University of Minnesota
Department of Pediatrics
Blood & Marrow Transplant Program

Biochemical Correction of Severe Epidermolysis Bullosa by Allogeneic Cell Transplantation and Serial Donor Mesenchymal Cell Infusions

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Principal Investigator/IND Sponsor
Jakub Tolar, MD, PhD

Co-Investigators:
Bruce R Blazar, MD*
Kathryn Dusenbery, MD*
Christen Ebens, MD, MPH
John A McGrath, MD*
Kristen Hook, MD*
David H McKenna, Jr., MD*
Paul J Orchard, MD
Mark J Osborn, PhD*
John E Wagner, MD
Angela Smith, MD
Troy Lund, MD
Kristina M. Nelson, PharmD*
Cathryn Jennissen, PharmD, BCOP*
*will not consent study participants

Biostatistician:
Todd E. DeFor, MS

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Revision History

Revision Number	Version Date	Summary of Changes	Consent change?
	07/09/2015	original to CPRC	n/a
	10/07/2015	In response to CPRC's initial review: • Corrected post-transplant CTX dose and schedule to make consistent throughout document • Added language to section 5 permitting the use of a known carrier as the donor in certain situations • Updated section 4.1 that sexually active patients must be on contraception Additional edits to section 10 including delete day 28 and day 60 skin biopsies and associated procedures, move several procedures from the research x chart to standard of care as SOC for this disease; clarifications in the donor chart – corrected chimerism sample language and clarified skin sample at point of BM biopsy will only be obtained on donors collected at the UMN, delete section 8.1 as mobilized apheresis is not used in this study	n/a
1	01/05/2016	Throughout protocol document – clarify collection of additional BM for MSC production at the time of collection for transplant is optional for the donor. Create two treatment arms, based on donor's consent status – no/refuses additional BM collection = Arm A transplant only, yes/agrees to additional BM collection = Arm B transplant + MSC infusions Section 7.5 – MSC Administration - remove language regarding collection of BM cells for MSCs and their manufacturing and storage and move to section 8 BM Harvest and MSC Production Section 8 – separate into subsections of bone marrow harvest for transplant, risks of BM donation, additional marrow harvest for MSC's in consenting donors and manufacture and storage of MSCs Section 10.2 – Research Related Procedures – move procedures associated with MSC infusions to new section and label this section as Arms A and B; delete reference to QOL as determined by play score/performance status in section 10.1 Section 10.3 insert new section titled Research Related Procedures (Arm B) to indicate MSC only related procedures and assessments Section 11.2.2 – clarify targeted toxicity assessment to be done on all patients at the day 60, 100, and 180 time points Title page, page 3 and section 12.3 – change IND sponsor from John Wagner to Jakub Tolar	yes

Revision Number	Version Date	Summary of Changes	Consent change?
		Other minor clarifications and edits through-out	
2	04/04/2016	Update design throughout protocol including synopsis and section 3 Study Design – creates two treatment arms to accommodate donors who refuse to consent to the collection of addition bone marrow for the manufacturing of MSC cells for the serial infusions: Arm A – HCT only, Arm B – HCT plus serial post-transplant MSC infusions Section 1.3, synopsis - Add correlative (research related, non-clinical) objectives section and move two of the secondary objectives to this section; Sections 14.1 and 14.2 – update endpoints to match objectives, update and expand statistical analysis Section 5.5 – delete lactating as a donor exclusion criteria – add rationale with associated citations Clarifications and Edits: Schema page – redesign presentation using symbols to improve understanding of treatment plan Section 5.6 – clarify donor match selection and priority Section 7.1.1 and Synopsis/Schema – clarify rabbit ATG (hymoglobulin®) to be used, replace Solumedrol with the generic name methylprednisolone as the pre-med Section 7.1.4 – update TBI to current language Section 7.4.1 – add a target dose range for tacrolimus Section 7.5 – clarify MSC will be thawed at “bedside”, delete sentence regarding study stopping rule Section 8.1 - update (decrease) targeted cell dose during BM harvest. Add unrelated donor targeted cell dose Section 8.3 – change marrow target for MSC production from a range (25-35 ml) to 30 ml Section 10.1 – clarify SOC clinical evaluations by adding footnotes, add carnitine testing and other minor edits Section 10.2 – add description of iscoreEB Other minor edits and clarifications	yes
3	07/06/2016	Add option for re-transplant in event of graft failure (lack of engraftment, autologous recovery, loss of graft) either on this protocol (using Arm C) or on the UMN BMT Graft Failure protocol (MT2013-06) Though-out document – increase the window (from ±7 to ±14 days) for each MSC infusion	Yes, plus new re-transplant consent

Revision Number	Version Date	Summary of Changes	Consent change?
		Synopsis, Section 1.2, Section 14.1.2 - correct wording for 2nd objective/endpoint from double chimerism to CD3+ and CD15+ and specify all time points to be done per x-chart in section 10.1 Synopsis, Section 4.1 and Appendix I – simplify disease related eligibility, simplify renal eligibility requirement Section 7.2 – edit for clarification infusion of BM cells Section 7.4.1 – expand tacrolimus administration details Section 10 – add baseline pregnancy testing for patients and donors Section 10.1 – move iscorEB and associated footnote from research x chart to SOC Section 10.4 – move baseline chimerism to screen Section 13 and 14.1.3 – indicate that skin samples may be collected at additional time points as clinically indicated Section 14.2 – clarify that Arms A and B will be combined for assessment of the primary, secondary and transplant related endpoints Section 14.4 – create separate stopping rules based on diagnosis (JEB vs RDEB) – clarify graft failure as aplastic and define TRM as transplant related mortality Section 15 – add 3 additional references Through-out document change iscorEB nomenclature (don't capitalize the "i") – not tracked Other administration edits and clarifications through-out	
5	08/23/2016	--Synopsis, sections 1.2 and 14.1.2: Expand secondary objective of comparing baseline iscoreEB to the year 2 iscoreEB, replace CD3+ and CD15+ with myeloid and lymphoid, respectively --Synopsis, sections 3 and 14: increase annual enrollment to up to 25 patients --Schema, sections 2.3, 3, 7, 7.4.1 and 10.1: omit tacrolimus for patients with an HLA identical donor --Schema, sections 3, 7.5, 7.8, 10.3: clarify that the time points for MSC infusions are the targeted time points but may be altered at the discretion of the treating physician without being considered a protocol deviations --Schema, sections 3, 7.8: clarify for a re-transplant (arm c), the original donor is preferred but not required --Section 4.1 and appendix I: replace pulmonary function requirement for oxygen saturation to adequate in the opinion of the enrolling investigator	yes

Revision Number	Version Date	Summary of Changes	Consent change?
		--Sections 5 and 10.4 and appendix II: clarify that unrelated donors will be assessed, consents and collected per local donor center; simplify donor criteria --Section 7.1.3 Remove IV hydration from pre-transplant Cytoxan dosing plan --Sections 7.5, 9.3,10.3 and 10.4 headers – include " and if applicable, Arm C" --Section 7.7: simplify duration of study participation language Add Drs Angela Smith, Troy Lund and Weston Miller as co-investigator	
5.1	10/24/2016	Administrative amendment (affects Section 7, Treatment Plan, only) • Allow infusion of MSCs through peripheral intravenous line (PIV) when a central line is not available. • Revise O2 monitoring guidelines to allow monitoring at regular intervals and if clinically indicated during the 2 hours post-MSC administration	No
6	11/02/2016	--Formally incorporates changes from administrative amendment 5.1 --Synopsis, Schema and Study Design added Arms D and E to capture data on subjects treated at the higher dose of TBI --Schema & Section 7.1.4 Increased dose of TBI to 400 cGy for first transplant --Section 3 added rationale for administering remaining MSC cells in the case of a second transplant and increasing the dose for TBI --Section 10.1 clarified daily assessments only until engraftment --Section 14.2, 14,3 and 14,4 clarified statistical analysis and safety monitoring plan for after TBI dose increased from 300 to 400 cGy	No
7	11/23/2016	--Schema, Sections 2.3, 3, 7, and 14 clarified that TBI administration will be separated into two fractions of 200 cGy each (on a single day); other minor edits	Yes
8	02/09/2017	Section 4.1, 5, 10.1, 10.4, eligibility checklist – PRA results added to donor screening Section 4.2, eligibility checklist - beta 3 laminin JEB mutants added to exclusions Section 5 – marrow may be supplemented with cryopreserved cord blood	No
9	09/14/2017	Synopsis, Schema, section 2.2, 3, 7, 7.5, 8.3, 10.1, 10.3 -- increased number of MSC infusions Schema, section 1.3, 3, 7, 7.6.1, 95, 10.1, 10.4, 14.1.3, Appendix II – added CelluTome skin grafting to supportive care Section 10.1 & 13 – clarified number of skin samples taken	Yes

Revision Number	Version Date	Summary of Changes	Consent change?
9A	10/31/2017	Synopsis; section 2.3 - Clarification requested by CPRC review of Amendment 9 (stipulation referred to change made in Amendment 8).	No
9B	10/31/2017	Correction of typographical errors in schema (arm names)	No
NA	12/11/2018	Consent revision as requested by IRB	Yes
10	2/05/2019	Updated investigator team Closure of Arms A and B to enrollment due to excess non-neutropenic graft failure and replacement by subsequent arms Addition of Arms F and G for HLA-mismatched stem cell recipients – the treatment regimens on these arms have increased intensity (addition of busulfan) to prevent graft failure Addition of Appendix VI regarding busulfan dose selection, AUC monitoring and algorithm for dose modification Addition of clinical monitoring of CelluTome skin grafting	Yes

PI/IND Sponsor Contact Information:

Jakub Tolar, M.D., Ph.D.
Professor
Hematology/Oncology and Transplantation
Department of Pediatrics
MMC 366 Mayo
420 Delaware St SE
Minneapolis, MN 55455
612 626-2912 (phone)
612 624-3913 (fax)
tolar001@umn.edu (email)

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SYNOPSIS

Study Design:	This is a single-institution, phase II study to determine the event-free survival at 1 year post allogeneic transplant with or without serial mesenchymal stem cell (MSC) infusions from a related donor (HLA identical, mismatched or haploidentical) or matched unrelated donor for the biochemical correction of severe epidermolysis bullosa (EB). A single marrow harvest is performed to collect the stem cells for the transplant procedure and, with the donor's consent, the collection of an additional 40-50 ml sample for MSC production for post-transplant infusion on month (± 14 days) 2, 4, and 6; and additionally offered at 8 and 10 months. If the donor refuses to consent for the extra marrow collection, the patient will receive only the transplant. The patient will be enrolled, based HLA-matching and on the donor's consent to one of the following arms: Arm A: stem cell transplant alone using 300 cGy TBI (closed to enrollment) Arm B: stem cell transplant plus serial MSC infusions using 300 cGy TBI (closed to enrollment) Arm D: stem cell transplant alone using 200 cGy BID TBI for 8/8 HLA-matched bone marrow recipients Arm E: stem cell transplant plus serial MSC infusions using 200 cGy AND TBI for 8/8 HLA-matched bone marrow recipients Arm F: stem cell transplant along using 200 cGy BID TBI plus addition of low dose Busulfan for HLA-mismatched bone marrow recipients Arm G: stem cell transplant plus serial MSC infusions 200 cGy BID TBI plus addition of low dose Busulfan for HLA-mismatched bone marrow recipients In event of graft failure (failure to engraft, autologous recovery or loss of graft), patients may be eligible for re-transplant on this study (Arm C) or through the University of Minnesota BMT protocol MT2013-06 (if <1 year from first BMT) or on this study (Arms D-G) (if >/=1 year from first BMT).
Primary Objective:	To estimate the event-free survival rate by 1 year post-transplant with an event defined as death or a 50% increase in a patient's iscoreEB from baseline
Secondary Objectives:	• To document the percentage change of a patient's iscoreEB at 1 and 2 years as compared with baseline • To determine the incidence of transplant-related mortality (TRM) at day 180 • To describe health quality of life at 1 and 2 years as compared to pretreatment results • To determine proportion of lymphoid and myeloid chimerism at various time-points (days 28, 60, 100, 180, and year 1 and 2) among surviving patients
Key Eligibility:	Diagnosis of severe form of EB with documented collagen, laminin, integrin, keratin or plakin deficiency 0-25 years of age An available healthy HLA matched or partially HLA matched related or unrelated donor for HSC graft and MSC Adequate renal, hepatic, cardiac and pulmonary function

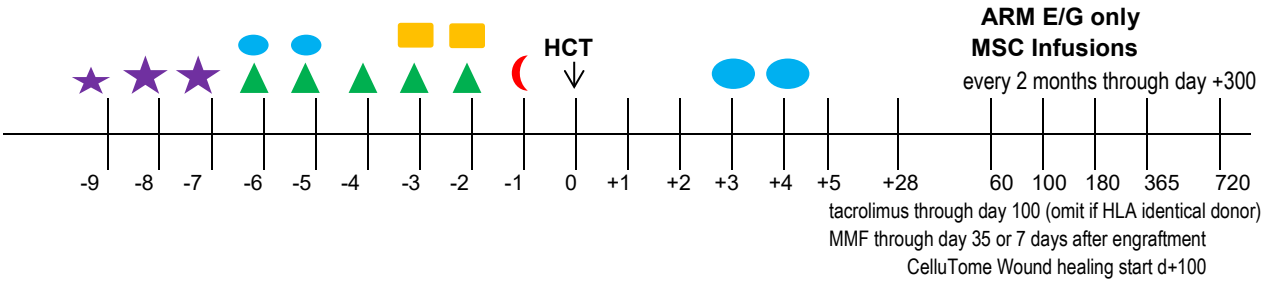
Key Exclusion:	Evidence of HIV infection or known HIV positive serology; current active serious infection; diagnosis of squamous cell carcinoma; beta-3 laminin JEB, donor with EB; pregnancy
Enrollment Plan:	Enroll up to 25 patients per year for a total of 69 patients An optimal interim analysis for futility without suspension of accrual due to the long primary endpoint of event-free survival at 1 year post HCT will be performed after 36 patients are enrolled. Given the change in conditioning (300 cGy TBI to 200 cGy bid TBI, then inclusion of low dose busulfan), enrollment will start over for the purpose of analyses; the currently enrolled 15 patients plus the planned new cohort of 69 patients for a total of 84.

SCHEMA

Treatment arms based on HLA-match and donor's level of consent

- **ARM D or ARM F: stem cell transplant** (if donor refused collection of additional BM cells for MSC production) Arm D: 200 cGy BID TBI effective November 2016 Arm F: Addition of busulfan for HLA-mismatched BM effective January 2019
- **ARM E or ARM G: stem cell transplant plus serial MSC infusions** (if donor consented to additional BM cell collection for MSC production) Arm E: 200 cGy BID TBI effective November 2016 Arm G: Addition of busulfan for HLA-mismatched BM effective January 2019

	HLA-matched	HLA-mismatched
Donor consents for MSC production	Arm E	Arm G
Donor refuses MSC production	Arm D	Arm F



Preparative Regimen

rabbit ATG (Thymoglobulin®) 0.5 mg/kg IV over 6 hours on day -9 with pre-meds (acetaminophen, diphenhydramine and methylprednisolone) and
rabbit ATG (Thymoglobulin®) 2 mg/kg IV over 4 hours on day -8 and day -7 with pre-meds (acetaminophen, diphenhydramine and methylprednisolone). Continue methylprednisolone taper through day -2.
cyclophosphamide 14.5 mg/kg IV over 1 hour on days -6 and -5 with mesna 2.9 mg/kg IV 5x/daily
fludarabine 30 mg/m^2 IV over 60 minutes on days -6 through day -2 See section 7.1.2 for dose adjustments based on renal function, age or weight.
busulfan IV over 3 hours on days -3 and -2 for HLA-mismatched BM recipients only (Arms F and G). See section 7.1.3 and Appendix VI for dose based on weight.
TBI: total body irradiation 200 cGy BID (400 cGy total) day -1 (reduce to 300 cGy if re-transplanted on Arm C)

Hematopoietic Cell Transplant

HCT: hematopoietic cell infusion

Post HCT chemotherapy

cyclophosphamide 50 mg/kg IV over 2 hours with mesna 10 mg/kg IV 5x/day on day +3 and +4

GVHD Prophylaxis

tacrolimus day +5 through day +100, then taper (omit if HLA identical donor – 8/8 match)
mycophenolic acid mofetil (MMF) day +5 through day +35 or 7 days after engraftment

Epidermal Grafting Using the CelluTome® Epidermal Harvesting System

Offered up to 3 sessions starting day +100 then additional sessions at 12 week intervals if clinically indicated

ARM E, and G only^

Serial MSC Infusions

MSC: donor mesenchymal stem cell infusions offered post-transplant every 2 months (±14 days) through 10 months post BMT; however these time points may be altered on an individual patient basis at the discretion of the treating investigator.

Re-Transplant Option for Graft Failure (not part of the above schema)

In the event of graft failure, time from first transplant should be considered when determining the intensity of conditioning required.

If <1 year since the initial BMT, two options are available:

1) Re-transplant the patient within this protocol (ARM C)
2) Re-transplant by enrolling patient on the University of Minnesota BMT protocol MT2013-06: Treatment of Graft Failure after HSCT (PI – T. Lund) while continuing follow-up per this protocol

If >/= 1 year since the initial BMT: Re-transplant on this protocol as a new subject on ARM D-G, whichever is appropriate.

