs) will be well tolerated. Further, we hypothesized that the changes made to the transplantation regimens would result in permanent hematopoietic donor engraftment that can serve as a source of renewable cells for the healing of mucocutaneous wounds and translate to long-term clinically meaningful benefits.

Hematopoietic engraftment was near-complete in those treated with MAC.[7] As expected with lower doses of conditioning chemotherapy, engraftment was significantly lower in those treated with RIC—on average 26% for CD15 cells and 55% for CD3 cells +100 days after HCT. In agreement with standard-of-care, when we observed declining CD3 chimerism, we infused additional donor T cells (donor lymphocyte infusions, DLI). In five of six individuals treated with DLI (**Table 2**), this intervention correlated with stabilization of grafted hematopoietic cells. Based on data from disease models of DEB[13] and observations of no blistering in human obligate heterozygotes of pathogenic *COL7A1* mutations (e.g., parents of children with generalized severe DEB), the relative number of wild-type skin cells capable of ameliorating blistering is likely to be less, perhaps significantly less, than 50%. We therefore assessed the proportion of donor cells in skin biopsies after HCT. The maximum skin chimerism in those subjects with successful hematopoietic engraftment averaged 31% in the MAC (HSC + MSC) cohort, compared to 14% in the RIC group (**Tables 1** and **2**). Thus skin chimerism correlated proportionally with hematopoietic cell findings.

We observed an increase in C7 immunolabeling in 2 of 7 DEB individuals treated with MAC (RII-1 and RII-7; in addition to those reported previously[7]), and in 4 of 13 individuals treated with RIC (RIII-1, RIII-2, RIII-5 and RIII-6). The increase in C7 was long-lasting. This finding supports a mechanistic model whereby engrafted bone marrow serves continuously as a source of cells capable of migration to injured skin and secretion of C7. In four other subjects in the RIC group, however, any changes in C7 expression after HCT were less obvious because, despite severe clinical phenotype, their baseline C7 immunoreactivity was bright before HCT (RIII-3, RIII-9, RIII-10, RIII-13), and thus any increase could not be picked up by a change in immunosignal intensity. Those subjects with graft failure or early death were not evaluable. Similar to the one individual reported previously,[7] after HCT several individuals in the current trials showed no detectable increase in C7

at the basement membrane zone, often despite clinical improvement (**Tables 1** and **2**) and favorable changes in functional skin stability. Of note, although we observed anti-C7 antibodies in serum before and after HCT, there was no evidence of anti-type VII collagen immune complexes (indirect immunofluorescence with binding to the base of salt split skin) after HCT (data not shown).

Reconstitution of AFs at the DEJ following HCT is regarded as a prime indicator of structural and functional (physiologic) correction of the inherited skin pathology. Therefore, we evaluated absence or presence of AFs in skin of the clinical trial subjects before and after HCT using transmission electron microscopy of fixed and C7 antibody-labeled skin biopsies. In some individuals (for example, RII-1) we saw no AFs despite increased immunohistochemical C7 expression after HCT; in others (for example, RIII-1 and RIII-10) AFs were seen after HCT that demonstrate at least two of the three defining criteria of normal AFs, i.e., insertion into the lamina densa, arching and/or looping profiles, and central cross-banding.[14,15] Moreover, for some of the more uncertain wisp-like fibrils seen inserting into the lamina densa in some subjects (for example, RIII-1 and RIII-10), we were able to demonstrate immuno-ultrastuctural localization of C7 (for both C7 non-collagenous domain NC-1 (antibody 185) and/or C7 non-collagenous domain NC-2 (LH24 antibody) at the sites of AF-like structures at the DEJ, providing evidence for the generation of at least rudimentary AFs. TEM evaluations were frequently problematic due to high concentrations of “ground substance” at the DEJ in the vicinity of where anchoring fibrils would be expected (for example RII-5, RIII-5 and RIII-9). This substance was commonly flushed away during extensive washes prior to fixation in the samples prepared for immuno-EM, making it more likely that anchoring fibrils would be identified (for example, RIII-1 and RIII-10) in samples prepared for immunocytochemistry.

Given that our ultimate goal for DEB individuals is functional skin correction, we assessed the skin’s integrity before and after HCT in a quantitative way. We employed a suction blister device whereby constant negative pressure is applied to the skin and the time to blistering is proportionate to the capacity of skin layers to adhere to each other. Since AFs mediate connectivity of the epidermis to the dermis, the length of time the skin is able to withstand the application of negative pressure is a measure of C7 expression, polymerization, and its functional integration in skin. Typically, individuals with DEB experience blistering within 10 minutes, healthy people in approximately 60 minutes, and the heterozygous carriers of *COL7A1* mutations in 30-50 minutes. Of note, in several representative examples from both the MAC and RIC trials, serial assessments of DEB subjects after HCT showed gradual increases in skin resilience under pressure.

Wound healing, the most striking and desired metric, has been dramatically improved in all but two surviving and engrafted individuals (**Tables 1** and **2**), as evidenced by both objective (serial clinical assessments and photographs) and subjective measures (Parental Statements). As described in the latest classification of inherited epidermolysis bullosa,[1] DEB individuals with the generalized severe form have a disorder that is systemic. Thus individual metrics of biochemical, structural, and physical changes in skin, including the compound measure of functional skin stability with the suction blister device, do not entirely capture the complex pathology. Nor do these measurements encompass the impact of RDEB on the health of individuals with it or the disease burden on their daily lives. To tackle this issue and determine the overall effect of HCT on the natural history of REB, we employed the composite, recently validated, iScorEB tool to assess disease severity and quality of life[10]. In addition, there were statistically significant decreases in total number of red blood cell transfusions in peri-transplant period (MAC versus RIC, 22.3 versus 6.3 transfusions, p-value 0.003), in number of days spent in the hospital (MAC versus RIC, 74.8 versus 38 days, p-value 0.01).

