UNIVERSITY OF MINNESOTA BLOOD & MARROW TRANSPLANTATION PROGRAM

MT2009-09

BIOCHEMICAL CORRECTION OF SEVERE EPIDERMOLYSIS BULLOSA BY ALLOGENEIC STEM CELL TRANSPLANTATION

Version Date: November 21, 2016

Study Committee

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FDA IND#14166

Amendment History

Version Date	Amendment Number	Modification(s)	Consent Modification
09/01/09	Original		NA
10/22/09	Revision 1	• Addition of IND Number • Deletion of references to intra-lesion mesenchymal stem cells throughout document • Subsection 4.5.6 pregnancy exclusion and requirement for birth control • Subsection 4.6.2 additional details on required assessment criteria • Subsection 6.5 reference to institutional SOPs for supportive care guidelines • Section 7.0 details on standard procedures for skin biopsies and GI endoscopy and mucosal biopsies • Section 9.0 reference to following FDA regulations for adverse event reporting and donor eligibility requirements	Yes (delete directed MSC donor consent); otherwise no changes
03/18/10	Revision 2	• Add opportunity for patients with a sibling donor and complete chimerism at > 6 months post transplant to receive up to two MSC infusions at least one month apart. Section 4.7-4.12 details eligibility criteria for sibling donor MSC's; Insert Section 6.5 detail of sibling MSC administration; Section 8.2 update with adverse event monitoring pre and 4 hours post MSC infusion; Insert new section 10.6 regarding statistical analysis of sibling MSC's; • Section 6.1 – Busulfan - replace phenytoin with Keppra as seizure prophylactic; • Update adverse event reporting (section 8), data and safety monitoring (section 9), and conduct of the study (section 11) with current U of MN language. • Clarifications, administrative, and formatting changes in treatment plan and study parameters. Delete eligibility checklist and add targeted toxicity form to the appendices.	Yes, update all consents, new patient/parent consent for sibling donor MSC infusions
05/21/10	Revision 3	Section 5.2 Staggering of Enrollment – After discussion with the FDA – patients with the JEB Herlitz variant of EB will be exempt from the enrollment delay (requiring at least 4 weeks between patients for the first 5 enrolled in each stratum); section 4.2.4 add normal EKG or approved by Cardiology for transplant; section 4.5.1 clarify "systemic" infection; Section 8 – update the definition of UPIRTSO; Section 9.1 DSMB added reference to section 5.2.	No
09/18/10	Revision 4	Section 4.3 Deleted references to use of HLA mismatched adult volunteer donors. Section 6.3.2 Deleted reference to T cell depletion of unrelated donor marrow.	No, consents do not specifically address use of T cell depletion or HLA matching of donor

Version Date	Amendment Number	Modification(s)	Consent Modification
04/21/11	Revision 5	Treatment schema (page 7) changed to reflect modified conditioning regimen, replacing the busulfan, cyclophosphamide and fludarabine myeloablative conditioning with a non –myeloablative regimen consisting of cyclophosphamide, fludarabine, low dose total body irradiation and antithymocyte globulin. Addition of Subsections 2.3.4 and 2.3.5 reviewing results of this trial to date and rationale/results with the proposed conditioning regimen. Section 6 and 6.1 modified to reflect the new treatment plan using cyclophosphamide, fludarabine, low dose TBI and ATGAM. Section 12 includes a change in the references to the literature, updated citation 42 updates the prior citation reflecting the Minnesota experience of the initial clinical trial and new citations 47 and 48 support the use of the new conditioning regimen. Appendix 5 changes reflect a more detailed plan for collection of autologous PBSC and addition of a monitoring plan for EBV reactivation. Section 8 – update to current IND safety reporting requirements	Yes, update patient transplant specific consents to reflect change in conditioning regimen
10/07/2011	Revision 6	At request of FDA, update to clarify unlicensed cord blood units may be used as a cell source (sections 4.3, 8.1, and 9.3), minor administrative changes/updates	No
02/14/2012	Revision 7	Remove staggering of enrollment – permission received effective 02/08/2012 from the FDA to proceed without the 1 month between patient enrollments; clarify QOL completion is not required for children < 2 years of age; restore sibling cell dose to section 4.3 that was deleted 2 versions ago in error;	Yes
03/26/2012	Revision 8	Section 8 clarify adverse event monitoring and documentation will end at day +100 except for in association with the late MSC infusion; Correct section numbering sections 5 and 6	No
09/24/2012	Revision 9	Section 4.3 – clarification of match criteria and order of donor preference Section 8.3 – update MCC SAE coordinator contact info	No
02/20/2014	Revision 10	Section 2.3 – clinical experience with non myeloablative transplant and noted increased incidence of partial engraftment, providing the rationale for higher dose TBI. Section 6 – shows TBI 300 cGy. Section 7.2 – added zinc, selenium, vitamin D3 levels at specific time points Appendix 11 – add iscorEB	Yes
12/03/2015	Revision 11	Title page – designate Jakub Tolar as principal investigator and IND sponsor; page header and section 10.7 – remove references to Dr. Wagner	No – previously updated
11/21/2016	Revision 12	Synopsis, section 4 – updated eligibility criteria Section 7.1, 7.2 updated clinical assessments Synopsis, section 7.2 – subject follow up will end after 2 years	Yes