Ideally the same donor would be used for both transplants; however it is recognized this is not always an option.

1 Study Objectives

1.1 Primary Objective

To estimate the event-free survival rate by 1 year after transplant with an event defined as death or a 50% increase in a patient's iscoreEB from baseline.

1.2 Secondary Objectives

- To document the percentage change of a patient's iscoreEB at 1 and 2 years as compared to baseline
- To determine the incidence of transplant related mortality (TRM) at day 180
- To describe health quality of life at 1 and 2 years as compared to pretreatment results based on Lansky Play Score (Karnofsky if > 16 years)
- To determine proportion of lymphoid and myeloid chimerism at various time-points (days 28, 60, 100, 180, and year 1 and 2) among surviving patients

1.3 Correlative Objectives

- To describe the pattern of biochemical improvement as measured by an increase in protein expression (collagen, laminin, integrin, keratin or plakin) and related structural and physical changes at days 100 and 180, then at 1 and 2 years
- To describe the pattern and durability of donor MSC engraftment in the skin (ARM B, E, and G)
- To describe wound healing for the patients who receive CelluTome as supportive care.

1.4 Transplant Related Objectives

- Estimate the probability of survival at 1 year
- Determine the incidence of grade II-IV and grade III-IV acute graft versus host disease (GvHD) at day 100
- Determine the incidence of chronic graft versus host disease at 1 year
- Time to neutrophil engraftment (defined as the first of three consecutive days after HCT that the patient's absolute neutrophil counts is $\geq 0.5 \times 10^9$ per liter)
- Determine the incidence of sustained neutrophil engraftment (neutrophil engraftment without autologous recovery)
- Time to platelet engraftment (defined as the first time after HCT that the patient can sustain platelet count $\geq 20 \times 10^9$ per liter for three consecutive days)
- Determine incidence of bacterial, viral and fungal infections at 1 year

2 Background

2.1 Dystrophic Epidermolysis Bullosa (DEB)

Dystrophic EB (DEB) is a group of heritable mechanobullous skin diseases characterized by skin fragility, blister formation, and scarring. The most severe forms of DEB 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 recessive dystrophic epidermolysis bullosa (RDEB) 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 DEB 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 DEB.

Type VII collagen (C7) is synthesized by both human keratinocytes and fibroblasts. It is secreted within 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. Genetic defects in the collagen type VII gene, *COL7A1*, result in RDEB. Ultrastructurally, abnormal morphology and low numbers or absence of AFs results in cleavage and detachment of the sublamina densa from the underlying dermis and extreme skin fragility (**Figure 1**). DEB can be inherited as an autosomal dominant or autosomal recessive 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.

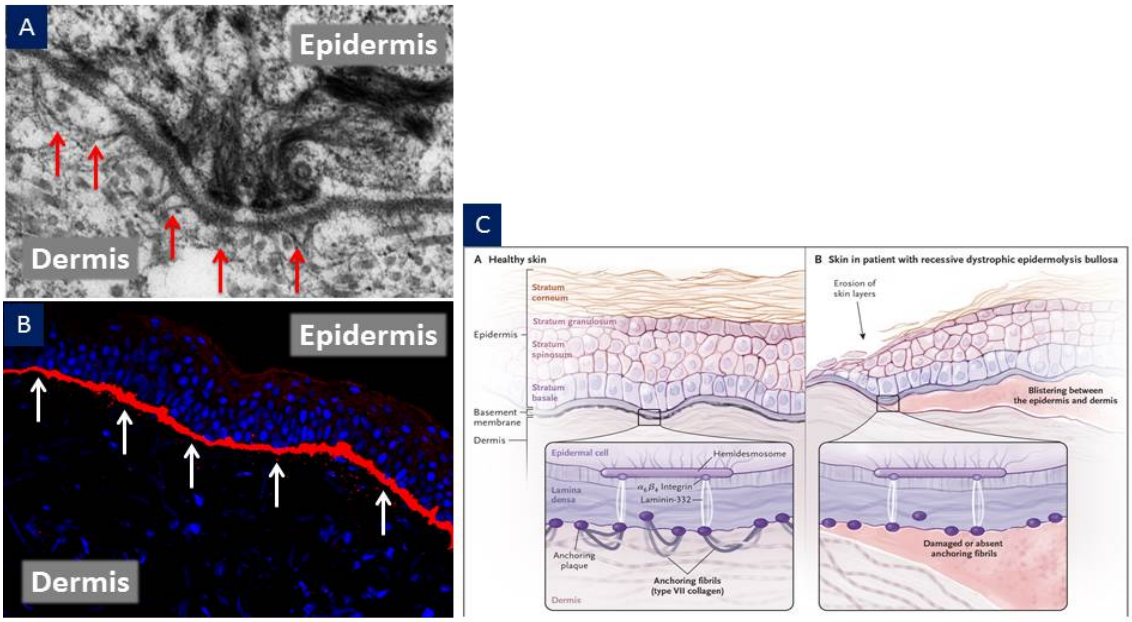


Figure 1. Structure of dermal-epidermal junction and phenotype of generalized severe RDEB. A. Ultrastructural features of normal human skin basement membrane zone with anchoring fibrils (arrows) extending from basement membrane to papillary dermis. B. Immune fluorescence-aided visualization of normal human type VII collagen (in red) at the dermal epidermal junction. Nuclei are blue (DAPI). C. Schema of healthy and RDEB skin (NEJM, 2015).

Several new therapies targeting wounds in RDEB have been developed, including local cell therapy with skin fibroblasts[4, 5] and mesenchymal stem/stromal cells,[6] gene therapy with gamma-retroviral[7] or lentiviral vectors,[8-10] and C7 protein replacement therapy.[11-13] These innovations emanated from decades of preclinical molecular and cellular studies by highly motivated teams around the globe, [3, 5, 14-20]. Despite the 'impossible-to-forget' appearance of a child with RDEB, the pathology of generalized severe RDEB is best understood as a global one, with multiple external and internal organ systems affected (cutaneous and gastrointestinal) and with whole body responses (such as compromised nutrition, anemia, pruritus, chronic inflammation, local and systemic infections) amplifying the injury of the underlying C7 deficiency. Therefore, as an alternative to *local* gene, cell and protein therapies, we sought *systemic* and possibly permanent cross-correction of C7 in the extracellular matrix using durable transplantation of self-renewing blood and marrow stem cells.

2.2 Allogeneic Hematopoietic Cell Transplantation in EB

To investigate a possibility of systemic stem cell therapy for generalized severe RDEB, we transplanted congenic wild-type bone marrow cells (among a variety of

other stem cell types) into neonatal mice with a targeted disruption of the *Col7a1* gene[21] that results in widespread blistering and early death due to the absence of C7 expression. We hypothesized that there was a stem cell population in bone marrow that homed to injured skin near the DEJ and produced cellular progeny which secreted wild-type C7 protein in the area where it was needed. While various non-hematopoietic and hematopoietic cell populations from bone marrow failed to successfully correct the disease, it turned out that an infusion of highly purified bone marrow progenitors (CD150+ CD48- cells),[22] migrated to injured skin and secreted C7. AFs were partially restored, and blisters on paws healed[23] within the 14 day window prior to death of untreated affected pups. Thus, donor cells capable both of secreting C7 and homing to the injured mucocutaneous membranes led to partial correction of the disease phenotype. With this functional correction of C7 in a murine model of human RDEB, data from Chino *et al*.[24] also demonstrating similar effects with prenatal CD90-depleted bone marrow, and absence of any other curative approach for severe RDEB, we sought to examine the safety and efficacy of allogeneic blood and marrow transplantation as a treatment for children with the severest forms of RDEB.

Hematopoietic cell transplantation. Our first clinical trial[25] of systemic cellular therapy (hematopoietic cell transplantation, HCT) for a genodermatosis demonstrated that a) donor cells home to injured skin and do so in unexpectedly high numbers, b) expression of C7 is increased and sustained for many years after blood and marrow transplantation, and c) AFs gradually appear and increase in numbers. Clinically, nearly all patient have experienced some improvement in the maintenance of overall skin integrity (**Figure 2**). However, 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 as myeloablative conditioning (MAC) or RDEB disease progression, or both. Therefore, with the ultimate aim of

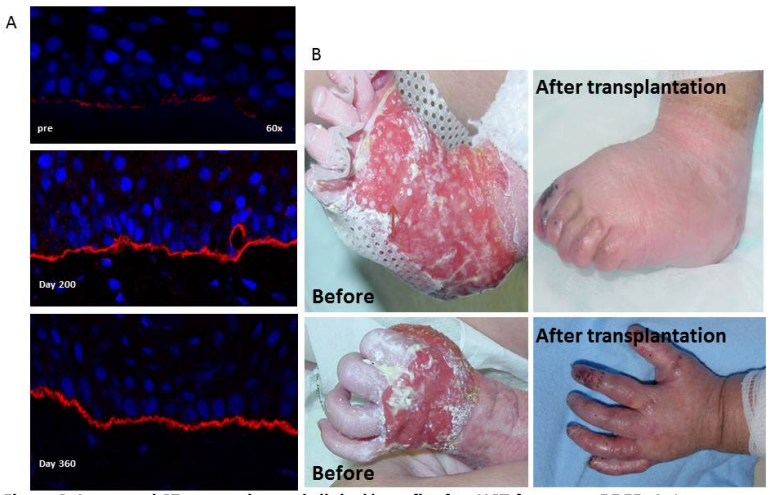


Figure 2. Increased C7 expression and clinical benefit after HCT for severe RDEB. A. Immune fluorescence-aided visualization of human type VII collagen (in red) at the dermal epidermal junction before HCT (top), 200 days after HCT (middle) and 360 days after HCT (bottom). B. Clinical comparison of extremity wounds before (left panels) and after HCT (right panels).

improving the efficacy and safety of HCT for the systemic and durable therapy of generalized severe RDEB, we sequentially modified the HCT treatment protocol (**Figure 3**) with the addition of supplemental cells and reduced intensity

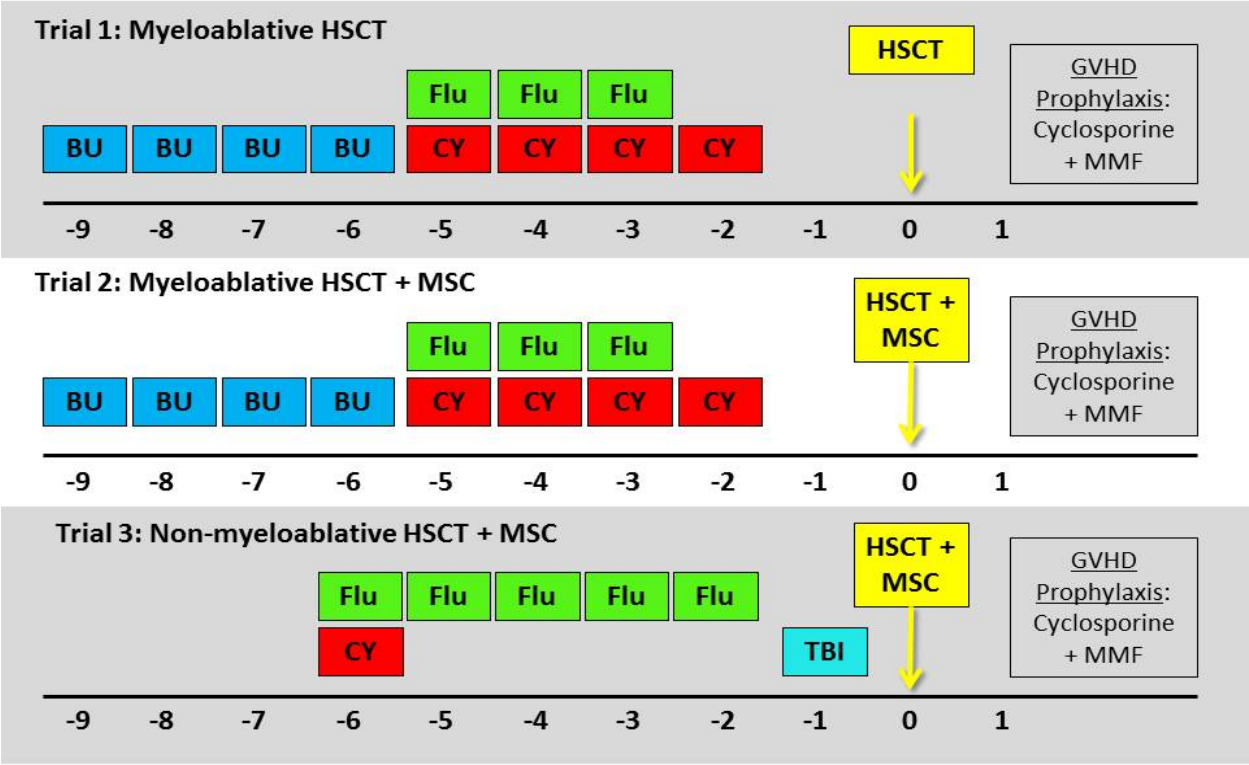


Figure 3. Evolution of the conditioning regimen for the treatment of generalized severe RDEB.

conditioning (RIC). The former, addition of mesenchymal stem/stromal cells (MSC), has been based on identification of non-hematopoietic donor cells in skin of HCT recipients (**Figure 4**), on the capacity of MSC to secrete C7, and on their immune-modulatory potential to mitigate graft-versus-host disease, one of the common immune complications of allogeneic HCT. The latter, RIC, has been introduced to assess whether less than complete hematopoietic chimerism can mediate adequate clinical response in the setting of conditioning regimen that is expected to result in less morbidity and mortality after allogeneic HCT.

Patients treated to-date. In total, between 2007 and 2014, 26 individuals (0.4-20y) with life-threatening, severe generalized form of RDEB have undergone allogeneic HCT. Of these, 13 were treated with a MAC (busulfan, fludarabine [FLU], and cyclophosphamide [CY]) and 13 were treated with RIC (CY, FLU, anti-thymocyte globulin, and low dose total body irradiation). RDEB subjects received HCT from related (N=15, all bone marrow grafts), or an unrelated donor (N=11; 6

bone marrow grafts and 5 umbilical cord blood grafts). For the recent 20 individuals (the first 6 have been reported in reference [25]), the patient characteristics, grafts and outcomes are summarized in **Table 1** for MAC and **Table 2** for RIC. In a cohort

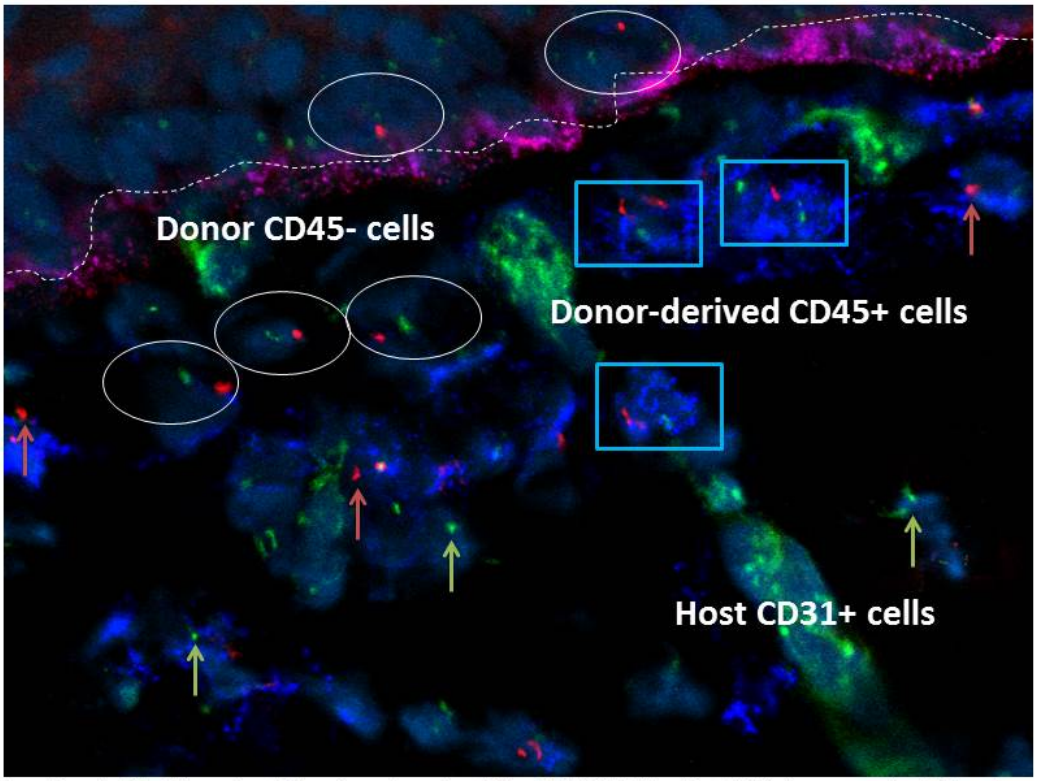


Figure 4. Engraftment of both hematopoietic and non-hematopoietic cells in RDEB skin after HCT. Y chromosome (red dot and arrow) and X chromosome (green dot and arrow) of male donor cells in skin of female recipient after sex-mismatched HCT. Some donor cells express pan-hematopoietic antigen CD45 (dark blue and light blue rectangles), other donor cells (non-hematopoietic cells in both epidermis and dermis) do not (white ovals). As expected in a recipient with an engrafted lympho-hematopoietic system, occasional donor hematopoietic cells were detected inside a dermal blood vessel (endothelium stained green with anti CD31 antibody). A linear band (fuchsia) at the dermal-epidermal junction (dotted line) shows type VII collagen.

of patients with another generalized severe form of EB, junctional EB (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 RDEB patients.