1.5 Interim Results as of September 2018

Interval assessment completed in September of 2018 included 9 DEB subjects enrolled on Arm A. No subjects have been treated on Arm B. Assessment of the initial round of CelluTome grafting for the first 9 subjects on Arm A included a total of 18 grafted wounds. The mean decrease in wound surface area at 12 weeks post-grafting was 85.6% (range 24.4-100%). Eight wounds assessed at 1 year post-grafting showed a mean decrease in wound surface area of 85.6% (range 44-100%). Donors universally had no scarring. Given these remarkable responses, we amended the protocol to allow for re-consenting and data collection on additional rounds of grafting for enrolled subjects lacking adverse events after 1 year on study.

1.6 Rationale for Homotypic Epidermal Grafting

The biological premise of this protocol is immune tolerant environment generated by hematopoietic cell transplantation (HCT) from related donor, either human leukocyte antigen (HLA) identical or HLA haplo-identical family member, followed by superficial epidermal grafting from the same donor. In an alternative, though less common (only 10-30% of EB patients have identifiable mosaic skin patches), scenario the same superficial epidermal grafting procedure will be used to transfer mosaic (naturally gene corrected) skin from one body site to another in the same individual, regardless of HCT. In both cases permissive immune system and skin

chimerism is expected to enable long-term epidermal engraftment and wound healing.

Such potential of long-term skin repair is based not only on the immune tolerance, however, but also on composition of the skin graft. As in bone marrow, in skin a small fraction of cells (typically less than 1%) are stem cells capable of symmetrical (which generates two stem cells) and asymmetrical cell division (which generates one stem cell and one differentiated cell). These tissue-specific hematopoietic and epidermal stem cells are the source of continual tissue regeneration and—after their respective transplantation—prerequisite of reconstitution in the new site. In skin stem cells reside in bulge of hair follicles, inter-follicular dermis and epidermis. The latter will be captured in the proposed harvesting of epidermis, as both basal and supra-basal layers of skin contain skin stem cells (quiescent progenitors) and transient-amplifying cells (proliferating progenitors).

1.7 Study Objectives

The **primary** study objective is to achieve > 50% wound closure as measured on a per wound basis within 12 weeks of skin grafting.

Secondary objectives are to:

1. assess safety, longevity and functionality of grafted skin in the recipient at 1 year
2. measure changes in quality of life (QOL) through pain, itching and general QOL questionnaire via iscorEB assessment score
3. safety and seamless, scar-free healing of the body sites of the donor from which epidermis has been harvested over the period of 1 year

2 CelluTome® Epidermal Harvesting System

The CelluTome® is a vacuum device that is applied to an area of skin and left on for approximately 30 minutes. Via gentle negative pressure, it slowly raises a series of small (millimeters in diameter), thin epidermal skin grafts that are then sheared off via a disposable blade within the apparatus. The grafts then adhere to a square of non-adhering silicone mesh dressing and are ready for grafting to another site. A similar piece of silicone mesh is then applied to the area of harvested skin as a wound dressing. Refer to appendix III for an overview of the process.

While similar devices have existed for years, the Cellutome has several novel transformative properties.[16-19] Harvesting causes only possible mild discomfort (warmth and pressure), infrequent, mild scarring and/or hypopigmentation , and results in minimal

(if any) bleeding for the donor. Further, the device is significantly more automated and yields much more standardized grafts than prior iterations of this device. The potential for operator error or suboptimal specimens during blister grafting has been significantly diminished.

3 Study Overview

This is a single-institution pilot study to determine the capacity of immune-syntonic epidermal grafting to achieve > 50% wound closure as measured on a per wound basis within 12 weeks of skin grafting in individuals with the generalized severe forms of epidermolysis bullosa, DEB and JEB. Up to 3 uninfected regions (or infected regions if previously treated prior to grafting) will be identified tor treatment with similar size wounds identified as controls if possible (no treatment).

Wound reassessments will be performed via weekly photographs through week 12 (as able) and at follow up visits (either remote or at the University of Minnesota) approximately 6 and 12 weeks after epidermal grafting. Whenever possible, assessments at the University of Minnesota will be scheduled as part of post-transplant follow-up visits. In addition, safety, longevity and functionality of grafted skin will be monitored in the recipient over the period of 1 year, as well as, safety and seamless, scar-free healing of the body sites of the donor from which epidermis has been harvested over the period of 1 year.

Although the procedures are identical, this study will have two treatment arms based on the source of the epidermis donor cells:

Arm A: Epidermis donor cells are from the original hematopoietic stem cell donor. This arm requires two separate consents – one for the donor and one for the patient

Arm B: The patient is his/her own donor for the epidermis cells. This arm requires a single consent explaining both the harvest procedures and the graft procedures.

Targeted enrollment over 3 years is 25 patients for Arm A and 10 patients for Arm B.