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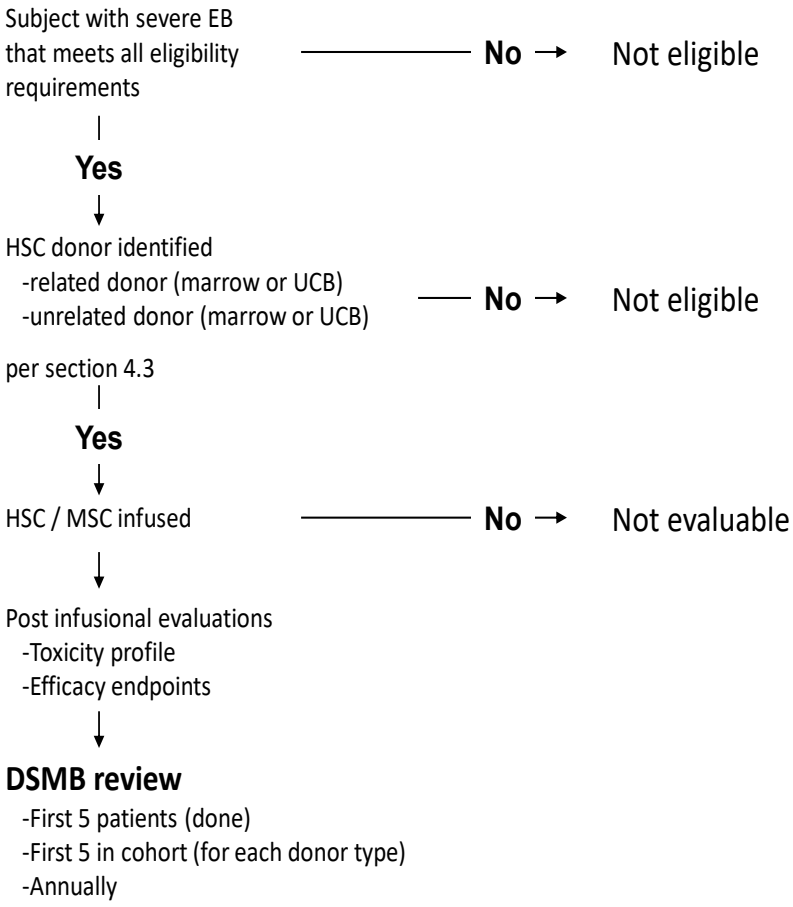
Protocol Synopsis