Table 1. Patient characteristics, grafts and outcomes for RDEB with myeloablative conditioning (MAC).

Patient	RII-1	RII-2	RII-3	RII-4	RII-5	RII-6	RII-7
Sex **Age at HCT**	Female 2 years	Female 9 years	Male 13 years	Female 1 years	Male 5 years	Male 4 years	Male 10 years
Clinical features[1]	Bloody emesis, corneal abrasions, anal fissure	Esophageal strictures	Pseudo-syndactyly, IDA, esophageal strictures, wheelchair bound	Neonatal seizure	Esophageal strictures, aspiration pneumonia	Neonatal sepsis, pneumonia, colitis, esophageal strictures, constipation	Neonatal intubation, esophageal strictures, Pseudo-syndactyly, corneal erosions, contractures, IDA
Donor: sex, HLA match	UCB, female, 5/6	1st UCB transplant, female, 5/6; 2nd UCB transplant, female, 5/6	UCB, female, 4/6	Sister BM, 8/8	Brother BM, 8/8	Unrelated BM, female, 8/8	Brother BM, 8/8
Cell dose[2]	11×10^7	5.5×10^7 4.4×10^7	8.17×10^7	5.23×10^8	1.41×10^8	3.1×10^8	3.5×10^8
HCT day	2/19/2010	3/25/2010 and 4/27/2010	4/29/2010	7/2/2010	9/10/2010	12/30/2010 and 7/14/12	3/11/2011
Severe adverse events	Respiratory failure, PTLD/EBV viremia	Graft failure, HHV6 viremia	Chronic GvHD (extensive), seizure, colitis, HHV6 viremia	RF: HD, VOD, mucositis: intubated, capillary leak syndrome, heart failure	Upper GI bleeding after esophageal dilatation	Fungemia, bronchopulmonary hemorrhage; RF: HD; PTLD: late graft failure, successful second transplant	RF: HD, acute GvHD (skin 3, grade 2), seizure, upper GI bleeding, respiratory failure, fungal sepsis
Skin donor chimerism[3]	30%	0%	33%	24%	21%	14%	97%
Biochemical correction	C7 increased	C7 increased[4]	C7 not increased	C7 not increased	C7 not increased	C7 not increased	C7 increased
Outcome: before → after HCT	75% → <25% BSA involved	Graft failure No change	No change	75% → <5% BSA involved	90% → <5% BSA involved	90% → 0% BSA involved	90% → <35% → 90% BSA involved[5]
Survival (as of 1/31/15)	Alive	Alive	Died of cGvHD on day 496	Died of VOD on day 101	Alive	Alive	Died of sepsis on day 212

[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 2×10^6 HLA-unmatched (3rd party) mesenchymal stromal cells. [3]Highest value shown. [4]The C7 increase occurred early after HCT (day 28) and later comparisons were not possible, or indicated. [5]The erosions healed between days 60 and 100, then progressively worsened.

Legend: BM, bone marrow; UCB, umbilical cord blood; BSA, body surface area; C7, collagen type VII; CB, cord blood; CSA, cyclosporine; EBV, Epstein-Barr virus; FTT, failure to thrive; GI, gastrointestinal; GvHD, graft-versus-host disease; HD, hemodialysis; HHV6, human herpes virus type 6; HLA, human leukocyte antigen; IDA, iron-deficient anemia; MA, myeloablative; PTLD, post-transplant lymphoproliferative disease; RDEB, recessive dystrophic epidermolysis bullosa; RF, renal failure; GI, gastrointestinal; VOD, veno-occlusive disease (sinusoid obstruction syndrome).

Table 2A. Patient characteristics, grafts and outcomes for RDEB with reduced intensity conditioning (RIC, 200cGy TBI).

Patient	**RIII-1**	**RIII-2**	**RIII-3**	**RIII-4**	**RIII-5**	**RIII-6**	**RIII-7**
Sex **Age at HCT**	Male 20 years	Female 2 years	Female 6 years	Female 7 years	Male 9 months	Male 3 years	Female 11 mo
Clinical features[1]	Severe contractures, wheelchair bound, pseudo-syndactyly, IDA, dental carries, depression	Bloody emesis, corneal abrasions	Esophageal strictures, corneal abrasions	Esophageal strictures, corneal abrasions	Hygroma, IDA, FTT, corneal abrasions	Esophageal strictures, corneal abrasions,	Bloody emesis
Donor: sex, HLA match	Unrelated BM, 8/8	Brother BM, 8/8	Brother BM, 8/8	Unrelated CB, 4/6	Unrelated BM, 8/8	Unrelated BM, 8/8	Unrelated BM, 8/8
Cell dose[2]	5.00×10^8	6.21×10^8	7.28×10^8	9.1×10^8	9.91×10^8	9.00×10^8	7.20×10^8
HCT day	6/7/2011	9/15/2011	2/23/2012	4/6/2012	7/13/2012	6/4/2013	7/16/2013
Severe adverse events	Colitis	Pneumonia, mixed chimerism (DLI)	Bacteremia	Graft failure	Bacteremia, mixed chimerism (DLI)	TEN, hemophagocytosis, fungal sepsis	Pruritus
Skin donor chimerism[3]	23%	2%	7%	0%	15%	11%	3%
Biochemical correction	C7 increased	C7 increased	Continuous expression of C7	C7 not increased	C7 increased	C7 increased	C7 not increased
Outcome: before→ after HCT	90% → <10% BSA involved	75% → <10% BSA involved	75% → <5% BSA involved	75% → <50% BSA involved	75% → <5% BSA involved	90% → <5% → >90% (secondary to TEN) BSA involved	75% → <5% BSA involved
Survival (as of 1/31/15)	Alive	Alive	Alive	Alive	Alive	Died of sepsis on day 152	Alive

Table 2B. Patient characteristics, grafts and outcomes for RDEB with reduced intensity conditioning (RIC, 300cGy TBI).

Patient	**RIII-8**	**RIII-9**	**RIII-10**	**RIII-11**	**RIII-12**	**RIII-13**
Sex **Age at HCT**	Male 3 years	Female 1 year	Male 11 months	Female 4 years	Female 4 months	Male 6 months
Clinical features[1]	Esophageal strictures	Severe oral mucosa blistering	Corneal abrasions	Esophageal strictures, corneal abrasions	Mild oral mucosa blistering	Moderate oral mucosa blistering
Donor: sex, HLA match	Sister BM, 8/8	Sister BM, 7/8	Mother BM, 8/8	Sister BM, 8/8	URD BM, 8/8	Sister BM, 8/8
Cell dose[2]	3.53×10^8 4.5×10^8	2.45×10^8	3.35×10^8	4.27×10^8	5.51×10^8	5.5×10^8
HCT day	8/20/2013 and 9/3/2014	5/7/2014	6/24/2014	9/24/2014	10/14/2014	12/02/2014
Severe adverse events	Graft failure (successful second transplant)	Acute GvHD (skin 3, grade 2), EBV; bacteremia	Mixed chimerism (DLI); bacteremia	Mixed chimerism (DLI); EBV viremia; bacteremia	Graft failure; pericardial effusion	Mixed chimerism (DLI); intussusception
Skin donor chimerism[3]	17%	22%	28%	0%	8%	13%
Biochemical correction	C7 not increased	Continuous expression of C7	Continuous expression of C7	C7 not increased	C7 not increased	Continuous expression of C7
Outcome: before→ after HCT	75% → <5% BSA involved	75% → <5% BSA involved	75% → <10% BSA involved	75% → <10% BSA involved	55% → <5% BSA involved	55% → <5% BSA involved
Survival (as of 1/31/15)	Alive	Alive	Alive	Alive	Alive	Alive

[1] All patients had: >55% skin surface covered with erosions and blisters; history of 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 2×10^6 HLA unmatched (3rd party) mesenchymal stromal cells. [3]Highest value shown.
Legend: BM, bone marrow; BSA, body surface area; C7, collagen type VII; FTT, failure to thrive; HLA, human leukocyte antigen; IDA, iron-deficient anemia; DLI, donor lymphocyte infusion; EBV, Epstein-Barr viremia; TEN, toxic epidermal necrolysis; RDEB, recessive dystrophic epidermolysis bullosa.

Safety 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 (N=13) cohort (p=0.3). Of the 20 recent RDEB 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 in comparison of related versus unrelated grafts, bone marrow versus umbilical cord blood grafts, and those who received additional MSC versus those who have not. One individual in each cohort (MAC: RII-7, RIC: RIII-9) developed acute GvH (grade 2, with stage 3 skin involvement - erythroderma); one individual developed extensive chronic GvH (MAC: RII-3).

Efficacy outcomes. Our primary efficacy measure was the event-free survival rate by 1 year after HCT with an event defined as death or failure to have demonstrable increase in C7 or other biochemical, structural or physical measure of improvement. The overall probability of 2-year event-free survival in the MAC cohort (N=13) was 62% (95% CI, 31-82 %) versus 82% (95% CI, 44-95%) for the RIC (N=13) cohort (p=0.3).

Donor-recipient chimerism. Hematopoietic engraftment was near-complete in those treated with MAC. As expected with lower doses of conditioning chemotherapy, engraftment was significantly lower in those treated with RIC: on average 26% in CD15 cells and 55% on CD3 cells 100 days after HCT. The maximum skin chimerism in those with successful hematopoietic engraftment was on average 31% in MAC cohort, 10% in RIC with 200cGy TBI, and 15% in RIC with 300cGy (**Tables 1** and **2**). Thus skin chimerism correlated proportionally with hematopoietic one, and provided structural and functional links between bone marrow and biochemical, structural and physical measures of mucocutaneous changes after HCT.

Biochemical measure - C7 expression. We observed increase in C7 immunofluorescence in two RDEB individuals treated with MAC (RII-1 and RII-7; in addition to those reported previously), and in four individuals treated with RIC (RIII-1, RIII-2, RIII-5 and RIII-6). In support of 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 individuals available for long term assessment, we were able to detect this increase at 1-3 years after HCT. In several others the change in C7 expression after HCT was less obvious as they showed,

despite severe clinical phenotype, baseline C7 immunoreactivity before HCT (RIC: RIII-3, RIII-9, RIII-10, RIII-13).

Structural measure - Anchoring fibrils (AFs). AFs are homotrimers of C7 and the most direct structural foundation of the C7 function at the DEJ. In some individuals (for example, RII-1) we saw no AFs despite increased C7 expression after HCT; in others (for example, RIII-1 and RIII-10) AFs have been seen after HCT.

Physical measure - Functional skin stability. Given that our ultimate goal for RDEB individuals is functional skin, we aimed to assess its integrity before and after HCT in a quantitative way. We employed a suction blister device whereby constant negative pressure is applied to skin and time to blistering is proportionate to capacity of skin layers to adhere to each other. As AFs mediate connectivity of epidermis and dermis the longer the time the skin is able to withstand the negative pressure applied to it is a measure of C7 expression, polymerization, and its functional integration in skin. Typically, individuals with RDEB experience blistering within 10 minutes, healthy people in approximately 60 minutes, and the obligatory heterozygotes (such as, the parents of RDEB children) in 30-50 minutes. Critically, in several representative examples from both MAC and RIC trials, serial assessments of RDEB subjects after HCT showed gradual increase in skin resilience under pressure (**Figure 5**).

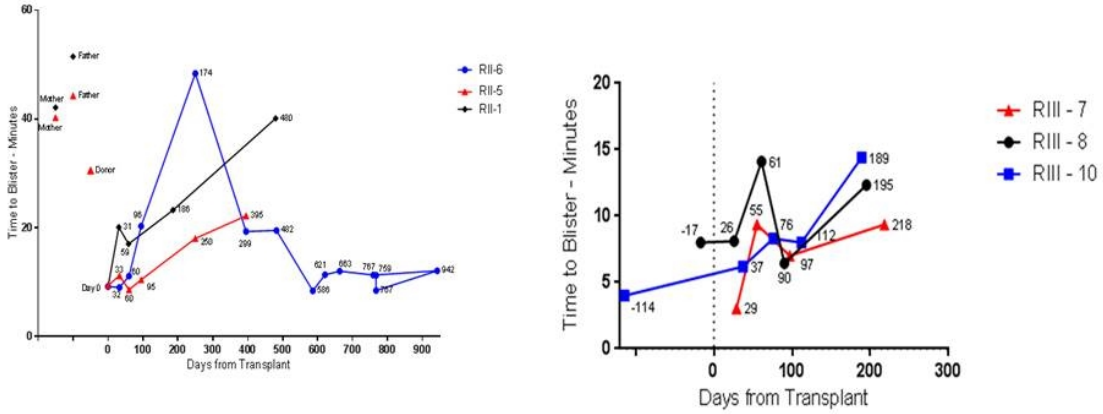


Figure 5. Functional skin stability. Individuals with RDEB treated with myeloablative conditioning (MAC) HCT (left panel). B. Individuals with RDEB treated with reduced intensity conditioning (RIC) HCT (right panel). Gentle negative pressure (12 mm Hg) is applied to the skin using a chamber with three 3 mm openings, and time to development of three full blisters is measured.

Clinical assessment. The individual metrics of biochemical, structural and physical changes in skin, including the compound measure of functional skin stability with suction blister device, while critical to local quantitative assessment of treatment intervention, do not capture entirely the complex pathology in its impact on health of individuals with RDEB. However, clinical photographs documenting skin responses as they differ among individuals with RDEB, unveil the impact of HCT in the most definitive way (**Figure 6**).

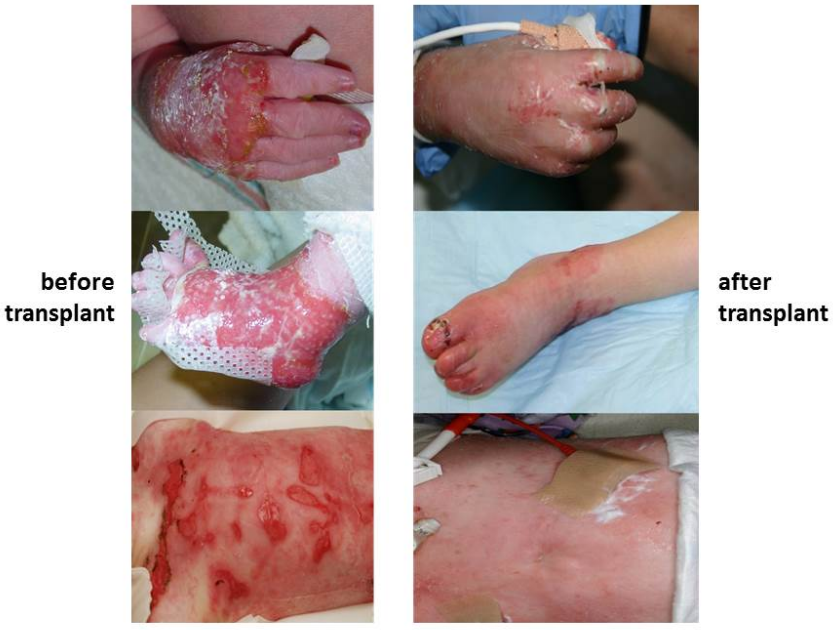


Figure 6A. Increased healing after HCT. Before (left panel) and after HCT (right panel).

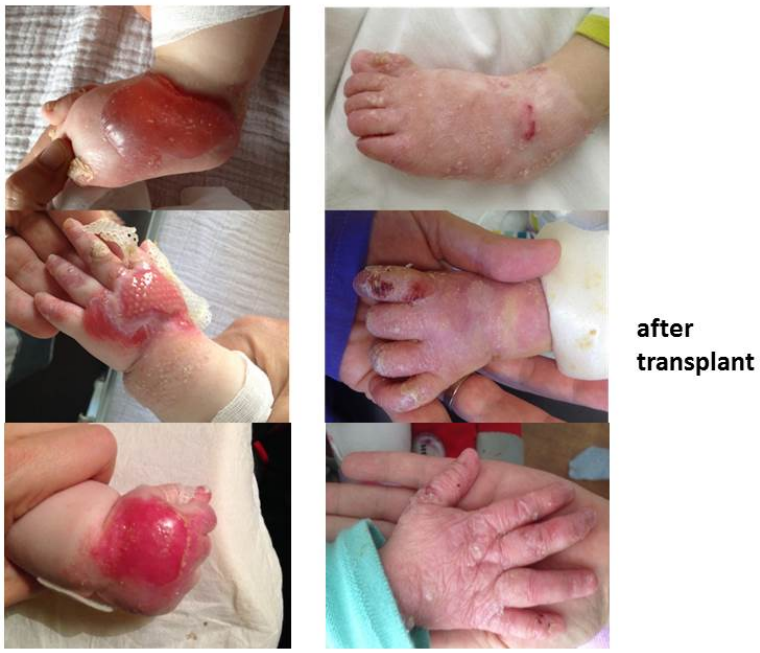


Figure 6C. Increased healing after HCT. Before (left panel) and after HCT (right panel).