Up to 25 additional patients may re-enrolled on the study. Re-enrolled allogenic graft recipients will be considered Arm C (treatment and follow up identical to Arm A) and re-enrolled self-donor recipients will be considered Arm D (treatment and follow-up identical to Arm B).

If at least 12 weeks after the initial grafting there are good outcomes and no unacceptable side effects we will offer to treat the "control" and/or other wounds in a serial fashion with up to three treatment sessions anticipated. Results of subsequent sessions and re-enrolled subjects will be reported within this study, but only the primary/first treatment session will count toward the primary and secondary endpoints. Refer to section 6.4 for additional details.

4 Patient/Donor Selection

4.1 Patient (Recipient)

- Diagnosis of Dystrophic Epidermolysis Bullosa (DEB) or Junctional Epidermolysis Bullosa (JEB) with at least one wound, visibly free from infection (or previously treated) and meets the eligibility for Arm A or Arm B based on the skin graft source:
- Cell harvest from previous HCT donor (Arm A) – not applicable if Arm B
  - At least 6 months after HCT with donor chimerism
    - Peripheral blood donor chimerism should be measured within 21 days of grafting and be >/= 5% and stable. Stability of chimerism will be determined by the protocol team and based on 3 peripheral blood chimerism values at least 1 month apart.
  - No history of pre-BMT autoimmune cytopenias
  - Off immune suppressive therapy
  - Original transplant donor is available and willing to be the epidermis donor
- Self-donation (Arm B) – not applicable if Arm A
  - Proven somatic reversion
  - Site for skin grafting free of cellulitis and any other clinically evident abnormalities
  - Meets donor eligibility (section 4.2)
- Insurance pre-authorization for procedure, if applicable
- Voluntary written consent (patient or parent/guardian for minors with assent) prior to any research related procedures or treatment.

4.2 Skin Graft Donor (either HCT donor for the EB patient [Arm A] or EB patient herself/himself [Arm B])

- Age > 2 years (based on prior safety testing of the device)
- Healthy on physical examination in the opinion of the evaluating provider
- Negativity for Hepatitis B and C, HIV, and HTLV1/2 within 30 days of donation.

- Voluntary written consent (donor or parent/guardian for minors with assent) prior to any research related procedures

5 Study Registration

Registration will occur after the patient and/or parent/guardian has signed the appropriate consent form and eligibility is confirmed, but before any study related procedures are performed. To be eligible for registration to this study, the patient must meet each of the criteria listed on the eligibility checklist (appendix I, and if Arm B, appendix II) based on the eligibility assessment documented in the patient's medical record.

Upon completion of the screening evaluation, eligibility confirmation and obtaining written consent, the study coordinator or designee will register the patient in OnCore.

At the time of registration, the patient will enrolled on one of the following study arms based on the epidermal cell source:

Arm A: Previous hematopoietic cell transplant (HCT) donor

Arm B: Patient (self-donation)

Donors for patients enrolled on Arm A will meet each criteria listed on the donor eligibility checklist (appendix II) and be registered in OnCore per Masonic Cancer Center guidelines.

6 Treatment Procedures

6.1 EB Wounds

We will identify up to 3 regions indicated for grafting with an equal number of control (no treatment) lesions (as able). Such wounds would be open erosions, apparently free of infection (as documented by physical exam—local redness, swelling, and pain—as microbiome is inevitable and physiologic on any muco-cutaneous surface). Whenever possible, we will seek symmetrical wounds (relative to median axis on the head and trunk, or relative to long axis on extremities) and plan to treat one side only in the first setting. In this fashion we will be able to compare the dynamics of healing of untreated ("control") and treated sites, while the systemic factors (such as, level of activity, inflammation, environmental influences) remain approximately the same. If there are no significant side effects, we will treat the "control" wounds and/or other wounds after a minimum 12-week period of observation after the initial grafting, and in serial

fashion after that with up to three sessions anticipated over the approximately one-year follow-up period from time of initial CelluTome session (refer to section 6.4).

6.2 Healthy Skin Grafts

We will identify optimal harvesting sites on the body of recipient. Although the epidermal collection is essentially painless (donor will feel warmth and mild pressure at the site) and leaves infrequent, mild scarring and/or hypopigmentation we will not choose intensely exposed locations, such as head, neck, palms and soles. The primary collection site is often the thigh, but as skin is developmentally, structurally and functionally different at different parts of the body we will attempt collection of epidermis from a general area on the recipient's body that corresponds to the site of the wound being treated. Of course, we are prepared to adapt to physical specifics and psychological choices of the recipient. For Arm B enrollees, grafts will be taken from sites without history of blistering.

6.3 Epidermal Transfer

The grafting will be done in real time. This FDA approved (Regulation number 878.4820) operating procedure will be followed in all details to collect the donor epidermis from a maximum of 3 sites at one sitting and to transfer it to up to 3 regions in the recipient location. Grafts will be secured with bandages and protected for at least 2 weeks, and preferably up to 3-4 weeks if possible. Patients are educated on how to re-apply grafts that have fallen off (i.e. cleanse with saline and re-apply if < 2 weeks and no obvious contaminate of the graft)., although it is not presumed to be detrimental if these grafts are not in place the full 2-4 weeks. Wounds will be observed for any signs of infection, photographed weekly (along with the respective "control" wounds) as able.