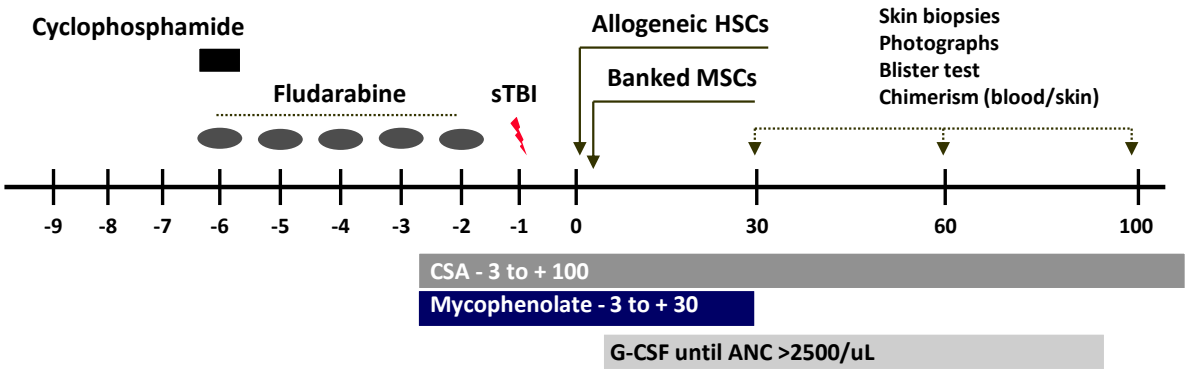
BIOCHEMICAL CORRECTION OF SEVERE EPIDERMOLYSIS BULLOSA BY ALLOGENEIC STEM CELL TRANSPLANTATION

Study design:	Open label, single institution, phase II study
Primary objective:	To estimate the event-free survival rate by 1 year post-transplant with an event defined as death or failure to have a demonstrable increase in collagen, laminin, intergrin, keratin or plakin deposition by 1 year post-transplant or other biochemical, structural or physical measure of improvement.
Secondary Objectives:	Determine the incidence of transplant-related mortality at 180 days; describe the pattern of biochemical improvement as measured by increase in protein expression (collagen, laminin, integrin, keratin or plakin); describe health quality of life at day 365 and 730 as compared to pretreatment results; evaluate the pattern and durability of hematopoietic stem cell (HSC) and third party mesenchymal stem cell (MSC) engraftment in the skin; determine the probability of survival at 1 year.
Eligible disease:	Severe forms of inherited blistering skin diseases, referred to as epidermolysis bullosa (EB).
Eligibility criteria:	Subjects must be 0-25 years of age and have adequate organ function, , an available healthy HLA matched or partially HLA matched related or unrelated HSC donor and MSC graft.
Exclusion Criteria:	Evidence of HIV infection or known HIV positive serology; current active serious infection; diagnosis of squamous cell carcinoma; donor with EB; pregnancy or breast feeding.
Accrual Objective:	75 subjects over 5 years.
Study duration:	Subjects will be followed for a minimum of 2 years after stem cell transplant.

Subject Screening and Enrollment Schema



Systemic Treatment Plan



Cyclophosphamide 50 mg/kg IV on day -6
Fludarabine 40 mg/m2 IV on days -6, -5, -4, -3, -2
Antithymocyte globulin (ATG) 30 mg/kg IV on days -4, -3, -2
Total body irradiation 300 cGy on day -1

After 6 months from transplant, patients with a sibling donor and complete chimerism may be eligible to receive up to two MSC infusions at least one month apart from the sibling donor.

1 HYPOTHESIS AND OBJECTIVES

The underlying hypothesis is that the infusion of bone marrow (BM) or umbilical cord blood (UCB) from a healthy unaffected donor will correct the collagen, laminin, integrin, keratin or plakin deficiency and reduce the skin fragility characteristic of severe forms of epidermolysis bullosa (EB). A secondary hypothesis are that fludarabine-based reduced intensity preparative regimen will be well tolerated and sufficient for donor engraftment, and that mesenchymal stem cells (MSC) from a healthy donor will enhance the safety and effectiveness of the allogeneic hematopoietic stem cell (HSC) transplant as well as serve as a source of renewable cells for the treatment of focal areas of residual blistering.

1.1 Primary Objective

To estimate the event-free survival rate by 1 year post-transplant with an event defined as death or failure to have a demonstrable increase in collagen, laminin, integrin, keratin or plakin deposition by 1 year post-transplant or other biochemical, structural or physical measure of improvement.

1.2 Secondary Objectives

1.2.1 Determine the incidence of transplant-related mortality (TRM) at 180 days.

1.2.2 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.