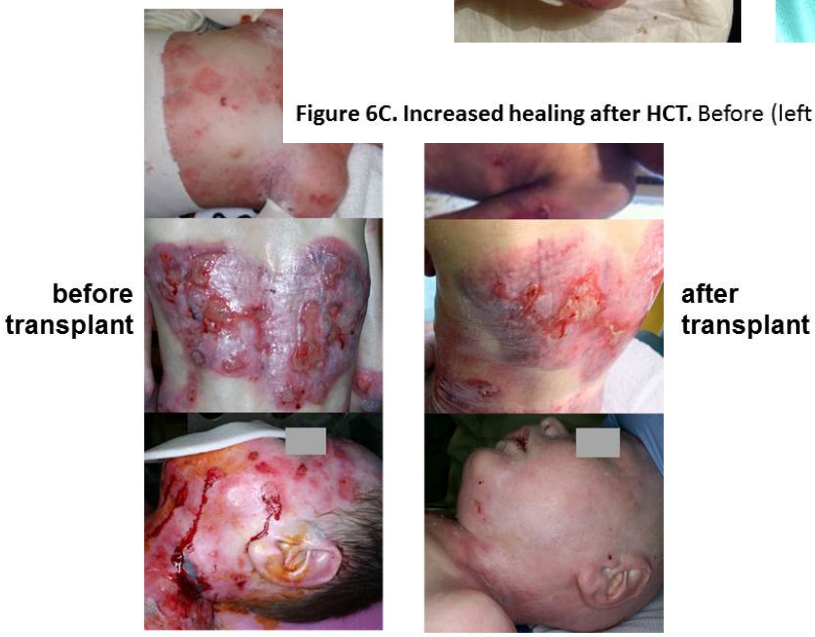


Figure 6B. Increased healing after HCT. Before (left panel) and after HCT (right panel).

2.3 Rationale for Treatment Plan

The key motivation for changing the HCT conditions in the individuals with EB is to improve efficacy and safety. The reduced intensity chemotherapy (RIC) approach has dramatically improved the safety (treatment-related morbidity and mortality). However, hematopoietic chimerism has been mixed and, expectedly, skin chimerism has been lower than that seen in the MAC cohort. Assuming that production of C7 (and other cutaneous adhesive molecules) is directly proportionate to the number of donor cells in skin, the higher skin chimerism is desirable, and—based on our comparison between MAC versus RIC groups—achievable with complete hematopoietic chimerism. The possibility of an acceptable safety profile together with near-complete hematopoietic engraftment has recently become possible with

improvements in related HLA-haploidentical HCT using high dose post-transplant cyclophosphamide[26-28].

The key benefits of this post-grafting strategy are [1] a dramatic reduction of severe GvHD (which has been limiting for early versions of haploidentical HCT), [2] the ability to limit post-HCT immunosuppression and thus allow for earlier and qualitatively superior immune reconstitution, and [3] the rapid availability of a donor[29-32]. For patients with EB specifically, this translates to [1] less skin injury (by GvH reaction) in an already damaged skin, [2] better and faster establishment of immune response in people with multiple open wounds, typically already colonized or infected with polymicrobial drug-resistant species, and [3] shortening the time between the decision to transplant and the HCT itself, which is particularly relevant in generalized severe JEB, which has a mean survival of less than 6 months of age.

Therefore, we propose to adapt the most robust and tested approach to deplete alloreactive cells *in vivo* by [1] administering high doses of cyclophosphamide in a narrow window after HCT (originally developed by scientists at the John Hopkins University School of Medicine but now adopted nationally and internationally for many neoplastic as well as non-malignant indications)[28, 33-35]. This plan uses combination chemotherapy (fludarabine and cyclophosphamide), immunotherapy (thymoglobulin), and radiotherapy (total body irradiation) prior to HCT. Post-transplant immunosuppressive therapy consists of high-dose cyclophosphamide with MESNA protective therapy, followed by tacrolimus (omit for persons with HLA identical (8/8) related donors) and mycophenolic acid mofetil (MMF) as described in detail in Section 7 (Treatment Plan).

Induction of tolerance with post-HCT cyclophosphamide is bidirectional: eradication of both donor and host alloreactive T cells reduces the incidence of both GvH and host versus graft (HvG) reactions[36, 37]. This is relevant for EB cohorts, as we have observed graft failure in several individuals (Tables 1 and 2). In order to advance from the state-of-art current platform, we will prioritize matched-sibling HLA-identical donors over T-cell-replete haploidentical donors and HLA-matched unrelated donors—using the same conditioning before and after HCT. Therefore, with or without crossing the HLA barrier, we expect to be able to rapidly identify a favorable donor for nearly all patients.

As RDEB and JEB are inherited genodermatoses, the haploidentical donor pool might be reduced but likely only minimally (in relatives with autosomal dominant mutations that are present in the compound heterozygosity state in the proband with autosomal recessive disease). Moreover, it is relevant that carriers of

autosomal recessive mutations (parents, the obligatory heterozygotes) are typically asymptomatic, and matched-sibling donors that are heterozygotic for these mutations have been used with success as HCT donors (with no discernible clinical difference in outcomes of such heterozygous-matched sibling HLA-identical HCT and homozygous wild-type matched sibling HLA-identical or HLA-matched unrelated donor HCT; Tables 1 and 2).

Moreover, systemic infusions of mesenchymal stromal/stem cells (MSCs) have been found safe in a large number of disorders, including RDEB[6, 38-44]. As they are not HLA-matched to the recipient, and sometimes not to the HCT donor, as expected these “third party MSCs” are rejected and thus their effects are limited, typically to several weeks. Our present approach is based on the advantage of most donors being related (matched sibling donors or haploidentical family members) to the recipient and the ability to culture MSCs from marrow at the time of HCT harvest. Accordingly, we will obtain, screen, and qualify these MSC cultures in time for systemic infusions at months 2, 4, and 6; and additionally offered at 8, and 10 months. The cell dose will be 1-2 million MSCs per kilogram of recipient weight; a dose that has repeatedly been shown to be safe in a clinical setting.

The sum of experimental and clinical data supports the conclusion that, contrary to the prevailing professional opinion of the last several decades, protein replacement therapy by allogeneic blood and marrow transplantation is not limited to freely diffusible molecules, such as enzymes, but also large structural proteins, such as collagens and perhaps laminins. While HCT improve the integrity of the skin and mucous membranes, future strategies will be built on the improvements of HCT (this protocol) and on concept of combination therapy (Figure 7).

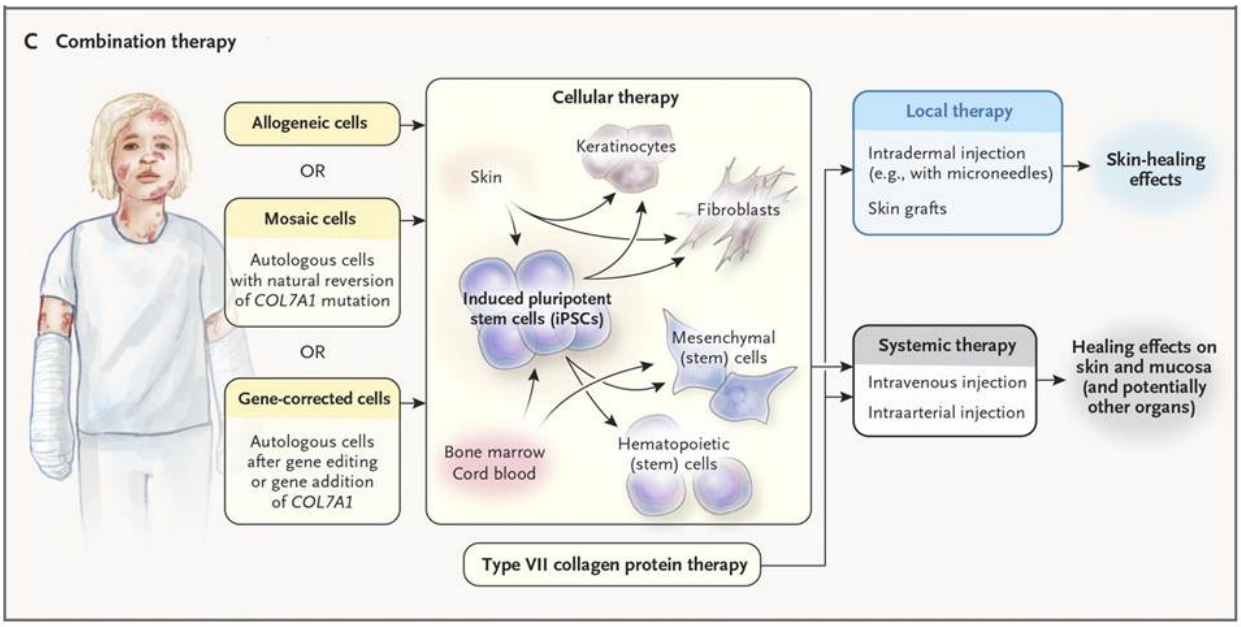


Figure 7. Combination Therapy for Epidermolysis Bullosa. In addition to systemic therapy with HCT, local therapies are possible. These include intradermal injection, use of microneedles, and skin grafts, but they do not reach all the physical manifestations of EB. The potential for a cure most likely lies in a combined approach of both local and systemic therapies, individualized to the specific patient (NEJM 2015).

Rationale for Re-Transplant in the Event of Graft Failure

Haploidentical hematopoietic cell transplantation (haplo-HCT) has become a new standard of care for selected malignant disorders[58,59]. This has been enabled by T cell depletion, either ex vivo (by T cell depletion of the graft) or in vivo (by post-transplant cyclophosphamide, PTCy, administration). Remarkably, this strategy has been shown to be reasonably safe and highly efficacious in non-malignant, typically genetic, disorders treatable by HCT. For example, sickle cell disease, thalassemia, mucopolysaccharidosis type I, dyskeratosis congenita, or Fanconi anemia have been treated successfully with haplo-HCT[60-68]. One of the limitations of haplo-HCT, especially with better tolerated reduced intensity conditioning, is failure to engraft. Fortunately, second transplants for either aplastic graft failure or recovery of autologous hematopoiesis have been almost always successful[69-72]. This is further supported by haplo-HCT data from diseases that present as "natural" graft failure-equivalents, that is, bone marrow failures such as seen in dyskeratosis congenita, Fanconi anemia or idiopathic severe aplastic anemia—all of which have been shown to respond favorably to allo-HCT. Therefore, our

approach of using second transplant after graft failure in patients with generalized severe EB, is expected to result in similar favorable safety and efficacy profile. This is likely to be further enhanced by the use of mesenchymal stromal/stem cells from allo-HCT donor after the second allo-HCT transplant. The plan for re-transplant includes administration of additional MSC doses after the second transplant, following the same schedule as the initial transplant. With depletion of marrow during the second transplant, the rationale for this repeated MSC administration is not different from the rationale for MSC administration in the initial transplant. MSC dosing at month 2, 4, 6, 8, and 10 may promote engraftment, skin healing and diminish the risk of graft versus host disease. In addition to this rationale, administration of MSCs following a second transplant will ensure consistency in the plan of care and outcome data.

Rationale for Increased Dose of TBI (300 cGy to 200 cGy BID)

As of November 2016, we have had incidences of autologous recovery after transplant (i.e. engraftment of the patient's own cells after transplant, rather than the donor cells). Although not unexpected, we do strive to reduce these occurrences as much as possible. Dr. Tolar, the study Sponsor-Investigator, consulted with other experts in BMT at the University of Minnesota, as well as international leaders in haploidentical transplant, to identify a plan to better promote engraftment of the donor cells. The resounding suggestion has been to increase the dose of TBI to 200 cGy BID (rather than 300 cGy). Leading BMT MDs in haploidentical transplants at Johns Hopkins (Drs. Symons, Luznik & Fuchs) have implemented this change in a similar patient population (i.e. non-malignant disease, marrow not previously prepped with chemotherapy/radiation), with much improved full donor engraftment (4/5 patients had full donor engraftment with 400 cGy TBI vs 11/34 patients with 200 cGy TBI) and no additional ill effects experienced by patients. (unpublished data, personal communication)

Rationale for Exclusion of beta-3 laminin JEB

Interim analysis of early subjects found no lasting benefit for the patients with beta 3 laminin 332 mutation.

Rationale for Addition of Low Dose Busulfan for HLA-mismatched stem cell recipients

As of November 2018, five of 14 (36%) enrolled patients exhibited graft failure with autologous recovery (no aplastic graft failure), including 2 of 8 patients receiving the increased dose of TBI (200 cGy BID as opposed to a single fraction of 300 cGy following the November 2016 protocol modification. A thorough interim evaluation of potential factors mediated autologous recovery was completed, considering

donor type, presence of recipient anti-HLA antibodies [both panel reactive antibodies (PRA) and donor specific antibodies (DSA)], pre-conditioning and day 0 absolute lymphocyte counts, and bone marrow CD34+ stem cell counts. Graft failure with autologous recovery predominated in haploidentical parent recipients (4 of 4, 100%; 1 of 3 in haploidentical sibling, 0 of 1 in HLA-matched related donor, and 0 of 5 in HLA-matched sibling recipients). Eight of 11 tested patients (3 receiving matched sibling bone marrow not tested) had positive PRA, though only 3 had DSA. Of those patients with DSA, the earliest two had graft failure with autologous recovery. The 3rd patient with DSA underwent a debulking protocol including immunosuppression to decrease production of antibodies and plasmapheresis to remove pre-existing antibodies to limit the risk of immune mediated graft rejection. This patient had successful donor engraftment. There were no statistically significant associations between engraftment and pre-BMT absolute lymphocyte count ($p=0.92$) or day 0 absolute lymphocyte count ($p=0.36$), or CD34+ stem cell dose ($p>0.99$).

The importance of DSA and potential role in immune mediated graft failure has been recognized and mitigation with debulking standardized. This is reflected in the current Donor Selection criteria. The engraftment barrier with haploidentical parent donors is likely the result of inadequate patient myeloablation. We strive to reduce the risk of this complication by escalating the intensity of conditioning for recipients of HLA-mismatched grafts with inclusion of low dose busulfan.

3 Study Design

This is a single-institution, phase II study to determine the event-free survival at 1 year post allogeneic transplant with or without serial mesenchymal stem cell (MSC) infusions from a related donor (HLA identical, mismatched or haploidentical) or matched unrelated donor for the biochemical correction of severe epidermolysis bullosa (EB).

A single marrow harvest is performed to collect the stem cells for the transplant procedure and, with the donor's consent; an additional 40-50 ml sample is collected at this time for the production of MSC for post-transplant infusions. If the donor refuses to consent for the extra marrow collection, the patient will receive only the transplant. The patient will be enrolled, based on the donor's consent to either:

Arm A: hematopoietic cell transplant alone using 300 cGY of TBI (closed to accrual)

Arm B: hematopoietic cell transplant plus serial MSC infusions using 300 cGY of TBI (closed to accrual)

Arm C: re-transplant using 300 cGy of TBI (regardless of original transplant arm) if <1 year from first BMT

Effective with the November 2016 version of the protocol:

Arm D: hematopoietic cell transplant alone using 200 cGY BID of TBI (400 cGy total) for recipients of 8/8 HLA-matched bone marrow

Arm E: hematopoietic cell transplant plus serial MSC infusions using 200 cGY BID of TBI (400 cGy total) for recipients of 8/8 HLA-matched bone marrow

Effective with the November 2018 version of the protocol:

Arm F: hematopoietic cell transplant alone using 200 cGy BID of TBI (400 cGy total) + addition of low dose busulfan for recipients of HLA-mismatched bone marrow

Arm G: hematopoietic cell transplant plus serial MSC infusions using 200 cGy BID of TBI (400 cGy total) + addition of low dose busulfan for recipients of HLA-mismatched bone marrow

A preparative regimen consisting of thymoglobulin, fludarabine, cyclophosphamide, and total body irradiation is administered prior to a T cell replete donor marrow infusion. Post-transplant cyclophosphamide is given on day 3 and 4 to deplete alloreactive cells.

Graft-versus-host-disease prophylaxis consisting of tacrolimus and mycophenolic acid mofetil (MMF) is started on post-transplant day 5 and continued through day 35 or 7 days after engraftment for MMF and day 100 for tacrolimus (start of taper). Tacrolimus is eliminated if a fully matched (HLA identical) donor is used.

Local wound therapy will be offered as clinically indicated using the FDA approved vacuum device (CelluTome®, Regulation number 878.4820). Patients will receive local wound therapy using epidermal skin grafting from the same donor (donor consent required) that provided the hematopoietic graft.

For patients enrolled in Arm E and G, MSCs will be infused every 2 months (± 14 days) through 10 months post-transplant (months 8 and 10 offered if patient is available); however these time points may be altered on an individual patient basis at the discretion of the treating investigator.

Disease reassessments will be performed at days 28, 60, 100, and 180, then at 12 and 24 months post-transplant.

In the event of graft failure (e.g. lack of engraftment, autologous recovery, loss of graft), time from the first transplant should be considered when determining the intensity of conditioning required.

If the 2nd transplant will occur <1 year since the first transplant, two options are available:

1) Re-transplant the patient within this protocol (Arm C)
2) Re-transplant the patient by enrolling on the University of Minnesota BMT protocol MT2013-06: Treatment of Graft Failure after HSCT (PI – T. Lund) while continuing follow-up per this protocol

If the 2nd transplant will occur >/=1 year following the first transplant: The 2nd BMT could be completed on this protocol as a new subject on ARM D-G, whichever is appropriate based on HLA-match and donor consent for MSC production.