6.4 Subsequent Wound Treatment

It is recognized that most participants will have additional wounds (including the control lesions from the initial treatment event) which could benefit from healthy tissue grafting. Patients will be offered treatment of up to 3 additional regions if at least 12 weeks has passed since the last treatment, if there were no unacceptable outcomes from previous graft procedures and if the original donor is available. Subsequent treatments would not contribute to the primary and secondary study endpoints, but data will be collected in a similar fashion including weekly photographs (as able) for clinical care and follow-up of wound BSA with wound BSA analyzed ideally at 6 and 12 weeks (as well as additional follow-up timepoints for other treatment sessions/clinical care) for publication.

Patients who have additional wounds that could benefit from CelluTome after one year of treatment with no significant adverse events attributable to the CelluTome procedure will be offered to extend treatment and re-enroll for an additional year. Re-consent would then occur with each annual follow-up if the patient wishes to extend treatment with the intent of collecting data for publication; otherwise subsequent sessions could be completed off-protocol as compassionate care without data collection for publication. If additional wounds are treated the original schedule (i.e. 1 year follow-up) will be adhered to as the 1st set of treated/control wounds are for the study endpoints. Subsequent wound treatment will be treated as an independent event (i.e. start as a new day 0) and the schedule in section 8 will serve as a guideline, therefore deviations from this schedule for subsequent treatment sessions will not be considered protocol deviations. Because subsequent treatments will not contribute to the study endpoints, follow-ups (weeks 6 and 12, at 1 year), may be performed remotely or at a medical facility closer to home with the results sent to our institution. Although it is recommended the patient return for follow-up assessment at approximately 12 weeks and 1 year post initial grafting session, remote or local follow-up will be allowed.

7 Expected Risks

7.1 Donor

We do not expect any significant side-effects as this procedure has been accepted as safe in the field and used for treatment of number of skin conditions, such as vitiligo and diabetic leg ulcers, with an excellent safety profile.[16-19] Of note, though the harvested tissue (epidermis) does not cross the basement membrane, the procedure is essentially painless (donor will feel warmth and mild pressure at the site) and there is infrequent, mild scarring and/or hypopigmentation, we will still examine the sites of epidermal harvest via weekly photographs until healed (as able) and at 12 weeks as able for any abnormalities detectable by physical examination.

7.2 Recipient

Even though the graft and recipient are uniquely and fully HLA matched, there can be local (and much less likely systemic) effects of grafting. Most prominent among these is non-engraftment (sloughing off the graft) because of subclinical infection, ongoing fibrosis and—related to that—inadequate vascular supply at the recipient site. This can be accompanied by local inflammation with systemic febrile reaction, though none would be expected to be serious adverse events.

8 Schedule of Events

8.1 Patient (Recipient – Arm A/re-enrollments Arm C)

	Prior to CelluTome Treatment	Day Of Harvest (Day 0)	Weekly Until 12 Week Visit[3]	6 Weeks (+/- 1 Week)[5]	12 Weeks (+/- 1 Week)[6]	1 Year (+/- 3 Months)[6]
Written consent	X					
Physical exam		X		X	X	X
Identification by physical exam and documentation of EB wounds[1]		X				
Donor chimerism	X[7]					
Photographs of the targeted lesions (and "control" lesions as able)	X anytime between baseline and harvest		X by parent or caretaker via iphone or similar device	X	X	X
Transfer of the epidermal micrografts from donor to the recipient sites		X				
Assessment of adverse events		X		X	X	X
Quality of life - itch and pain scales, (iscorEB)[2]	X			X	X	X
Research skin biopsies[8]	X					

1 – a maximum of 3 EB regions will be identified for treatment with additional wounds considered controls (as able). If there are no significant side effects, control wounds and/or other wounds may be treated after a minimum of 12 weeks of observation after the initial grafting per section 6.4.

2 –testing/procedures may be waived, if performed recently as part of follow-up on an EB treatment protocol (i.e. MT2009-09 or MT2015-20) or as standard of care, if the results are available for this study. Will attempt collection of full iscorEB at annual visits in accordance with standard of care, but at minimum, patient portion is requested for analysis of pain and itch.

3 – If possible

4 – footnote deleted

5 -May be conducted remotely through UMN provider or the patient's home/local provider if physical assessment indicated and the patient is unable to return to UMN

6- Ideally at UMN site unless patient is absolutely unable to return, in which case local or remote assessment should be completed with results sent to the PI and study staff

7- Peripheral blood donor chimerism should be measured within 21 days of grafting and be >/= 5% and stable. Stability of chimerism will be determined by the protocol team and based on 3 peripheral blood chimerism values at least 1 month apart

8 – Three 4mm punch biopsies (optional) at a grafted site to examine immunofluorescence, electron microscopy, and donor engraftment taken at time of routine sedated procedure during the 1-year follow-up time period.

8.2 Donor (Arm A)

	Prior To CelluTome Treatment	Day Of Harvest (Day 0)	Weekly Until The Week 12 Visit[1]	6 Weeks And 12 Weeks (+/- 1 Week)[2]	1 Year (+/- 3 Months)[2]
Written consent	X				
Brief assessment	X			X	X
Donor infectious disease panel	X[3]				
Identification and documentation of harvesting sites (maximum of 3 sites)		X			
Epidermal harvesting		X			
Photographs of harvest sites		X (pre)	X by parent or caretaker via iphone or similar device	X	
Assessment of adverse events		X		X	X

1 – if possible; not needed once harvest site is healed per PI.