1.2.3 Describe health quality of life at day 365 and 730 as compared to pretreatment results.

1.2.4 Describe the pattern and durability of HSC and third party MSC engraftment in the skin.

1.2.5 Determine the probability of survival at 1 year.

2 BACKGROUND

2.1 Dystrophic Epidermolysis Bullosa (DEB)

2.1.1 Clinical Manifestation and Natural History of DEB

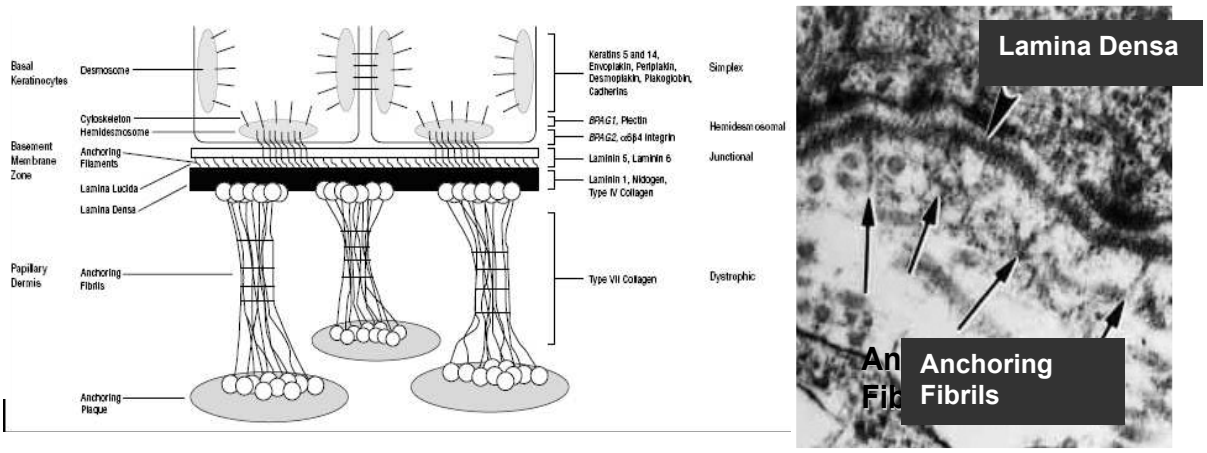
Dystrophic EB (DEB) is a group of heritable mechanobullous skin diseases characterized by skin fragility, blister formation, and scarring. The BMZ is characterized by a paucity or diminutive size of AF (Figure 1)[1-3]. The most severe form of DEB is generalized recessive DEB, characterized by mutilating scarring, blisters (up to 70-80% of the body surface), joint contractures, strictures of the esophagus, corneal erosions, renal disease and aggressive squamous cell carcinoma (SCC).

The high incidence and aggressive nature of the SCC is the number one cause of mortality among DEB patients. It is unknown what causes the fast growing, multiple appearance and early metastasizing of the SCC in DEB patients, but the risk of developing the carcinoma increases with age. SCC can appear as early as 13 years of age and by age of 40 years, one in every two patients will suffer and die from SCC. At 60 years, the cumulative risk of SCC is 76.5%, a much higher cumulative risk than the average person in USA (4-14%). The prognosis of a DEB patient with SCC is poor with death in most before age 30[4, 5].

Recently a study conducted with the National EB Registry showed that renal failure was a significant cause of mortality among DEB patients. Patients with severe form of DEB have a 12.3% incidence of renal failure by age 35 years, making renal failure the second most common cause of mortality among adult patients surpassed only by SCC mortality incidence[5].

Patients with severe DEB are also affected by profound physical disabilities with social and psychological implications. Daily activities (toileting, feeding, bathing, walking, etc.) are major challenges for these patients with many requiring complete assistance. For example one study demonstrated that only 24% of children with DEB can walk without assistance [6]. The quality of life of children with DEB worsens with age. One third of the parents of a DEB patient based their marriage relationship exclusively to the day to day care and logistic of the affected child. In addition, the majority of divorced families with DEB children blame the family disintegration on the disease[7].

Figure 1



From Uitto and Pullkinen. *Arch Dermatol.* 2001;137:1458-1461 (3).