Ideally the same donor will be used for the re-transplant; however it is recognized that this will not always be possible.

At the discretion of the treating physician and with the donor's permission, extra marrow for MSCs may be collected at the time of the bone marrow harvest. If the patient has donor MSCs available (either from the original transplant or the re-transplant), they may be infused in the same manner and schedule as detailed after the original transplant using day 0 of the second transplant. Targeted toxicities will be assessed at time of all MSC infusions.

All other tests/procedures and follow-up milestone visits would continue to be based on the initial transplant day for statistical analysis purposes. For those that are re-transplanted on arm C, standard of care and research testing will restart at second transplant day 0 in addition to the remaining follow-up milestones from initial transplant day. The window for these assessments may be expanded so these time points coincide if in the best interest of the patient.

4 Patient Selection

External Advisory Panel: Hematopoietic cell transplantation (HCT) for EB has spanned dermatology and transplantation biology in a novel fashion, and unquestionable RDEB expertise has been critical for success of the study. Therefore, we will continue to work with our External Advisory Panel (EAP): Professor John A. McGrath (St. John's Institute of Dermatology, King's College, London, United Kingdom), Professor Alain Hovnanian (Institut Federatif de Recherche Necker Enfants Malades, Paris, France) and Professor Katsuto Tamai (Department of Dermatology,

Osaka University, Osaka, Japan). The EAP reviewed data prior to and following HCT, and make recommendations on whether the EB phenotype of each individual considered for HCT is severe enough to make the potential risks of HCT acceptable.

4.1 Inclusion Criteria

- 0 through 25 years of age
- Diagnosis of a severe form of EB with documented collagen, laminin, integrin, keratin or plakin deficiency (by immunofluorescence staining with protein specific antibodies or Western blotting or by mutation analysis)
- Adequate organ function within 4 weeks of study registration defined as:
  - Renal: glomerular filtration rate within normal range for age
  - Hepatic: bilirubin, AST/ALT, ALP < 5 x upper limit of normal
  - Pulmonary: adequate pulmonary function in the opinion of the enrolling investigator
  - Cardiac: left ventricular ejection fraction ≥ 45%, normal EKG or approved by Cardiology for transplant
- Sexually active participants must agree to use adequate birth control for the during the study period (from before the start of the preparative chemotherapy through 1 year post-transplant)
- Available donor per section 5: targeted MFI < 1,000 (MFI exceeding 1000 must be approved by the PI and treatment team.)
- Voluntary written consent – adult or parent (with information sheet for minors, if applicable) prior to any research related procedures or treatment

4.2 Exclusion Criteria

- beta 3 laminin JEB mutants
- Active untreated systemic infection at time of transplantation (including active infection with Aspergillus or other mold within 30 days)
- History of HIV infection
- Evidence of squamous cell carcinoma
- Pregnant or breast feeding. Females of child-bearing potential must have a negative pregnancy test prior to study registration as the agents administered in this study are Pregnancy Category C and D.

5 Donor Selection

Unrelated donors will be screened and consented per usual by the Donor Center using the Donor Match Selection Criteria found below.

Donors assessed at the University of Minnesota must meet the following criteria in addition to the Donor Match Selection Criteria.

5.1 Be medically, socially, and psychologically fit to donate in the opinion of the evaluating medical personnel

5.2 Does not have epidermolysis bullosa*

5.3 Adequate hematologic, hepatic and renal function to undergo the donor procedure in the opinion of the evaluating medical personnel

5.4 Not pregnant with confirmation of non-pregnancy status within 7 days of donation. Lactation is not a contra-indication to donation. Transmission of anesthetic medications via breast-milk is variable, dependent upon fat solubility of the medication, doses provided to the lactating donor, and infant enteral absorption. Transmission of anesthetic medications via breast milk is negligible and general anesthesia not a contraindication to continued breast feeding / pumping.[51-54]

5.5 Voluntary written consent - adult or parent (with assent for minors, if applicable)

*At the discretion of the primary BMT physician, donors with known positive carrier status for EB may be considered if in the opinion of the primary BMT physician, the donor is the best available.

Donor Match Selection Criteria:

HLA-matched sibling (or 1 allele HLA-mismatched related): HLA-A, B, C, DRB1 genotypic identical donor, or HLA-A, B, C, DRB1 phenotypic identical donor, or 7/8 HLA-matched donor at HLA-A, B, C, DRB1

HLA mismatched or haploidentical related donors (including 1st degree relatives and half siblings): The donor and recipient must be identical at least one allele of each of the following genetic loci: HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. A minimum match of 5/10 is therefore required, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype.

Matched unrelated donors: Unrelated volunteer donor matched for HLA-A, -B, -C and -DRB1 defined by high resolution molecular typing.

Donor Source In Decreasing Order Of Priority

1. Related Donor (Marrow)[1, 2]

a. HLA-A, B, C, DRB1 genotypic identical (sibling) donor
b. HLA-A, B, C, DRB1 phenotypic identical donor
c. 7/8 HLA-matched donor at HLA-A, B, C, DRB1
d. Haploidentical matching at least one allele at HLA-A, HLA-B, HLA-Cw, HLA-DRB1 & HLA-DQB1

2. Unrelated Donor (marrow)[1]
   a. HLA-A, B, C, DRB1 phenotypic identical donor
   b. 7/8 HLA-matched donor at HLA-A, B, C, DRB1

[1]Targeted MFI <1,000 for any anti-donor HLA antibody; MFI > 1,000 may be considered by PI if no alternative. The PI can approve use of a certain donor even if DSA+ and debulk or not based on his/her preference or institutional guidelines.

[2]May be supplemented with cryopreserved UCB if available

If there is more than one donor with the same degree of HLA match, the following prioritization will be used unless there is HLA cross-match incompatibility or a medical reason to select otherwise, in which case donor selection is the responsibility of the PI. We will prioritize the lowest number of mismatches in the HVG direction (to potentially minimize graft rejection risk).

3. ABO compatibility (in order of priority)
   a. Compatible or minor ABO incompatibility
   b. Major ABO incompatibility
4. CMV status
   a. For a CMV seronegative recipient, use a CMV seronegative donor
   b. For a CMV seropositive recipient, use a CMV seropositive donor
5. Other donor characteristics
   a. The younger (if over 18) and lighter weight donor should be preferred.
   b. If all else is equal, male donors may be preferred over nulliparous female donors who may be preferred over multiparous female donors.
   c. Other factors such as donor age and health history will be integrated into the donor selection process per standard practice and may be prioritized over HLA, ABO and CMV status. Children donors may be used if appropriate.

6 Patient and Donor 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 based on the eligibility assessment documented in the patient's medical record. A copy of the eligibility checklist is in appendices I and II and under attachments within the study in OnCore the Cancer Center's clinical database.

Donors will be registered in OnCore per Masonic Cancer Center guidelines.

6.1 Registration with the Masonic Cancer Center Clinical Trials Office

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

The patient will be assigned to a statistical arm based on transplant only or transplant plus serial MSC infusions and TBI dose.

6.2 Patients Who Are Enrolled and Do Not Receive Study Treatment

If a patient is registered to the study and is later found not able to begin study treatment, the patient will be removed from study and treated at the physician's discretion. The study staff will update OnCore of the patient's non-treatment status. Study data will be collected until the time the patient is taken off study. The reason for removal from study will be clearly indicated in OnCore. The patient will be replaced to complete enrollment.

If a patient receives any study therapy and then is discontinued, regardless of the reason, the patient must be followed per section 7.8.

6.3 Re-Transplant for Graft Failure

Patients being re-transplanted due to graft failure within in this study reassigned to Arm C (if <1 year from 1st transplant) or Arm D-G as appropriate (if >/=1 year from 1st transplant) to release a new calendar and set of case report forms (CRFs). For patients re-transplanted on MT2013-06, no reassignment on this study is necessary.

7 Treatment Plan

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, G-CSF, antimicrobials, etc.).

Enrollment is open to both pediatrics and adults; however, the vast majority or all patients will qualify for care through the University of Minnesota Masonic Children's Hospital and the treatment plan is written as such. If an adult (>25 years of age) is enrolled, he/she would receive all of their care at the appropriate facilities within the University of Minnesota Medical Center, Fairview and the adult outpatient clinic(s).

Day	Drug Or Procedure	Refer To Section:
-9	Rabbit ATG (Thymoglobulin®) 0.5 mg/kg IV over 6 hours with pre-meds Continue methylprednisolone per taper through day -2	7.1.1
-8	Rabbit ATG (Thymoglobulin®) 2 mg/kg IV over 4 hours with pre-meds	7.1.1
-7	Rabbit ATG (Thymoglobulin®) 2 mg/kg IV over 4 hours with pre-meds	7.1.1
-6	fludarabine 30 mg/m^2 IV over 60 minutes* cyclophosphamide 14.5 mg/kg IV over 1 hour mesna 2.9 mg/kg IV 5x/daily	7.1.2 7.1.3
-5	fludarabine 30 mg/m^2 IV over 60 minutes* cyclophosphamide 14.5 mg/kg IV over 1 hour mesna 2.9 mg/kg IV 5x/daily	7.1.2 7.1.3
-4	fludarabine 30 mg/m^2 IV over 60 minutes*	7.1.2
-3	fludarabine 30 mg/m^2 IV over 60 minutes* busulfan IV over 3 hours**	7.1.2
-2	fludarabine 30 mg/m^2 IV over 60 minutes* busulfan IV over 3 hours**	7.1.2
-1	TBI 200 cGy BID (400 cGy total) or 300 cGy if re-transplanted on Arm C	7.1.4
0	Hematopoietic Cell Infusion (Transplant)	7.2
1	rest	-
2	rest	-
3	cyclophosphamide 50 mg/kg IV over 2 hours mesna 10 mg/kg IV 5x/daily	7.3
4	cyclophosphamide 50 mg/kg IV over 2 hours mesna 10 mg/kg IV 5x/daily	7.3
5	tacrolimus (continue until day 100 then taper) – omit if HLA identical related donor mycophenolic acid mofetil (continue until day 35 or 7 days after engraftment)	7.4.1 7.4.2
Serial MSC Infusions- Arm E, Arm G, and if applicable, Arm C (± 14 days).		
60	MSC Infusion #1	7.5
120	MSC Infusion #2	7.5
180	MSC Infusion #3	7.5
240	MSC Infusion #4***	7.5
300	MSC Infusion #5***	7.5
Wound Healing with CelluTome Epidermal Harvesting System		
At least 100	Offered up to three sessions at least 12 weeks apart	7.6

*See Section 7.1.2 for dosing in patients with renal dysfunction and/or < 10 kg body weight.
**See Section 7.1.3 and Appendix VI for dosing. Only for patients enrolled on Arms F or G.
***Offered if patient is available for additional infusions

7.1 Preparative Therapy (day – 9 through day -1)

The preparative therapy will be administered as written with no plans for dose modifications except as described below.

7.1.1 Rabbit ATG (Thymoglobulin®)

Thymoglobulin (rATG) will be infused through a 0.22 micro filter. The dose will be 0.5 mg/kg IV on day -9 over 6 hours and 2 mg/kg IV on days -8 and -7 over 4 hours.

Pre-medications:

- Acetaminophen 15 mg/kg/dose (Max Dose = 650 mg) by mouth or feeding tube 30 minutes prior to rATG
- Diphenhydramine 1mg/kg/dose (Max Dose =50 mg by mouth or feeding tube 30 minutes prior to rATG
- Hypersensitivity orders per standard guidelines during rATG administration.

A steroid taper will be given to prevent reactions to rATG as follows:

- On day -9 to -7, methylprednisolone 1mg/kg IV 1 hour prior rATG. This dose may be repeated once, 3 hours after the first dose.
- On day -6 and -5, methylprednisolone 0.75 mg/kg/ IV as a single dose;
- On days -4 and -3, methylprednisolone 0.5 mg/kg/ IV as a single dose;
- On day -2 methylprednisolone 0.25 mg/kg/ IV as a single dose.

7.1.2 Fludarabine

Fludarabine 30 mg/m^2/dose IV every 24 hours (adjusted for renal function per standard guidelines). Administer over a 60 minute IV infusion on Days –6 through –2 (maximum cumulative dose =150 mg/m^2).

For children < 10 kg, fludarabine dosing will be 1 mg/kg IV over 1 hour. If > 1 year of age, consider converting to mg/kg dosing after discussion between the protocol PI and pediatric BMT pharmacist.

If the adjusted creatinine clearance < 70 ml/min, a 20% dose-reduction of fludarabine may be considered after discussion between the protocol PI and pediatric BMT pharmacist.

Fludarabine dose adjustments for the purpose of poor renal function will not be considered a protocol deviation, but will be noted in OnCore.

7.1.3 Busulfan (Arms F and G only)

Busulfan compounding, administration and monitoring should be performed per institutional guidelines and infused over 3 hours on days -3 and -2. Busulfan dosing will be determined based on the weight of the patients upon admission and per our institution busulfan standard of practice dose calculator and nomogram (Appendix VI).

Seizure and VOD prophylaxis are prescribed according to institutional guidelines. Concomitant administration of azole anti-fungal agents (except fluconazole) and acetaminophen should be avoided within 72 hours before and after busulfan administration.

7.1.4 Pre-Transplant Cyclophosphamide

Cyclophosphamide 14.5 mg/kg/day will be administered as an IV infusion over 1 hour. Cyclophosphamide dosing is calculated based on actual weight (ABW) unless ABW is >150% above Ideal Body Weight (IBW) per institution guidelines.

Mesna 2.9 mg/kg IV 5 times daily will be administered per institutional guidelines.

7.1.5 Total Body Irradiation (TBI)

For patients undergoing transplant on Arm D, E, F, or G:

TBI will be given in two fractions on day -1 at least 6 hours apart. Each fraction will consist of 200 cGy given with right and left lateral beams prescribed to the midplane at the umbilicus at a dose rate of 10-19 cGy/minute. The total TBI dose will be 400 cGy for patients on either of these arms.

For patients undergoing second transplant on 2015-20 Arm C:

The dose of TBI will be 300 cGy in a single fraction on day -1 with right and left lateral beams prescribed to the midplane at the umbilicus at a dose rate of 10-19 cGy/minute.

7.2 Hematopoietic Cell (Bone Marrow) Infusion (day 0)

Refer to section 8 for donor collection.

Bone Marrow (T cell replete graft) will be harvested for infusion on day 0.

In general therefore:

- No T cell depletion

Infusion of fresh, unirradiated bone marrow after filtration of bone fragments

7.3 Post-Transplant Cyclophosphamide

Cyclophosphamide 50 mg/kg IV over 2 hours on day +3 and day +4

Mesna and IV fluid hydration will be administered per institutional guidelines.

Cyclophosphamide dosing is calculated based on actual weight (ABW) unless ABW is >150% above Ideal Body Weight (IBW) per institution guidelines.

7.4 GVHD Prophylaxis

On day 5, patients will begin prophylaxis with Tacrolimus and Mycophenolic Acid Mofetil (MMF). Patients will not be eligible for other GVHD prophylaxis studies.

7.4.1 Tacrolimus (Omit if 8/8 HLA- matched donor)

Tacrolimus will be initiated on day +5 as a continuous infusion at a starting dose of 0.03 mg/kg/day. The patient will be converted to oral dosing when appropriate. Tacrolimus will continue until day +100 with goals of 5-10 ug/L. Starting on day 101, the dose will be tapered 10% per week to discontinue at approximately day +180. Therapy with tacrolimus may continue past day +100 if GVHD is present or if patient is still a mixed chimera.

Tacrolimus may be discontinued earlier than Day 100 (with an appropriate taper plan) in the context of graft failure, or prohibitive toxicity. It is suggested that patients with suspected graft failure remain on tacrolimus until at least the ~Day 60 chimerism assessment.

7.4.2 Mycophenolic Acid Mofetil (MMF)

Mycophenolic acid mofetil will be given at a dose of 15 mg/kg IV (based upon actual body weight) every 8 hours with the maximum total daily dose not to exceed 3 grams (1 g IV TID). BMT patients have unpredictable oral bioavailability of MMF. Consider consulting the pediatric BMT pharmacist when changing MMF doses or routes of administration. MMF pharmacokinetics are not required on this protocol, but may be obtained when clinically indicated. Consult with the pediatric BMT pharmacist for AUC interpretations and dose adjustments when necessary.

If no acute GVHD, MMF prophylaxis will be discontinued after the last dose on day 35, or 7 days after engraftment, whichever is later. Definition of engraftment is 1st day of 3 consecutive days of absolute neutrophil count (ANC) >0.5 x10^{9}/L.

7.5 Serial MSC Infusions (months 2, 4, 6; and optionally at 8, and 10) – Arms E, G and if Applicable, Arm C

Refer to section 8 regarding MSC production and storage.

Month 2, 4, 6, 8, and 10 post-transplant are the "targeted" infusion time points; however, these time points may be adjusted to best fit individual patient situations as deemed most appropriate by the treating investigator. Adjustments to the MSC schedule may be made without being considered a protocol deviation. Doses at 8 and 10 months are optional and would be based on patient availability to return to UMMC.

MSC will be administered at a dose of 1-2 x 10^6/kg at the months (± 14 days) 2, 4 and 6: and offered at 8 and 10 months post-transplant in those patients whose donor consented to the additional marrow collection per section 8. The cells will be thawed at the "bedside".