2 - Donors may be assessed remotely if unable to return to primary site

3 - Within 30 days of harvest

8.3 Patient (Self Donor – Arm B/re-enrollments Arm D)

	Prior To CelluTome Treatment	Day Of Harvest (Day 0)	Weekly Until The Week 12 Visit[3]	6 Weeks And (+/- 1 Week)[5]	12 Weeks (+/- 1 Week)[6]	1 Year (+/- 3 Months)[6]
Written consent	X					
Physical exam				X	X	X
Identification by physical exam and documentation of EB wounds[1]		X				
Baseline skin biopsy of unaffected (possibly revertent or mosaic) site and affected site to confirm somatic reversion[2]	X					
Identification and documentation of harvesting sites	X					
Photographs of the targeted lesions, (“control” lesions, and harvest sites as able)	X anytime between baseline and harvest		X by parent or caretaker via iphone or similar device	X	X	X

	Prior To CelluTome Treatment	Day Of Harvest (Day 0)	Weekly Until The Week 12 Visit[3]	6 Weeks And (+/- 1 Week)[5]	12 Weeks (+/- 1 Week)[6]	1 Year (+/- 3 Months)[6]
Epidermal harvest and transfer of the epidermal micrografts to the targeted lesions		X				
Assessment of adverse events		X		X	X	X
Quality of life , itch and pain scales (iscorEB)[2]	X anytime between baseline and harvest			X	X	X
Research skin biopsies[7]	X					

1 – a maximum of 3 EB regions will be identified for treatment with additional wounds considered controls (as able). If there are no significant side effects, control wounds and/or other wounds may be treated after a minimum of 12 weeks of observation after the initial grafting per section 6.4.

2 –testing/procedures may be waived, if performed recently as part of follow-up on an EB treatment protocol (i.e. MT2009-09 or MT2015-20) or as standard of care, if the results are available for this study. Will attempt collection of full iscorEB at annual visits in accordance with standard of care, but at minimum, patient portion is requested for analysis of pain and itch.

3– If possible

4 – footnote deleted

5 - May be conducted remotely through UMN provider or the patient's home/local provider if physical assessment indicated and the patient is unable to return to UMN

6- Ideally at UMN site unless patient is absolutely unable to return, in which case local assessment should be completed with results sent to the PI and study staff

7 – Two 4mm punch biopsies (optional) at a grafted site to examine immunofluorescence, electron microscopy, at time of routine sedated procedure during the 1-year follow-up time period.

9 Adverse Event Monitoring, Recording and Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE events can be downloaded from http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

9.1 Event Monitoring/Documentation of Donor Sites and Recipient Sites

Event monitoring will focus on: 1) the areas from which the skin cells are harvested and 2) the sites to which the skin cells are applied for signs of poor healing (inflammation, sloughing of skin) with or without systemic fever.

Any adverse event, CTCAE v 4 ≥ grade 3 and felt to be at least possibly related to the epidermal grafting procedure will be documented in OnCore. The trial will halt enrollment and be re-evaluated if a grade 4 (CTCAE v4) skin related event occurs in any one of the treated lesions per section 12.4 and is at least possibly related to the epidermal grafting procedure.

9.2 Event Reporting

Certain events, in addition to recording on the study's eCRF's, will require prompt reporting to the University of Minnesota according to the table below:

Agency	Criteria for reporting	Timeframe	Form to Use	Submission information	Copy to:
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); any unanticipated adverse device effect, new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm refer to http://www.research.umn.edu/irb/forms.html	within 5 business days of discovery	Report Form	irb@umn.edu	SAE Coordinator mcc-saes@umn.edu

10 Data Collection and Study Monitoring

10.1 Data Management

This study will report clinical data using The Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database utilizing study specific electronic case report forms. Key study personnel will be trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore forms. Patient demographics, patient specific study treatment calendars, adverse events, reporting of deaths, and other information will be placed in OnCore. The iScorEB results will be recorded in the BMT database. All biopsy results and photographs will be maintained and stored under the supervision of the PI.

10.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRFs) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use.

10.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at https://z.umn.edu/dsmp

For the purposes of data and safety monitoring, this study is classified as moderate risk. Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the trial's progress twice yearly.
- The PI will comply with at least twice yearly monitoring of the clinical protocol by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in section 9.2 to the Masonic Cancer Center's SAE Coordinator and the University of Minnesota IRB.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

11 Outcome Measures

Multiple wounds may be treated for each patient. The primary outcome measure is the percentage of grafts successfully treated. Therefore, the experimental unit for this pilot study will be the wound, not the patient. If the body surface area affected by the wound is at least 50% lower at 12 weeks relative to baseline, the graft will be considered successful. For the purposes of this study only the initial treatment session (of up to 3 wounds for treatment and an equal number of control wounds) will be used for fulfilling the primary and secondary study endpoints.

Healing of wounded skin is a fairly stereotypic process in simple wounds, but becomes complicated and complex in the setting of multiple factors converging on the status of a wound at specific time. This includes: type of EB, gene mutation causing the RDEB or JEB, polymorphisms in modifier genes (such as enzymes that recycle skin extracellular matrix), infection and inflammation (local and systemic), HCT factors (age, graft, type of conditioning, physical and immune complications), and resulting hematopoietic and skin chimerism. [20-24]

Therefore, we will use several measures in the recipient (the patient with EB) to establish a template for the most accurate and unbiased evaluation of benefits associated with epidermal grafting, as follows:

[1] **Physical exam**. Comprehensive evaluation will be performed at 0 (i.e., before) grafting, at 6 (remote visit via phone assessment possible if physician and family agree travel to U of MN not clinically indicated) and 12 weeks, and 1 year after grafting.