2.1.2 Recessive Dystrophic EB (RDEB*)*

As shown in Figure 1, RDEB is due to a deficiency of collagen type VII (Col7). Col7 is synthesized and secreted by both human keratinocytes

and fibroblasts. It is secreted within the basement membrane zone (BMZ) lying between the epidermis and dermis of skin[6]. Col7 is the major component of BMZ attachment structures known as anchoring fibrils (AFs), which provide epidermal-dermal adherence[1, 2, 3]. Genetic defects in the Col7 gene, designated *COL7A1* result in RDEB[8, 9, 10].

DEB can be inherited in an autosomal dominant or autosomal recessive pattern. The most severe form of recessive disease (referred to as RDEB-severe generalized) is associated with extensive scarring and blistering. The skin blistering is usually present at birth and scarring starts within the first few months of life. The blisters and scarring are present at birth or become visible the first days after birth. The blisters in the neonate can cover the whole body, including oral and esophageal mucosa[11]. The blistering and scarring continues throughout life, resulting in disfigurement of both hands and feet, hallmarks of the disease. Other complications develop over time that contribute to the clinical profile including growth retardation, anemia, osteoporosis, renal dysfunction, lung and cardiac problems[6, 12-14].

Ultrastructural and genetic evidence identified the cause of the clinical symptoms profile through life. Is known that mutations in the *COL7A1* gene result in abnormal morphology, low numbers or absence of the AFs[2, 3] which in turn result in cleavage or detachment of the sublamina densa to the underlying dermis with subsequent extreme skin fragility[2, 3].

2.1.3 Junctional EB (JEB)

As shown in Figure 1, J JEB is due to a deficiency of laminin 332 (LAM5) caused by autosomal recessive mutations in the *LAMA3*, *LAMB3*, or LAMC2 genes (encoding the α3, β3 and γ2 polypeptides of laminin 332, respectively),. Alternatively, some forms of JEB result from mutations in collagen type XVII (Col17) encoded by the gene *COL17A1*. In all forms of JEB, there is splitting within the lamina lucida of the basal keratinocytes. In the Herlitz subtype, the hemidesmosomes are reduced in number and are hypoplastic and anchoring filaments may also be reduced or absent. In the non-Herlitz type, hemidesmosomes may be reduced or hypoplastic., IF staining reveals abnormal or absence of laminin 332 (LAM5) or collagen XVII depending on the underlying gene pathology in individual cases [15].

All forms of JEB are inherited in an autosomal recessive manner. The most severe form is the Herlitz type which manifests at birth with severe blistering with little or no trauma and granulation tissue involving the trachea. Congenital malformations of the urinary tract may also occur. Blistering and scarring continues throughout life, resulting in early death in many affected children. In contrast, the non-Herlitz type may be less severe although it can be severe and often life-threatening in neonates.

Some forms of JEB can also be caused by mutations in the *ITGA6* and *ITGB4* genes encoding the hemidesmosome-associated protein $\alpha 6\beta 4$ integrin. In some affected individuals the severity and prognosis can resemble Herlitz JEB. In addition, there typically extracutaneous abnormalities, specifically pyloric atresia, which can be a life-threatening complication, the non-Herlitz type may be less severe and may not show up during the neonatal period[16].

2.1.4 Other Severe Forms of EB

The classification of EB has recently been expanded to include intra-epithlial junctional blistering diseases, notably involving structural components of desmosome cell-cell junctions. Recent mouse data have demonstrated that BMT has the potential to restore genetically defective desmosomal proteins, with recovery of epidermal integrity in desmoglein 3-null mice[17]. From a human disease perspective, pathogenic mutations have been reported in 9 different desmosomal proteins, several of which can involve severe skin fragility (e.g. desmoplakin and plakophilin 1). Of particular interest, mutations in desmoplakin can also be associated with extracutaneous abnormalities such as cardiomyopathy which may start in childhood or in young adult life. Thus, if BMT is able to correct an inherited disorder od desmoplakin, there is the potential to carry our therapeutic BMT in the clinical window between onset of skin fragility (at birth) and the later development of life-threatening cardiomyopathy. Although less common than RDEB and JEB, inherited disorders of desmosomes and keratins represent a subtype of EB that, in some cases, may also warrant consideration for BMT.