Premedication (with acetaminophen, diphenhydramine, anti-emetic) and/or additional hydration are not required. The product is infused via IV drip directly into the central line as tolerated by the patient without a needle or pump. MSCs can also be given via a peripheral IV (PIV) when a central line is not available. While adverse events are rare based on substantial past experiences with MSCs, infusional toxicity will be evaluated by continuous monitoring of the subject's vital signs and O^2 saturation by pulse oximetry during the MSC administration. For two hours after MSC administration, O^2 saturation will be monitored at regular intervals and when clinically indicated. Infusional toxicity will be indicated by a decrease in oxygen saturation during the infusion period. After the product bag empties, the bag and IV tubing will be flushed with sterile normal saline. Saline may be either injected directly into the bag or a 50 cc saline IV bag may be attached to the product bag. Post infusion assessments will be done per section 11.2.

7.6 Concurrent Therapy and Supportive Care

Supportive care will be provided per institutional guidelines. Guidelines may be updated based on current data/drugs without requiring a protocol amendment or being considered a protocol deviation.

Due to the risk of squamous cell carcinoma associated with voriconazole, it is preferred that itraconazole be used for antifungal prophylaxis in this patient population. Additionally, empiric vancomycin therapy should only be used as appropriate according to the circumstances outlined in our institutional infection prophylaxis and therapy guidelines.

7.6.1 Epidermal Grafting Using the CelluTome® Epidermal Harvesting System

For patients with persistent wounds, local wound therapy will be offered using epidermal skin grafting from the same donor that provided the hematopoietic graft with donor consent/assent. We will use FDA approved vacuum device (CelluTome®, Regulation number 878.4820) that enables 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.

EB Wounds

We will identify regions indicated for grafting. Such wounds would be open erosions and 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).

Healthy Skin Grafts

We will identify optimal harvesting sites on the body of the donor. Although the epidermal collection is essentially painless (donor will feel warmth and mild pressure at the site) and leaves no scar, we will not choose intensely exposed locations, such as head, neck, palms and soles. 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 donor'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 donor, and we will not collect twice from exactly the same site.

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 transferred to open wound areas. Grafts will be secured with bandages and ideally protected for at least 2 weeks, and preferably up to 3-4 weeks if possible. Wounds will be observed for any signs of infection and photographed weekly as able.

Sessions will be offered to patients at least 100 days post-transplant, with up to three total sessions occurring at least 12 weeks apart.

Donor Eligibility

- The HCT donor for the EB patient

- Age > 2 years (based on prior safety testing of the device)
- Healthy on physical examination in the opinion of the evaluating provider
- Known negativity for Hepatitis B and C, HIV, and HTLV1/2
- Voluntary written consent (donor or parent/guardian for minors with assent)

7.7 Duration of Treatment

All patients will undergo a hematopoietic stem cell transplant on day 0 with standard of care post-transplant follow-up. In addition, if the donor agreed to the collection of additional cells for MSC production, the patient will receive MSC infusions post-transplant unless any of the following occurs:

- consent is withdrawn or patient is noncompliant
- patient experiences unacceptable toxicity
- administering the MSCs would not be in the best interest of the patient

7.8 Re-Transplant For Graft Failure

Patients with graft failure (e.g. lack of engraftment, autologous recovery, loss of graft) may be re-transplanted with consideration of time from 1st BMT informing intensity of conditioning.

If the 2nd transplant will occur <1 year since the 1st transplant, two options are available:

1) Re-transplant on this study (Arm C)
2) Re-transplant on University of Minnesota Blood and Marrow Transplantation (BMT) protocol MT2013-06C while maintaining post-transplant follow-up visits based on the original transplant date

If the 2nd transplant will occur >/=1 year following the first transplant: The 2nd BMT could be completed on this protocol as a new subject on ARM D-G, whichever is appropriate based on HLA-match and donor consent for MSC production.

Re-transplant is done out of medical need. All aspects of the procedure will be within the standard of care for re-transplant at this institution and charged to the patient and/or 3rd party payer in the usual manner,

At the discretion of the treating physician and with the donor's permission, extra marrow for MSCs may be collected at the time of the bone marrow harvest. If the patient has donor MSCs available (either from the original transplant or the re-transplant), they may be infused in the same manner and schedule as detailed

after the original transplant using day 0 of the second transplant. Targeted toxicities will be assessed at time of all MSC infusions.

All tests/procedures and follow-up milestone visits in association with the 1st transplant would continue.

A formal statistical analysis in association with the re-transplant will not be used as these patients are already being analyzed within the context of the initial transplant. A descriptive analysis may also be completed for those transplanted on Arm C or retransplanted on Arms D-G, in regards to the standard of care and research testing that will restart at second transplant day 0; the window for these assessments may be expanded so these time points coincide if in the best interest of the patient.

7.9 Duration of Study Participation

Evaluable patients will be followed for response, toxicity, and survival for 24 months from the transplant unless consent is withdrawn.

After 2 years, routine post-transplant follow-up will continue. Survival status and other routine clinical information may be abstracted from the medical record or other data sources for up to 5 years from study registration.

8 Bone Marrow Harvest and MSC Production

Prior to the bone marrow collection it will be established if the donor has consented to the collection of additional BM for the production of MSCs.

8.1 Bone Marrow Harvest for Transplant

Unrelated donor bone marrow will be collected in the usual manner using established parameters determined by the National Marrow Donor Program.

Related donor bone marrow (BM) will be collected according to current institutional guidelines. The bone marrow harvest will be performed on the transplant day (Day 0). The BM will be infused the same day of collection. The nucleated cell target range will be between 2 and 10 x 10^8/kg of recipient ideal body weight with the volume not to exceed 20 mL/kg of donor's weight once the minimal target of 2 x 10^8/kg has been reached. Given that there is always uncertainty about the feasibility of harvesting certain patients, the harvesting team will have the ability to stop the collection if the minimum target of cells has not been reached and it is believed that it will be unsafe or technically unfeasible to reach the mentioned target if harvest was to continue after a reasonable effort has been made. In this

case, the study PI will be notified immediately and efforts should be made to assure at least a cell count of 2 x 10^8/kg of recipient ideal body weight. For unrelated donors, a target nucleated cell dose of 5 x 10^8/kg will be requested.

8.2 Risks of Donating Bone Marrow

The most serious risk associated with donating bone marrow involves the use and effects of anesthesia during surgery. Other risks associated with bone marrow donation include soreness at the site(s) of collection, pain with walking and generalized weakness after the procedure.

8.3 Additional Marrow Harvest for MSC Production with Donor's Consent

At the time of BM harvest, an additional 40-50 mL BM will be taken from the donor for MSC culture in donors who have agreed to the extra cell collection (embedded in the donor consent as a yes (agree)/no (refuse) checkbox). If the donor has refused to the additional bone marrow collection, the transplant will proceed as plan, but the MSC infusions will not be done.

8.4 MSC Manufacture and Storage

BM adherent cells are cultured under standardized conditions. After expansion culture, cells are removed for testing while the remainder is cryopreserved in aliquots for future use. MSCs will be banked prior to use and will not be released to a patient until the results of all quality control assays (sterility, epitope analysis, and differentiation potential) satisfy lot-release criteria (as defined in the IND). Details on the manufacturing procedure, including lot-release testing and specifications, can be found in the Chemistry, Manufacturing and Controls (CMC) section of the Investigational New Drug (IND) application. Only MSCs meeting lot-release criteria will be available for clinical use.

9 Expected Risks of Study Treatment

9.1 Preparative Regimen

Anti-Thymocyte Globulin (rATG)		
Common occurs in more than 20% of patients	**Less Common** occurs in 5 to 20% of patients	**Rare** occurs in fewer than 5% of patients
• fever • chills • low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • pain • headache • abdominal pain • diarrhea	• feeling poorly (malaise) • dizziness	• severe allergic reaction (anaphylaxis)

Anti-Thymocyte Globulin (rATG)		
Common occurs in more than 20% of patients	**Less Common** occurs in 5 to 20% of patients	**Rare** occurs in fewer than 5% of patients
• high blood pressure (hypertension) • nausea • swelling of hands and/or feet (peripheral edema) • shortness of breath (dyspnea) • loss or lack of strength (asthenia) • high levels of potassium in the blood (hyperkalemia) • rapid heartbeat (tachycardia)		

Fludarabine		
Common occurs in more than 20% of patients	**Less Common** occurs in 5 to 20% of patients	**Rare** occurs in fewer than 5% of patients
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness • tiredness (fatigue) • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding, severe anemia, skin blistering, or other causes

Cyclophosphamide		
Common occurs in more than 20% of patients	**Less Common** occurs in 5 to 20% of patients	**Rare** occurs in fewer than 5% of patients
• nausea/vomiting • mucositis • sterility • severe suppression of • blood counts • diarrhea • fluid weight gain/edema • alopecia	• hemorrhagic cystitis	• cardiomyopathy • skin rash • SIADH (Syndrome of Inappropriate Anti-diuretic Hormone)

Busulfan (ARMS F, G only)		
Common	**Less Common**	**Rare**
• Nausea/vomiting • Mucositis • Rash • Severe suppression of blood counts • Diarrhea	• Veno-occlusive disease • Alveolar hemorrhage • Pulmonary fibrosis • Interstitial pneumonitis	• Seizures (low frequency with anti-seizure prophylaxis) • Hepatic fibrosis/liver failure

Busulfan (ARMS F, G only)		
Common	**Less Common**	**Rare**
• Fluid weight gain/edema • Alopecia • Hyperpigmentation		

Total Body Irradiation		
Common occurs in more than 20% of patients	**Less Common** occurs in 5 to 20% of patients	**Rare** occurs in fewer than 5% of patients
• nausea and vomiting • diarrhea • cataracts • sterility • endocrinopathies • growth failure • intestinal cramps • mucositis	• parotitis • interstitial pneumonitis • generalized mild erythema • veno-occlusive disease	• dysphagia • vertebral deformities • nephropathy • risk of 2nd malignancy years later (when given along with chemotherapy)

9.2 Transplant Procedures

With The Cell Infusion

- nausea and vomiting
- possible allergic reaction (including itching, hives, flushing [red face], shortness of breath, wheezing, chest tightness, skin rash, fever, chills, stiff muscles, or trouble breathing)

General Transplant Related Risks

- slow recovery of blood counts
- graft failure
- Graft-Versus-Host Disease (GVHD)
- other complications including:
  - damage to the vital organs
  - serious infections
  - relapse of disease or a new blood cancer
  - risk to the unborn

9.3 MSC Infusions (Arm E, Arm G and, if applicable, Arm C)

- allergic reaction to MSC (rare)
- shortness of breath (rare)
- ectopic tissue formation (theoretical)
- malignancy (one reported case)
- opportunistic infection (rare)

9.4 GVHD Prophylaxis

Mycophenolate mofetil (MMF)		
Common	**Less Common**	**Rare, but may be serious**
• miscarriage • birth defects • diarrhea • damage to unborn baby • limited effectiveness of birth control • stomach pain • upset stomach • vomiting • headache • tremors • low white blood cell count with increased risk of infection • increased blood cholesterols • swelling of the hands, feet, ankles or lower legs	• anemia • rash • difficulty falling asleep or staying asleep • dizziness • uncontrollable hand shakes	• difficulty breathing • unusual bruising • fast heartbeat • excessive tiredness • weakness • blood in stool • bloody vomit • change in vision • secondary cancers, such as lymphoproliferative disease or lymphoma • Progressive Multifocal Leukoencephalopathy

Tacrolimus		
Common	**Less Common**	**Rare, but may be serious**
▪ kidney problems ▪ loss of magnesium, calcium, potassium ▪ high blood pressure ▪ tremors ▪ increases in cholesterol and triglyceride	▪ nausea ▪ vomiting ▪ liver problems ▪ changes in how clearly one can think ▪ insomnia ▪ unwanted hair growth ▪ confusion	▪ seizures ▪ changes in vision ▪ dizziness ▪ red blood cell destruction

It is very important that grapefruit or drinks with grapefruit juice are not consumed while taking Tacrolimus. Grapefruit has an ingredient called bergamottin, which can affect some of the treatment drugs used in this study**.** Common soft drinks that have bergamottin are *Fresca*, *Squirt*, and *Sunny Delight*.

9.5 Epidermal Grafting Using the CelluTome®

9.5.1 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. Of note, the harvested tissue (epidermis) does not cross the basement membrane, so the procedure is essentially painless (donor will feel warmth and mild pressure at the site) and there is not typically residual visible scarring unless there is a malfunction of the device or other unanticipated event.We will still encourage examination the sites of epidermal harvest via weekly photographs until healed and at 12 weeks (as able) for any abnormalities detectable by physical examination.

9.5.2 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.

10 Clinical Evaluations

Days and testing/assessment frequency may be altered as clinically appropriate (e.g. in the event of graft failure some procedures may be waived).

10.1 Clinical Evaluations – All Patients Standard of Care

	Screen	Pre-BMT	Day 0 to 30			Day 31 to 100				Follow-up
		Days -3, -2	Daily[11]	Weekly	Day +28 (± 3 days)	Weekly	Day +60 (± 14 days)	Day +100 (± 14 days)	Day +180 (± 14 days)	1 and 2 years (± 30 days)&
	Patient's General Assessments									
Informed consent	X									
Medical history	X		X			X			X	X
Physical exam	X		X			X			X	X
iscorEB[a]	X							X	X	X
Play Score/Performance status[1]	X				X			X	X	X
Height/Weight[12]	X							X	X	X
GVHD evaluation[2]				X		X			X	X
Adverse event notation			X			X			X	X
	Patient's Laboratory Assessments[3]									
CBC, diff[4]	X		X			X			X	X
Platelet	X		X			X			X	X
PT/PTT, CH50, C3 & C4	X							X		
Serum chemistries[5]	X			X		X			X	X
Iron studies[6]	X							X	X	X
Zinc, Selenium, Vitamin D3, Carnitine	X								X	1 year only
Viral serology[7]	X							X	X	X
Pregnancy test for females of childbearing potential	X									
Blood DNA to Molecular Diagnostics Lab for Chimerism Assessment	X				X		X	X	X	X
Skin DNA to Molecular Diagnostics Lab for Chimerism Assessment								X	X	X
Immune Panel and Ig levels[8]	X				X		X	X	X	X
PRA: initial (for all donors except fully matched)	X									
PRA: Confirmatory w/in 21 days of treatment (for all donors except fully matched)	X									
Busulfan TDM[9]		X								
	Patient's Procedures									
EKG	X									
ECHO	X									
Chest Xray or CT	X									
Oxygen saturation	X									
Renal Function (eGFR)	X									
Ophthalmology consult*								X	X	X
GI endoscopy and biopsy*	X				X		X	X	X	X
Skin biopsy specimens[10]	X							X	X	X

	Screen	Pre-BMT	Day 0 to 30			Day 31 to 100				Follow-up
		Days -3, -2	Daily[11]	Weekly	Day +28 (± 3 days)	Weekly	Day +60 (± 14 days)	Day +100 (± 14 days)	Day +180 (± 14 days)	1 and 2 years (± 30 days)&
Skin fragility testing	X							X	X	X
Photographs of skin	X							X	X	X
Wound Healing Using the CelluTome® Device[12, 13]								X	X[13]	X day 240[14]
Photographs of treated wounds[15]								X[16]		

*Only if clinically indicated

^Arm B and E only (if donor agreed to the collection of additional bone marrow for MSC production – refer to sections 7.5 and 10.3)

& After 2 years, patient will continue with standard of care post-transplant follow-up from which information may be abstracted from the medical record or other sources for the purpose of this study

[a] iscorEB surveys are a validated, standard of care tool used to assess disease status in all patients with Epidermolysis Bullosa. Patients &/or patient representatives may be asked to complete the patient portion of the survey corresponding with additional standard of care assessments as determined by the treating provider. All completed iscorEB surveys are intended to be used to further understand and describe changes in disease severity of epidermolysis bullosa throughout the trajectory of treatment.

[1] Assessment tool in Appendix III. The Lansky scale will be used for patients less than or equal to 16 years of age and the Karnofsky scale will be used for patients greater than 16 years of age.

[2] Assessment tool in Appendix IV.

[3] A sample that is not obtained or obtained outside the protocol window will not be considered a deviation from protocol if this is related to daily blood volume limitations.

[4] Complete blood count with leukocyte differential will be measured.

[5]Basic metabolic panel to be completed daily through engraftment and comprehensive metabolic panel will be measured weekly from day 0 – 30. Comprehensive metabolic panel will be measured at all additional specified time points.

[6] Ferritin, TIBC, and serum iron will be measured.

[7] Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (HBcAb), Hepatitis C virus (HCV), CMV & EBV will be measured. CMV and EBV may be assessed at additional intervals per institutional guidelines.

[8] IgA, IgE, IgG, IgM & IgG subclasses will be measured.

[9] Busulfan therapeutic drug monitoring (TDM) per Appendix VI (Arms F and G only)

[10] 5 samples: DNA for chimerism assessment; IF or Western to assess protein expression (i.e. collagen, laminin, integrin, keratin or plakin); EM to assess structural changes

[11] Daily until engraftment

[12]As able

[13] The donor must provide consent/assent

[14] Up to 3 sessions at least 12 weeks apart

[15] For patients who elect wound healing with CelluTome

[16] At minimum, photographs will be taken at baseline grafting and ideally also at wk 6 (+/- 1 wk), *wk 12 (+/- 1 wk), and *1 year (+/1 3 months).[*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] . Photographs are also requested weekly between day of grafting and 12-week evaluations; photographs not received at these target dates or outside of these target dates that are outside of the study team's control (i.e. patient unable to return to study site and photographs not sent via email despite request from study team), will not be considered reportable deviations.