[2] **Photographs**. In addition to photographs taken with an iPad or similar device, a specialized camera or photograph with standard measuring device in picture may be used at these four time-points (0, 6 and 12 weeks and 1 year after grafting) per PI discretion to allow for quantitative assessment of wound size over time. Weekly photographs will also be requested as able until the week 12 visit to assess healing and for any adverse events.

[3] **iscorEB.** A validated, EB-specific scale used to capture data regarding quality of life, pain and itching will be obtained at 0, 6 and 12 weeks and 1 year after grafting. [20,23,24]

[4] **Donor assessments**. Follow up physical assessments, adverse event assessments, and photos can be performed remotely at donor's home clinic (if physical exam is clinically indicated) or with UMN physician (i.e. telephone call, skype, etc.), if donor is not able to return.

Of note, all these procedures are fully operational and have been used extensively to assess clinically relevant and actionable data in more than 50 individuals from families with RDEB and JEB evaluated at the UMN over last 8 years. As we have established immune tolerance after successful grafting of bone marrow or umbilical cord blood, we have been developing approaches to leverage this immune tolerance in local therapy. We plan to use Cellutome® as a complementary therapeutic measure for difficult to heal erosions after hematopoietic cell transplantation (Arm A) or in autologous setting for transfer of mosaic skin (Arm B). As the immune system of the recipient will "see" such skin grafts as "self" this is an elegant way of clinical application of acquired immune tolerance in transplantation biology.

12 Statistical Considerations

This is a pilot study with the purpose of determining preliminary efficacy and safety measures of epidermal skin grafting in EB patients. Arm A (graft from hematopoietic stem cell donor) and Arm B (self-donor) will be analyzed separately.

After 1 year on study, patients may re-enroll on the trial. These will be considered new subjects (each enrollment counts separately). Allogeneic recipient re-enrollments will be considered Arm C (treatment plan identical to Arm A) and self recipient re-enrollments will be considered Arm D (treatment plan identical to Arm B). Arm C and Arm D will not contribute to primary or secondary outcomes, but a descriptive summary of Arms C and D will be performed separately.

12.1 Trial Size

Target accrual is 25 patients on arm A and 10 subjects on arm B over three years. Analysis of the primary endpoint can occur 12 weeks after the last subject begins treatment. Some secondary safety endpoints will be collected for 1 year.

Up to additional 25 patients may re-enroll in the study – these patients count toward a total study enrollment goal of 60 but only the initial enrollments will contribute to primary and secondary outcomes.

12.2 Analysis of Primary Endpoint

Each wound will be photographed before treatment, and at 6 and 12 weeks after treatment. If the body surface area affected by the wound is at least 50% lower at 12 weeks relative to baseline, the graft will be considered successful. The primary outcome measure is the percentage of grafts successfully treated as evaluated by a "blinded" reviewer.

Multiple wounds may be treated for each patient. The degree of correlation in response is unknown, but we expect that different wounds on the same patient could respond differently. Therefore, the experimental unit for this pilot study will be the wound, not the patient. If a mean of 2 wounds are treated for each enrolled patient, the 95% confidence interval of the response proportion will be as follows:

Observed response	95% CI in Arm A (50 lesions)	95% CI in Arm B (20 lesions)
25%	(14%, 39%)	(9%, 49%)
50%	(36%, 64%)	(27%, 73%)
75%	(61%, 86%)	(51%, 91%)
90%	(78%, 97%)	(68%, 99%)

12.3 Analysis of Secondary Endpoints

The iscorEB (which includes measures of quality of life questionnaire, pain and itching) will be summarized by means and standard deviations. Changes over time will be compared using Wilcoxon signed-rank tests.

12.4 Safety Monitoring

The trial will halt enrollment and be re-evaluated if a grade 4 (CTCAE v4) or higher skin related event at least possibly related to the CelluTome device or skin transplant procedures occurs in any one of the treated lesions.

13 Ethical and Regulatory Considerations

13.1 Conduct of the Study

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the participants. The study will only be conducted after IRB approval has been obtained. The protocol, written consent document, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent (if minor - parents/guardians with minor assent) to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the decision is made to participate in the study, the appropriate consent documents will be signed. Participants who refuse to participate or who withdraw from the study will be treated without prejudice.

14 References

1. Bruckner-Tuderman L. Dystrophic epidermolysis bullosa: pathogenesis and clinical features. *Dermatol Clin* 2010; 28(1): 107-14.
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Appendix I – Eligibility Checklist – Patient/Recipient

Study of Epidermal Grafting Using the CelluTome® Epidermal Harvesting System for the Treatment of Individual Lesions in Persons with Epidermolysis Bullosa

Eligibility Checklist – page 1 of 1

Participant initials ☐☐☐☐ Patient ID ☐☐☐☐ (assigned in OnCore)

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the participant from study entry.