2.2 Treatment of Severe EB

2.2.1 Supportive Care

Care for patients with severe EB is entirely supportive with the aim of maximizing mucosal and skin integrity, reducing infection and minimizing pain and scar formation[18]. The mainstays of supportive care applicable to most children and adults with severe EB are:

- Protection of the skin and skin care with bandages to prevent shearing of the skin with friction and minimal trauma as well as infection prevention
- Treatment of growth failure by maximizing nutrition that may require gastrostomy tube or nasogastric feeding
- Esophageal dilatation to treat esophageal strictures secondary to repeated trauma and gastroesophageal reflux
- Treatment of gastroesophageal reflux with H2 blockers or proton pump inhibitors
- Prevention and treatment of chronic constipation
- Treatment of severe iron deficiency anemia secondary to chronic blood loss from mucosal and cutaneous blisters

- Surgical correction of scarring, contractures and syndactyly from repeated blistering
- Correction of severe dental problems, corneal and conjunctival adhesions and osteoporosis prevention/treatment.

2.2.2 Skin Grafting in EB

A subpopulation of patients with particularly severe areas of involvement requires skin grafting. Numerous reports suggest transient benefit of allogeneic skin grafts or artificial skin equivalents to treat the cutaneous manifestations of EB. Results have been limited by the allogeneic immune response and rejection as well as local infections.

The main allogeneic skin graft used to treat RDEB patients is Graftskin (Apligraf, Organogenesis Inc., Canton, MA) approved by FDA in 1998 to treat venous ulcers on the lower extremities and in 1999 to treat diabetic foot ulcers. Apligraft is a bilayer skin construct derived from human neonatal foreskin keratinocytes and dermal fibroblast cultures in a collagen matrix[19]. Apligraft provides the proper environment, growth factors, and components to stimulate healing and reconstitution of antigen expression in cases of DEB skin treatments in newborns[20] and children with severe DEB[21]. In the latter study 79% of the wounds healed as early as 7 days up to 18 weeks of follow up. In a recent Graftskin replacement therapy, the healing in five children with severe EBD was noted to have decreased itching and pain at the Graftskin treatment sites. However milia formation and erythema occurred for up to one year at sites prone to mechanical friction like the elbows, knees and ankles[22]. In general the quality of life improved but what is not clear is the long-term survival of the graft and health outcome of treatments.

In an attempt to enhance skin graft survival, combined use of allogeneic skin and BM transplantation has been performed[23]. A 4-month-old child with Herlitz type JEB was treated with BM transplant followed by transplant of skin grafts from the same donor. In this case, peripheral blood stem cells obtained from the child's haploidentical father (mismatched at 3 HLA antigens) were infused after conditioning with cyclophosphamide (CY) 120 mg/kg, melphalan 140 mg/m2 and anti-thymocyte globulin (ATG) 60 mg/kg. Four weeks after BM transplant, rapid progression of skin blistering occurred, resulting in loss of 2/3 of the total epidermis. While GVHD was not proven, excess sensitivity to melphalan as well as the natural course of Herlitz type-JEB likely resulted in the intensified loss of the epidermal surface. Skin transplant was performed using split thickness skin grafts (2 mm) from the thigh of the father. Grafts were placed on the dorsal trunk and gluteal region of the child. The remaining wounds were covered with glycerolized allogeneic skin to reduce fluid and protein losses. One week and 2 weeks afterwards, temporary grafts were replaced with donor skin grafts. All skin

grafts healed completely with excellent biomechanical and cosmetic results. Presence of skin derived from the donor was verified 17 and 67 days after skin grafting, supporting the use of this strategy for permanent large scale skin exchange in the setting of allogeneic BM transplantation. While engraftment was successful, presence of Pseudomonas colonization prior to transplantation, excessive chemosensitivity to melphalan and/or delayed immune recovery after HLA mismatched BM transplantation contributed to the development of systemic opportunistic infection and death.

2.2.3 New Therapeutic Interventions

New strategies for severe EB have focused on gene-, protein-, and cell-based therapies. However, to date, clinical trials in patients with RDEB have principally been limited to skin grafting with autologous skin grafts, autologous cultured keratinocytes, allogeneic keratinocytes, and skin bio-equivalents. None of these approaches have resulted in any long term success.