10.2 Research Related Assessments and Procedures (All Arms)

	Screen	Day +28 (± 3 days)	Day +60 (± 14 days)	Day +100 (± 14 days)	Day +180 (± 14 days)	1 and 2 years (± 30 days)
Monitor for Early Stopping Rules per section 14.4	Through day 100					
Research related bloods for Tolar Lab: ≥40kg patient weight: draw One 5 mL red top tube (serum) and One 5mL purple top tube (plasma/PBL) <40kg patient weight: draw One 0.2 mL/10kg red top tube (serum) and One 0.2 mL/10kg purple top tube (plasma/PBL)	X	X	X	X	X	X

10.3 Research Related Assessments and Procedures (Arms where MSCs are given)

	Day +60 (± 14 days)	Day +120 (± 14 days)	Day +180 (± 14 days)	Day +240 (± 14 days)*	Day +300 (± 14 days)*
MSC infusion	X	X	X	X	X
Targeted Toxicity at pre and 4 hours (+/- 30 minutes) post MSC infusion	X	X	X	X	X

*Optional based on patient availability

Day 60, 120, 180, 240 and 300 post-transplant are the “targeted” infusion time points; however, these time points may be adjusted to best fit individual patient situations as deemed most appropriate by the treating investigator. Adjustments to the MSC schedule may be made without being considered a protocol deviation.

10.4 Donor

Unrelated donors are screened, consented and collected per their usual local donor center procedures.

	Screen	After Consent
Donor’s General Assessments		
Informed consent	X	
Medical history	X	
Physical exam	X	
Donor’s Laboratory Assessments		
CBC, diff	X	
Platelet	X	
PT/PTT	X	
Viral serology	X	X[1]
donor blood to molecular diagnostics for their baseline chimerism	X	
PRA*	X	

	Screen	After Consent
Pregnancy test if of child bearing potential	X	X[1]
Donor's Procedures		
BM harvest		X
Skin specimen from BM site (UMN related donors only)[2]		X
For Donors who consent to Skin Cell Harvest		
Donor infectious disease panel within 30 days of Harvest		X
Skin Cell Harvest Using the CelluTome® Device**		X
1- Repeat if not done in previous 7 days 2- As able		

* Not required for fully-matched related donors per institutional SOP

**as clinically indicated

11 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

11.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Treatment-Emergent Adverse Event: Any event not present prior to the initiation of the treatment or any event already present that worsens in either intensity or frequency following exposure to the treatment. A treatment emergent AE refers to an event temporally related to the study treatment regardless of the causality assessment by the investigator.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. Note: a life-threatening event does not necessarily equate to a CTCAE grade 4.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. Thus, as defined by the FDA adverse events that occur as part of the disease process or underlying medical conditions are considered *unexpected;* however, for the purposes of this study they will not be documented or reported.

11.2 Adverse Event Monitoring, Recording and Reporting

11.2.1 Event Monitoring

Monitoring for adverse events will begin with the start of the preparative regimen and continue through 1 year post-transplant anniversary visit.

11.2.2 Event Recording/Documentation

Due to the intentional clearing of the marrow with chemotherapy as preparation for the HCT, it is expected that all patients will experience severe depression of their blood counts and other related toxicities as

detailed in section 9. Therefore, adverse event documentation in OnCore for the purposes of this study will focus on:

- All serious non-hematologic, non-infectious, non-dermatologic adverse events through neutrophil engraftment or Day 42, whichever is shorter
- All serious unexpected suspected adverse reactions after Day 42 (or engraftment) through 1 year post-transplant upon knowledge
- Targeted toxicities (appendix V) and unexpected suspected adverse reactions will be recorded for patients receiving MSCs at the following time points:
  - Prior to each MSC infusion
  - 4 hours (± 30 minutes) after each MSC infusion

Transplant related outcomes will continue to be collected as routine by the BMT Database.

11.2.3 Event Reporting to the U of MN IRB, the FDA, and MCC

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

Agency	Criteria for reporting	Timeframe	Form to Use	Submission information
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); 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
FDA	Unexpected fatal or unexpected life threatening suspected adverse reaction	As soon as possible but no later than 7 Calendar-Days	UMCC SAE form	Submit as an amendment to IND
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	As soon as possible but no later than 15 Calendar-Days		
	Note: Events due to the disease under treatment or an underlying medical condition will not require expedited reporting to the FDA for the purposes of this study			
SAE Coordinator	Study stopping rule events per section 11.3: Aplastic graft failure by day 42 Transplant related mortality by day 100	At time of reporting	Event Form	SAE Coordinator mcc-saes@umn.edu

At the time of continuing review/IND annual report, relevant events recorded in OnCore will be reported in summary format by those persons responsible for such reporting.

11.3 Monitoring for Stopping Rule Events

In addition to the event monitoring and reporting described in the previous section, the following events will be considered excess toxicity per section 14.4 and be reported as a stopping rule event using the Event Form found in OnCore:

- Aplastic graft failure by day 42 post-transplant
- Transplant related mortality (TRM) by day 100 post-transplant

Stopping rule events only apply to the initial transplant procedure.

A copy of the completed Event Form must be sent to the SAE Coordinator (mcc-saes@umn.edu) at the time it is submitted to the study statistician.

As these events are outcomes of the treatment, not toxicity as found in CTCAE, they should only be recorded/reported as an adverse event, if they meet the criteria found in section 11.2.

12 Data Collection and Monitoring

12.1 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 required for IND annual reporting will be placed in OnCore and other research databases maintained by MCC IT.

12.2 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. The Study Coordinator or designee will be responsible for completing e-CRFs based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

12.3 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 http://z.umn.edu/dmsp

For the purposes of data and safety monitoring, this study is classified as high risk (investigator initiated protocol under an IND). Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the trial's progress quarterly.

- 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 11.2.3 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

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).

IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the IND sponsor (Dr. Tolar) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

12.4 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at least 6 years after the study file is closed with the IRB and FDA.

In addition, the University of Minnesota Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the U of MN CTO before destroying any study related records.

13 Disease Outcome Measures

Five skin samples for tissue analysis will be obtained before the HCT and at day 100, 180, and 1 year then annually after HCT (additional skin samples may be taken as clinically indicated), from areas approximately 1 mm from a wound border. Histological changes will be assessed with light microscopy and the expression of C7 with a panel of anti-C7 antibodies;[25] ultrastructural changes assessed with transmission electron microscopy and presence of C7-containing anchoring fibrils with immuno-electron microscopy;[45] and donor engraftment in skin assessed by competitive polymerase-chain-reaction analysis of polymorphic variable-number tandem-repeat regions.

For functional assessment of skin stability, the time needed to create blisters using suction with a vacuum device (NP2 model, Electronic Diversities, Finksburg, USA)

will be measured. To test for anti-C7 antibodies in blood samples, a quantitative enzyme-linked immunosorbent assay will be used with the assessment of serum reactivity with the dermal side of the salt-split human skin[46].

For clinical evaluations, we will used instruments validated for scoring treatment outcomes in individuals with epidermolysis bullosa: disease severity tool (iscorEB)[47].

Photographs. A specialized camera or photograph with standard measuring system will be used at these time-points (day of harvest, 6 weeks, 12 weeks, and 1 year after grafting) to allow for quantitative assessment of wound size over time in correspondence with CelluTome treatments. Weekly photographs will also be requested as able until the week 12 visit to assess healing.

14 Statistical Considerations

14.1 Statistical Endpoints

14.1.1 Primary Endpoint

Event-free survival by 1 year post-transplant. An event will be defined as death or a 50% increase in a patient's iscoreEB from baseline.

14.1.2 Secondary Endpoints

- Percentage change in iscoreEB at 1 year and 2 years as compared to baseline calculated using each iscoreEB total score
- Probability of transplant-related mortality (TRM) at 180 days
- Change in health quality of life pre-transplant as compared to at 1 and 2 years post-transplant as measured by the Lansky or Karnofsky score (10-100)
- Proportion of lymphoid and myeloid chimerism at various time-points (days 28, 60, 100, 180, and year 1 and 2) among surviving patients

14.1.3 Correlative Endpoints

- Levels of biochemical improvement as measured by protein expression (collagen, laminin, integrin, keratin or plakin) and related structural and physical changes as derived from biopsy samples at days 100 and180, then at 1 and 2 years using a binary result provided by Dr. Tolar's lab
- Chimerism results from skin biopsy samples at days 100 and 180, then at 1 and 2 years (and additional time points if collected as clinically indicated) as performed by the Molecular Laboratory

- Wound healing will be evaluated at milestone clinic visits (day 180, 1 and 2 years post-transplant and as clinically indicated) by relative change of wound area. It is measured as number of square cm of open wound area at the each time point/open wound area before skin grafting. It is also included in the total body surface are as noted in the iscorEB.
- For patients who receive CelluTome, wound healing over time will be described by photographs taken prior to graft, at 6 weeks, 12 weeks, and 1 year after treatments.

14.1.4 Transplant Related Endpoints

The following endpoints are abstracted from the BMT database:

- Survival by 1 year
- Grade II-IV and grade III-IV acute graft versus host disease (GvHD) at Day 100
- Chronic graft versus host disease at 1 year
- Time to neutrophil engraftment (defined as the first of three consecutive days after HCT that the patient's absolute neutrophil counts is ≥ $0.5x10^9$ per liter)
- The incidence of sustained neutrophil engraftment (neutrophil engraftment without autologous recovery)
- Time to platelet engraftment (defined as the first time after HCT that the patient can sustain platelet count ≥ $20x10^9$ per liter for three consecutive days)
- Incidence of bacterial, viral and fungal infections at 1 year

14.2 Statistical Analysis

Arms A and B will be closed after enrollment of seven patients. Analyses of these patients will remain descriptive. Arms D and E will remain open but we expect to enroll approximately 8 additional patients due to the eligibility restriction to HLA matched siblings. The total enrollment on these two arms should be approximately 16 so analyses will also primarily be descriptive. Due to the change/justification in conditioning (addition of Busulfan) for future patients receiving an HLA-mismatched or haploidentical transplant as described in section 2.3, arms F and G will now be combined for assessment of the primary endpoint, secondary endpoints and the transplant related endpoints since we do not expect the addition of MSCs to alter these endpoints based on our large past experience of more than 1,000 patients[42,44,55-57]. Arms F and G may be separated, however, when assessing levels of biochemical improvement since MSCs may assume a role in collagen and laminin expression. The planned 69 patients in Arms F and G will be analyzed as follows: Kaplan-Meier curves will be used to estimate event-free

survival and survival. Cumulative incidence will be used to estimate the probability of TRM, treating time to events as a competing risk. Ninety-five percent confidence intervals will be estimated from respective standard errors. The proportion of donor chimerism or donor cells (including MSCs) at various time-points will be estimated among patients who have survived to the evaluated time-point along with descriptive plots. Medians, ranges and inter-quartile ranges will be given for actual chimerism values and percentage change and absolute values of iscoreEB between baseline and 1 year along with descriptive box-plots. iscoreEB assessments will be evaluated among patients surviving to 1 year post transplant. The Lansky or Karnofsky score will be measured by the proportion of patients with a score < 90 at each time-point. Specific focus will be on survivors at 1 and 2 years post-transplant. Simple proportions from binary measures will be used to describe the patterns of biochemical improvement over time from 5 different protein expressions. Wound healing will be assessed at 6 months, 1 year and 2 years descriptively by use of box-plots and estimating the average or median percent change in open wound area in relation to pre-skin grafting. In addition to primary and secondary endpoints, the transplant-related endpoints of acute and chronic GvHD, primary and sustained neutrophil and platelet engraftment and infections will be estimated by cumulative incidence treating non-event death as a competing risk. Analyses and descriptive plots will be performed with SAS 9.3 (SAS Institute, Cary, NC) and/or R 3.0.2.

14.3 Design and Sample-Size Justification

This study is designed as a two-stage phase II trial to estimate event-free survival (EFS). Since the goal is to estimate the EFS at a long-term time-point (1 year post-transplant), our design is a generalization of the Simon design. Rather than suspension of the trial for evaluation after stage 1, this design uses an optimal interim analysis for futility without suspension of accrual as proposed by Huang, Talukder and Thomas[48]. The goal of this design minimizes the expected sample size under the assumed parameters.

Our trial assumes that 40% 1 year EFS is unacceptably low and 60% 1 year EFS is a rate worthy of further study. Both the null and alternative hypotheses assume that the EFS follow an exponential distribution. Given an overall one-sided type I error of 5%, 69 total patients with an interim analysis for futility after accrual of 36 patients will provide 90% statistical power. These 69 patients do not include the currently enrolled 7 patients on Arms A and B nor the expected 16 patients on arms D and E as enrollment is starting over for purposes of analyses. Therefore total enrollment will be approximately 92 patients.

When 36 patients have been enrolled at the new TBI dose of 200 cGy bid with the inclusion of Busulfan, accrual will stop if the 1-year Kaplan-Meier estimate of EFS is less than 41.6%. The probability of stopping under the null hypothesis is 56%. The probability of stopping if the alternative hypothesis is true is 5%. If the trial reaches the end of stage II, significant clinical activity would require at least a 1 year Kaplan-Meier estimate of EFS of 52%. The R package 'OptInterim' with functions 'OptimDes' and 'SimDes' were used to create design parameters[49].

We do not expect the February 2019 amendment to alter the outcome of EFS as any differences will be in degree not in kind. Stated differently, differences will not be qualitative. Therefore, enrollment will continue as planned using only patients in arms F and G in the interim decision to continue enrollment as well as in the final estimate of EFS at the conclusion of the trial.

As many as 25 patients may be enrolled per year allowing study enrollment to be completed in approximately 3 years.

14.4 Toxicity Monitoring and Stopping Rules

Monitoring guidelines are developed to monitor excess toxicity using a continuous monitoring strategy based on an adaptation of Pocock stopping boundaries[50]. In the event that a stopping rule is triggered, enrollment will be halted. The study will be reviewed by the full study committee and if appropriate, by the IRB, prior to re-initiating enrollment. Although we do not expect different toxicity rates for the diagnoses of JEB and RDEB, monitoring guidelines will be applied separately based on EB diagnosis (JEB versus RDEB). We expect that 1/3 (n=23) of the patients will be transplanted for JEB and 2/3 (n=46) for RDEB. Continuous monitoring will start over with the use of the new conditioning regimen (adjustment from 300 cGy TBI to 200 cGy bid TBI plus the addition of Busulfan. Neither monitoring boundary was triggered in the 1st fifteen patients. Arms D and E will be monitored separately but with both diagnoses combined due the small expected enrollment (n=16).

14.4.1 Aplastic Graft Failure

14.4.1.1 JEB

Stopping rules were developed for excessive graft failure by day 42 post-transplant. The goal is to construct a boundary based on graft failure such that the probability of early stopping is at most 10% if the true graft failure rate is equal to 10% and our sample size is 23. Given these parameters, the upper stopping boundary for graft failure is 2 failures out of 3 patients, 3 out of 8, 4 out of 14, 5 out of 21 or 6 anytime. Graft failure will be defined

as failure to reach an ANC ≥ 500 neutrophils/uL for three consecutive days prior to day 42. If the actual rate of graft failure is 20% and 30% respectively, the probability of early stopping is 49% and 84% respectively.

14.4.1.2 RDEB

Stopping rules were developed for excessive graft failure by day 42 post-transplant. The goal is to construct a boundary based on graft failure such that the probability of early stopping is at most 10% if the true graft failure rate is equal to 10% and our sample size is 46. Given these parameters, the upper stopping boundary for graft failure is 2 failures out of 3 patients, 3 out of 7, 4 out of 12, 5 out of 18, 6 out of 24, 7 out of 31, 8 out of 38 or 9 anytime. Graft failure will be defined as failure to reach an ANC ≥ 500 neutrophils/uL for three consecutive prior to day 42. If the actual rate of graft failure is 20% and 30% respectively, the probability of early stopping is 66% and 97% respectively.

14.4.1.2 Arms D & E

Stopping rules were developed for excessive graft failure by day 42 post-transplant. The goal is to construct a boundary based on graft failure such that the probability of early stopping is at most 10% if the true graft failure rate is equal to 10% and our sample size is 16. Given these parameters, the upper stopping boundary for graft failure is 2 failures out of 4 patients, 3 out of 9, 4 out of 14, or 5 anytime. Graft failure will be defined as failure to reach an ANC ≥ 500 neutrophils/uL for three consecutive days prior to day 42. If the actual rate of graft failure is 20% and 30% respectively, the probability of early stopping is 40% and 73% respectively.

14.4.2 Transplant Related Mortality by 100 Days

14.4.2.1 JEB

Stopping rules were developed for excessive TRM by day 100 post-transplant. The goal is to construct a boundary based on mortality such that the probability of early stopping is at most 10% if the true TRM is equal to 10% and our sample size is 23. Given these parameters, the upper stopping boundary for TRM is 2 events out of 3 patients, 3 out of 8, 4 out of 14, 5 out of 21 or 6 anytime. The probability of early stopping of TRM is same as that for graft failure.