		Yes	No
1	Diagnosis of Dystrophic Epidermolysis Bullosa (RDEB) or Junctional Epidermolysis Bullosa (JEB) with at least one wound, visibly free from infection and meets the eligibility for Arm A or Arm B	☐	☐
2	☐ Cell harvest from previous HCT donor (Arm A) – meets all of the following: - At least 6 months after HCT with donor hematopoietic chimerism - Peripheral blood donor chimerism should be measured within 21 days of grafting and be >/= 5% and stable. Stability of chimerism will be determined by the protocol team and based on 3 peripheral blood chimerism values at least 1 month apart off immune suppressive therapy - Original transplant donor is available and willing to be the epidermis donor	☐	☐ or check if not applicable ☐
3	☐ Self-donation (Arm B) – meets all of the following: - Proven somatic reversion - Site for skin grafting free of cellulitis and any other clinically evident abnormalities - Meets eligibility for Donor (appendix II)	☐	☐ or check if not applicable ☐
4	Insurance pre-authorization for procedure	☐	☐ or check if not applicable ☐
5	Voluntary written appropriate consent (Arm A patient or Arm B)	☐	☐

Having obtained consent and reviewed each of the inclusion/exclusion criteria, I verify that this participant is eligible.

Signature of enrolling researcher _____ Date _____

Appendix II – Eligibility Checklist – Donor

Study of Epidermal Grafting Using the CelluTome® Epidermal Harvesting System for the Treatment of Individual Lesions in Persons with Epidermolysis Bullosa

Eligibility Checklist – page 1 of 1

Donor initials ☐☐☐☐ Patient ID ☐☐☐☐ (assigned in OnCore)

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the participant from study entry.

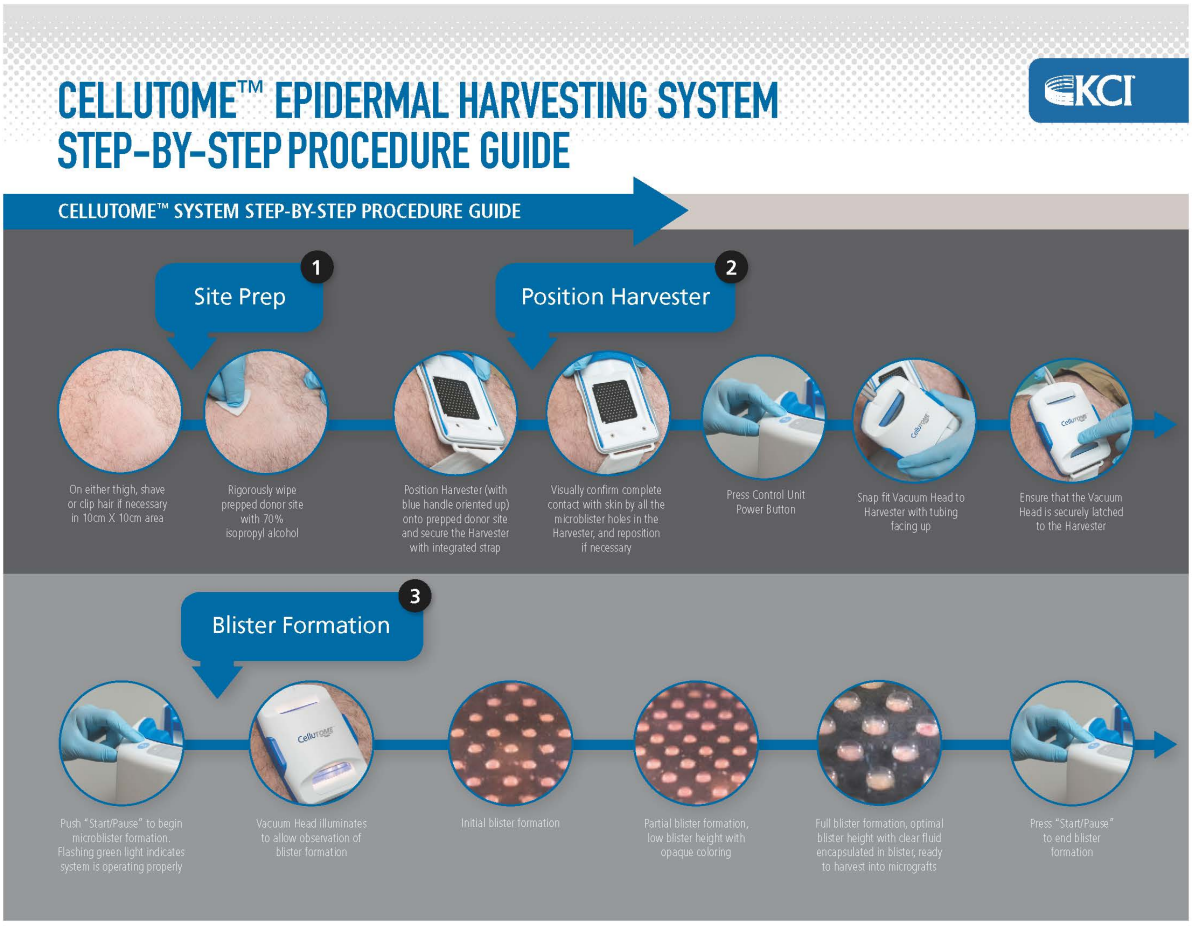
		Yes	No
1	Age > 2 years (based on prior safety testing of the device)	☐	☐
2	Healthy on physical examination in the opinion of the evaluating provider	☐	☐
4	Negativity for Hepatitis B and C, HIV, and HTLV1/2 within 30 days of donation	☐	☐
5	Voluntary written consent (donor or parent/guardian for minors) prior to any study related procedures (Arm A donor) Note: Patients enrolling on Arm B (Non-HCT autologous recipient), the Arm B consent form covers both donor and recipient information in a single document	☐	☐

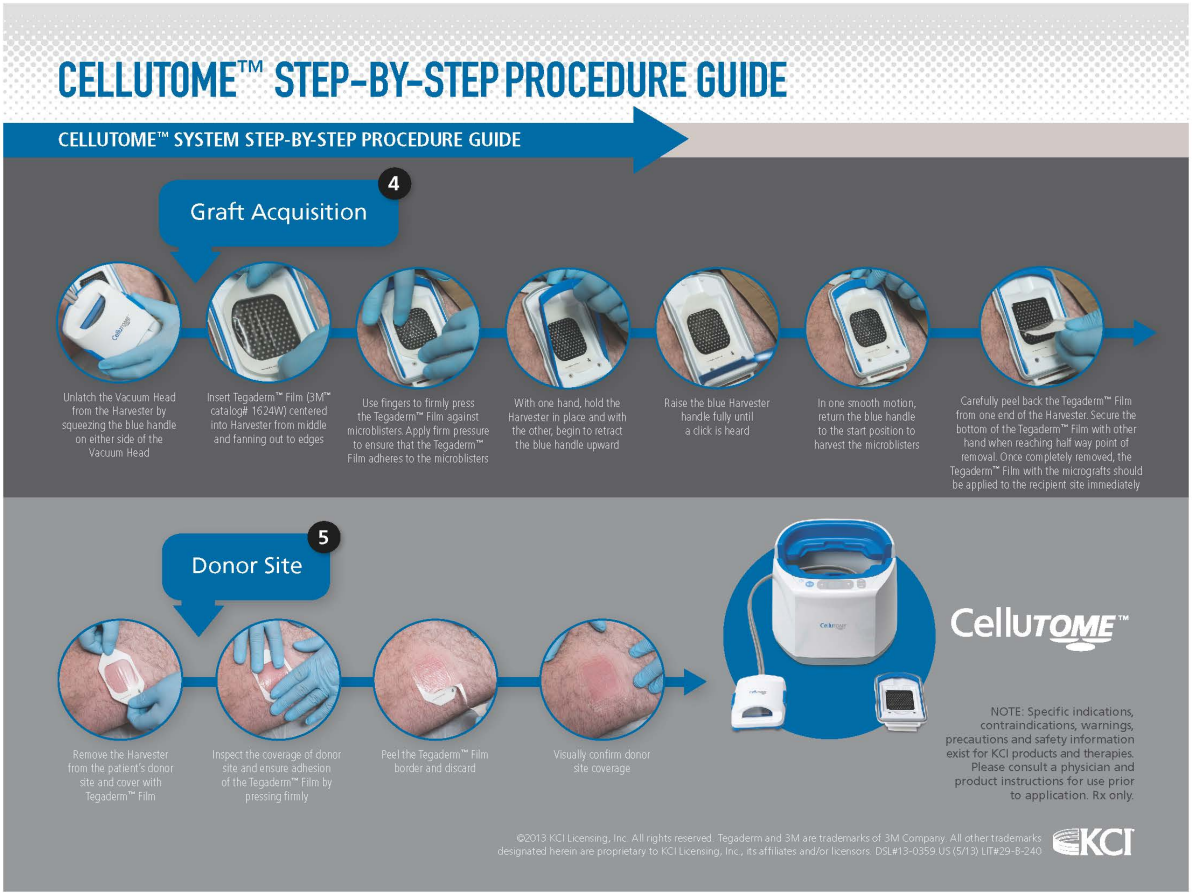
Having obtained consent and reviewed each of the inclusion/exclusion criteria, I verify that this donor is eligible.

Signature of enrolling researcher _____ Date _____

Appendix III – CelluTome Procedure Overview

Note: It is recognized in this CelluTome Procedure Overview that an adhesive dressing, Tegaderm® is used; however in this study a non-adhesive silicone mesh dressing is used and referenced as such through the protocol document.




CELLUTOME™ STEP-BY-STEP PROCEDURE GUIDE
CELLUTOME™ SYSTEM STEP-BY-STEP PROCEDURE GUIDE
4
Graft Acquisition
Unlatch the Vacuum Head from the Harvester by squeezing the blue handle on either side of the Vacuum Head
Insert Tegaderm™ Film (3M™ catalog# 1624W) centered into Harvester from middle and fanning out to edges
Use fingers to firmly press the Tegaderm™ Film against microblisters. Apply firm pressure to ensure that the Tegaderm™ Film adheres to the microblisters
With one hand, hold the Harvester in place and with the other, begin to retract the blue handle upward
Raise the blue Harvester handle fully until a click is heard
In one smooth motion, return the blue handle to the start position to harvest the microblisters
Carefully peel back the Tegaderm™ Film from one end of the Harvester. Secure the bottom of the Tegaderm™ Film with other hand when reaching half way point of removal. Once completely removed, the Tegaderm™ Film with the micrografts should be applied to the recipient site immediately
5
Donor Site
Remove the Harvester from the patient's donor site and cover with Tegaderm™ Film
Inspect the coverage of donor site and ensure adhesion of the Tegaderm™ Film by pressing firmly
Peel the Tegaderm™ Film border and discard
Visually confirm donor site coverage
CelluTOME™
NOTE: Specific indications, contraindications, warnings, precautions and safety information exist for KCI products and therapies. Please consult a physician and product instructions for use prior to application. Rx only.
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