14.4.2.2 RDEB

Stopping rules were developed for excessive TRM by day 100 post-transplant. The goal is to construct a boundary based on TRM such that the

probability of early stopping is at most 10% if the true TRM rate is equal to 10% and our sample size is 46. Given these parameters, the upper stopping boundary for TRM is 2 events out of 3 patients, 3 out of 7, 4 out of 12, 5 out of 18, 6 out of 24, 7 out of 31, 8 out of 38 or 9 anytime. The probability of early stopping of TRM is same as that for graft failure.

14.4.2.3 Arms D & E

Stopping rules were developed for excessive TRM by day 100 post-transplant. The goal is to construct a boundary based on mortality such that the probability of early stopping is at most 10% if the true TRM is equal to 10% and our sample size is 16. Given these parameters, the upper stopping boundary for TRM is 2 events out of 4 patients, 3 out of 9, 4 out of 14, or 5 anytime. The probability of early stopping of TRM is same as that for graft failure.

Due to additional stopping rules and re-initiation of the study, it is understood that the overall power may be slightly reduced for this study.

15 Conduct of the Study

15.1 Good Clinical Practice

The study will be conducted in accordance 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.

15.2 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 patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, progress reports, and any revisions to these documents will be provided to the IRB by the Investigator.

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Appendix I – Eligibility Checklist - Patient

Biochemical Correction of Severe Epidermolysis Bullosa by Allogeneic Cell Transplantation and Serial Donor Mesenchymal Cell Infusions (MT2015-20)

Eligibility Checklist – page 1 of 2

Patient initials ☐☐☐☐ Patient ID ☐☐

1st 2 initials of first name + 1st 2 initials of last name Seq # (i.e. 01, 02, 03, etc.)

INCLUSION CRITERIA

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

		Yes	No
1.	0 through 25 years of age	☐	☐
2.	Diagnosis of severe form of EB with documented collagen, laminin, integrin, keratin or plakin deficiency (by immunofluorescence staining with protein specific antibodies or Western blotting and by mutation analysis)	☐	☐
3.	Adequate organ function within 4 weeks of study registration defined as:	☐	☐

test	requirement
glomerular filtration rate	within normal range for age
total bilirubin	< 5 x UNL
AST	< 5 X UNL
ALT	< 5 X UNL
ALP	< 5 X UNL
oxygen saturation	adequate pulmonary function in the opinion of the enrolling investigator
left ventricular ejection fraction	≥ 45% normal EKG or approved by Cardiology

4.	Available donor PRA results (Not applicable for fully matched donors): ☐ Target MFI level <1,000 or approved by PI	☐	☐	☐ N/A
5.	Sexually active participants must agree to use adequate birth control for the during the study period (from before the start of the preparative chemotherapy through 1 year post-transplant) check if not applicable ☐	☐	☐	
6.	Voluntary written consent (patient or parent/guardian for minors) prior to any research related procedures or treatment	☐	☐	

Biochemical Correction of Severe Epidermolysis Bullosa by Allogeneic Cell Transplantation and Serial Donor Mesenchymal Cell Infusions (MT2015-20)

Eligibility Checklist – page 2 of 2

Patient initials ☐☐☐☐ Patient ID☐☐

EXCLUSION CRITERIA

A "YES" response to any of the following disqualifies the patient from study entry.

		Yes	No
1.	Beta 3 laminin JEB mutants	☐	☐
2.	Active untreated systemic infection at time of transplantation (including active infection with Aspergillus or other mold within 30 days)	☐	☐
3.	History of HIV infection	☐	☐
4.	Evidence of squamous cell carcinoma	☐	☐
5.	Pregnant or breast feeding. Females of child-bearing potential must have a negative pregnancy test prior to study registration as the agents administered in this study are Pregnancy Category C and D	☐	☐

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

_____ _____

Signature of enrolling investigator Date

Appendix II – Eligibility Checklist – Donor

Biochemical Correction of Severe Epidermolysis Bullosa by Allogeneic Cell Transplantation and Serial Donor Mesenchymal Cell Infusions (MT2015-20)

Eligibility Checklist – page 1 of 1

Donor initials ☐☐☐☐ Patient ID☐☐-D

1st 2 initials of first name + 1st 2 initials of last name

INCLUSION CRITERIA

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

		Yes	No
1.	Be medically, socially, and psychologically fit to donate in the opinion of the evaluating medical personnel	☐	☐
2.	Does not have epidermolysis bullosa (or does have but will be used as the donor per section 5)	☐	☐
3.	Adequate hematologic, hepatic and renal function to undergo the donor procedure in the opinion of the evaluating medical personnel	☐	☐
4.	Not pregnant with confirmation of non-pregnancy status within 7 days of donation	☐	☐
5.	Voluntary written consent - adult or parent (with assent for minors, if applicable)	☐	☐
6.	PRA results (Not applicable for fully matched donors): targeted MFI level within protocol bounds or approved by PI (The PI can approve use of a certain donor even if DSA+ and debulk or not based on his/her preference or institutional guidelines.)	☐	☐ ☐ N/A

Additional Eligibility to donate skin graft

A "NO" response to any of the following disqualifies the donor from the CelluTome Procedure.

		Yes	No
1.	Age > 2 years (based on prior safety testing of the device)	☐	☐
2.	Known negativity for Hepatitis B and C, HIV, and HTLV1/2	☐	☐
3.	Voluntary written consent (donor or parent/guardian for minors with assent)	☐	☐

Did donor consent to collection of additional BM cells for MSC production?

☐ no – enroll patient in Arm D or F: transplant only

☐ yes – enroll patient in Arm E of G: transplant plus serial MSC infusions

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

Signature of person verifying eligibility _____ Date _____

Appendix III – Lansky Play Score and Karnofsky Performance Status

For patients < 16 years of age:

Lansky Score	Play Score
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of and less time spent in play activity
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play but able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

For patients 16 years of age and older:

Karnofsky Performance Scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

Appendix IV – GVHD Scoring

Acute GVHD

Organ involvement will be staged using the criteria outlined in the table below. Biopsy of each organ site at diagnosis or major change in disease activity will be performed unless clinical circumstances make it impossible.

Consensus Clinical Stage and Grade of Acute GVHD (Przepiorka *et al*, 1995)

Stage	Skin	Liver	Lower Gastrointestinal Tract	Upper Gastrointestinal Tract
1	Maculopapular rash <25% of body surface	Bilirubin 2.0 – 3.0 mg/dl	Diarrhea 500 – 1000 mL/day or 280 – 555 mL/m^2	No protracted nausea and vomiting
2	Maculopapular rash 25-50% body surface	Bilirubin 3.1 – 6.0 mg/dl	Diarrhea 1000 – 1500 mL/day or 556 – 833 mL/m^2	Persistent nausea, vomiting or anorexia
3	Generalized erythroderma	Bilirubin 6.1 – 15.0 mg/dl	Diarrhea >1500 mL/day or >833 mL/m^2	
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dl	Severe abdominal pain, with or without ileus, or stool with frank blood or melena	

Grading for Treatment Criteria:

Mild GVHD Skin stage I-II only (Equivalent to Seattle Grade I).

Moderate GVHD Skin stage I-III and/or liver I-IV and/or Gastrointestinal tract (GI) I-III and/or Upper GI (UGI). (Equivalent to Seattle Grade II, III).

Severe GVHD Any stage IV along with severe clinical illness.

Late Acute and Chronic GVHD

Late acute and chronic GVHD will be assessed using the National Institutes of Health (NIH) Consensus Criteria.

Appendix V – Targeted Toxicities (For MSC Infusions Only)

MT2015-20 Refer to section 11.2.2 for time points

Patient Initials: _____ **Date of Assessment:** _____ **Assessment Time point:** _____

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Infusion related reaction	None	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae	Life-threatening consequences; urgent intervention indicated
Sinus Bradycardia	None	Asymptomatic, intervention not indicated	Symptomatic, medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated
Sinus Tachycardia	None	Asymptomatic, intervention not indicated	Symptomatic; non-urgent medical intervention indicated	Urgent medical intervention indicated	----------
Bleeding/ Hemorrhage		Mild; intervention not indicated	Moderate symptoms; medical intervention or minor cauterization indicated	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated
Hypertension	None	Pre-hypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg); medical intervention indicated; recurrent or persistent ≥ 24 hrs); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated	Stage 2 hypertension (systolic BP ≥ 160 mm Hg or diastolic BP ≥ 100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated.	Life-threateningconsequences (e.g.,malignant hypertension,transient or permanentneurologic deficit,hypertensive crisis); urgent intervention indicated.
Hypotension	None	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated
Fever	None	38.0 - 39.0◦ C (100.4 - 102.2◦ F)	> 39.0 - 40.0◦C (102.3 - 104.0◦F)	> 40.0 ◦ C (>104.0◦ F) for ≤ 24 hrs	> 40.0 ◦ C (>104.0◦ F) for > 24 hrs
Chills	None	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	----------
Dyspnea	None or no change	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Hypoxia	None	……	Decreased O_2 saturation with exercise (e.g., pulse oximeter < 88%) intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter < 88% or PaO_2 ≤ 55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated
Rash	None	Covering < 10% body surface area (BSA)	Covering 10-30% body surface area (BSA)	>30% body surface area (BSA)	Generalized exfoliative, ulcerative, or bullous dermatitis

Person Completing Form: _____ ADL = activities of daily living

Appendix VI – Low Dose Busulfan Dose Selection (For Arms F and G Only)

Busulfan Dose Selection, AUC Monitoring and Algorithm for Dose Modification Using Once Daily IV Dosing

Goal AUC 7560 [31 (mg · h)/L]

This protocol will use once daily (every 24 hour) intravenous busulfan dosing. Two (2) total doses of busulfan will be given over 2 days in the preparative regimen. AUC determination after doses #1 and #2 (AUC1 and AUC2). For all patients, each busulfan dose will be administered over 3 hours by central venous line per University of Minnesota BMT Program standard guidelines.

THE TOTAL regimen busulfan AUC (AUC_{cum}) targeted will be = **6950-8170 (µM·Min)/L [28.5-33.5 (mg · h)/L] with ideal target AUC_{cum} of 7560 (µM·Min)/L [31 (mg · h)/L]** in order to best optimize engraftment while minimizing toxicity. If at any time upon interim analysis and review of patients transplanted on this protocol with the principal investigators and pharmacy team, the subsequent busulfan levels following dose #2 (AUC2) are deemed reasonable and consistent, the decision may be made to only do kinetics with the dose#1 for all remaining patients treated on this protocol. In that event, kinetics for a dose#2 (i.e. AUC monitoring) may be performed whenever a dose change is required but will not be mandatory. These protocol changes (if enacted) will not require a treatment deviation or protocol amendment.

Steady State Concentration (Css) equivalent to 6950-8170 (µM·Min)/L = **600-700 ng/ml**

DETERMINATION OF FIRST BUSULFAN DOSE (DOSE #1)

The initial empiric busulfan dosing (Dose #1) for ALL patients will be determined as follows:

a. For patients weighing <12 kg: initial dose will be based on the formula according to the population PK model developed by Long-Boyle.
b. For patients weighing ≥12kg and <66 kg: initial dose will be determined by the nomogram adapted from Bartelink.
c. For patients weighing ≥66kg: initial busulfan dose = 3 mg/kg IV Once Daily

1. **For patients <12 kg: the first busulfan dose will be based on the population PK formula developed by Long-Boyle which calculates the first dose as a function of weight and age:**
    - Use the Dosing Calculator (Long-Boyle Busulfan 1st Dose) on the BMT website to determine the precise dose for the patient;
    - Age: enter the age in years (to the hundredths place) by dividing the patient's age in days by 365
    - Weight: enter the weight in kg (to the tenths place)
    - The Calculator is constructed using the following function:
        - Dose (mg) = $AUC_{(target)}$ x CL_i
        - $AUC_{(target)}$ = 15.6 mg·hr·L-1 (equivalent to 3800 µM·min·L-1)
        - CL_i is a function of weight and age
            - CL_i = 2.3 L/h x (Mat_{mag} + (1- Mat_{mag}) x [1-$e^{(-age \times Kmat)}$]) x (weight/8 kg)$^{0.75}$
            - Maturation magnitude (Mat_{mag}) = 0.46; Maturation rate constant (k_{mat}) = 1.4

- See Table A-1 below for EXAMPLE first Busulfan doses for hypothetical children under 12 kg; this table can be used to ensure your patient's calculator output does not carry significant error. However, the patient's first dose should be determined directly from the calculator.

Table A-1: Sample First Busulfan Doses for Various Ages and Weights Using the Long-Boyle Method for Determination of First Busulfan Dose for patients < 12 kg & for ideal target AUC_{cum} of 7560 (μM·Min)/L [31 (mg · h)/L]. Use this table to check against error from the calculator.

Sample Age	Sample Weight (kg)	1st Busulfan Dose Per Long-Boyle Method (mg)
1 month (0.08 years)	4	11
	4.4	11.9
	5	13
3 months (0.25 years)	5.8	17.4
	6.4	18.7
	6.8	19.6
6 months (0.50 years)	7.2	24.1
	7.8	25.5
	8.4	27
9 months (0.75 years)	8.2	29.4
	9	31.6
	9.6	33.1
12 months (1 years)	9.6	35.4
	10.2	37.1
	11	39.2
15 months (1.25 years)	10.2	38.8
	11	41
	11.8	43.3

2. **For patients >/= 12 kg to <66 kg:** the first busulfan dose will be according to weight-based dosing nomogram developed by Bartelink, which determines the first dose as a function of **weight** only.
    - Determine the first dose directly from the nomogram chart (no calculator is used)

- Round the patient's weight to the nearest kg. For weights in kg ending in "x.5", round UP to the nearest kg. For instance, for a weight of 43.5 kg, use 44 kg to determine the first busulfan dose from the nomogram.
- See Table A-2 for nomogram

Table A-2: Nomogram for the Determination of First Busulfan Dose for Patients ≥ 12 kg and < 66kg for ideal target AUC_{cum} of 7560 (μM·Min)/L [31 (mg · h)/L]. (Adapted from Bartelink).
Round the patient's weight to the nearest kg in order to determine the first busulfan dose from this nomogram

Weight (kg)	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)
		20	64.7	30	86.3	40	103.5	50	117.8	60	130.5
		21	67.1	31	88.2	41	105	51	119.1	61	131.6
12	41.8	22	69.5	32	90.2	42	106.5	52	120.5	62	132.6
13	45.1	23	71.9	33	92.1	43	108	53	121.8	63	133.7
14	48.2	24	74.3	34	94.1	44	109.5	54	123.2	64	134.7
15	51.2	25	76.5	35	96	45	111	55	124.5	65	135.8
16	54.1	26	78.5	36	97.5	46	112.4	56	125.7		
17	56.8	27	80.4	37	99	47	113.7	57	126.9		
18	59.5	28	82.4	38	100.5	48	115.1	58	128.1		
19	62.2	29	84.3	39	102	49	116.4	59	129.3		

3. **For patients weighing ≥ 66kg:** first busulfan dose = 3 mg/kg IV.

BUSULFAN THERAPEUTIC DRUG MONITORING (TDM)

Area under the curve (AUC) analyses will be calculated in-house per University of Minnesota BMT standard guidelines for all patients. The AUC will be calculated for each dose using serum busulfan concentrations (in ng/ml) performed by the University of Minnesota Medical Center Drug Analysis Lab and obtained at the following time-points:

- Immediately prior to busulfan infusion (*ONLY to be drawn if obtaining levels with doses 2)
- Immediately after the end of the busulfan infusion
- 15 minutes after the end of the busulfan infusion
- 1 hour after the end of the busulfan infusion
- 3 hours after the end of the busulfan infusion
- 5 hours after the end of the busulfan infusion
- 7 hours after the end of the busulfan infusion

* “trough” or pre-dose serum busulfan level will only be obtained when performing AUC on 2nd or higher doses, as serum busulfan level prior to dose #1 is assumed to be zero.

After determination of the patient's clearance and AUC, subsequent doses will be calculated linearly to achieve a goal cumulative AUC exposure.

For patients receiving 2 total busulfan doses the goal AUC_{cum} = 6950-8170 (μM·Min)/L [28.5-33.5 (mg · h)/L] with ideal target AUC_{cum} of 7560 (μM·Min)/L [31 (mg · h)/L]

GUIDELINES FOR CHANGING SUBSEQUENT BUSULFAN DOSES BASED ON A PATIENT'S TDM RESULTS

Subsequent busulfan dose changes will be made according to the following guidelines:

1. The **goal cumulative AUC range** (AUC_{cum} for the entire 2-dose course) **will be 6950-8170 (μM·Min)/L [28.5-33.5 (mg · h)/L] with ideal target AUC_{cum} of 7560 (μM·Min)/L [31 (mg · h)/L]**
2. Cumulative AUC is defined as the sum of each individual dose AUC:
   AUC_{cum} = AUC1 + AUC2
3. Changes will be made to subsequent doses only if the **projected AUC_{cum}** falls outside of the goal range.
   a. After the first busulfan dose, **projected AUC_{cum} = AUC1 x 2**
   b. If dose adjustment is necessary, new doses will be calculated based on **an ideal target AUC_{cum} of exactly 7560 (μM·Min)/L [31 (mg · h)/L]**
4. Total busulfan exposure will be recorded from busulfan AUC analyses following both doses:
   AUC_{cum} = AUC1 + AUC2

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