Allogeneic Fibroblasts. More recently, Wong et al[2] used intradermal injections of allogeneic fibroblasts in patients with RDEB. In 5 patients, an increase in Col7 was documented at the DEJ at 2 weeks and at 3 months following injection with increased numbers of AF, although none had normal morphology. While no adverse effects were reported, there was a beneficial effect albeit not permanent or systemic. The investigators hypothesized that the major effect of allogeneic fibroblasts is to increase the recipients' own COL7A1 mRNA levels leading to greater deposition of mutant Col7 resulting in the formation of additional rudimentary AF and partial functional improvement. Nonetheless these results provide the ‘proof of principle’ that allogeneic fibroblasts can have a positive effect and may be useful in particular for treating local areas that are repeatedly traumatized.

Mesenchymal Stem/Stromal Cells (MSC). Clinical investigation with MSC is expanding exponentially. MSC are a well-characterized population of adult stem cells found in the BM, capable of forming fat cells, cartilage, bone, tendon and ligaments, muscles cells, nerve cells and skin cells. MSC have been studied in great detail and have been used in hundreds of patients worldwide administered both systemically and locally[24-30]. Importantly data suggest that MSC can repair damaged skin even in humans[30].

MSCs can be obtained in quantities appropriate for clinical applications, making them good candidates for use in tissue repair. Techniques for isolation and amplification of MSCs in culture have been established and the cells can be maintained and propagated in culture for long periods of time, without losing their capacity to form all the above cell types.

Furthermore, MSC can be frozen and when thawed they function normally, thus allowing for future "off-the-shelf" therapy approaches. At a dose of 1-5 x 10^6/kg (the MSC dose range used in most reported series) toxicities have rarely been reported[28].

BM-MSC are the most advanced population of MSC with regard to clinical development. Osiris (Columbia, MD) has launched multiple clinical trials using MSC for enhancing engraftment and prevention and treatment of acute GVHD after allogeneic HSC transplantation. In addition, Osiris has trials in the treatment of myocardial diseases and type I diabetes among others[27]. The safety profile is established with few reports of toxicity. While theoretical, there have been no reports of ectopic tissue formation[31-33]. Notably, there has been one report of malignant transformation (MSC derived sarcoma) observed in one animal model [34].

2.3 Rationale for Evaluating BMT for Severe EB

It has long been known that BM derived cells play a critical role in wound healing. As wounds enroll many mature inflammatory cells which orchestrate the healing process, many less well characterized BM derived cells also migrate to wounds. Recent reports have illustrated that BM contains stem and progenitor cells capable of differentiating into non hematopoietic tissues, including skin[35]. Wounding is likely one mechanism by which BM-derived cells are recruited to organs. Data suggest that BM-derived cells promote wound healing particularly in settings where resident skin stem cells are deficient or defective.

In addition, allogeneic HSCT is an established approach for the correction of various metabolic diseases, such as mucopolysaccharidoses (ie, Hurler, Maroteaux–Lamy, and Sly syndromes), leukodystrophies (ie, childhood and adolescent cerebral X-adrenoleukodystrophy, globoid cell leukodystrophy and metachromatic leukodystrophy), fucosidosis, mannosidosis, Gaucher disease, Niemann–Pick type B, and malignant infantile osteopetrosis[36]. The mechanism of action is through the transfer of lysosomal and perioxisomal proteins from BM-derived cells into other cells missing the critical protein. Together, these observations led to the possibility that allogeneic HSCT might correct the dermatological manifestations of DEB and serve as a treatment of other diseases due to defects of secreted matrix proteins.

2.3.1 Preclinical Studies-“Proof of Concept”

Animal Model of EB. With the information on the molecular culprit of DEB, the next logical step was to develop a DEB animal model in which the experimental hypothesis about pathological mechanisms and possible therapeutic approaches can be tested.

The animal was generated by replacing exons 46-69 with antibiotic resistant genes. This led to a truncated messenger with eventual lack of a functional Col7 protein in the mice. Heterozygous results in normal mice, null mice results in mice with extensive cutaneous blistering. The null knockout mice show all the hallmark of the disease. Extensive blistering, fusion of fingers and toes, sublamina densa detachment from underlaying dermis, absence of AF and lack of immunostaining of the Col7 at the membrane basement[37].

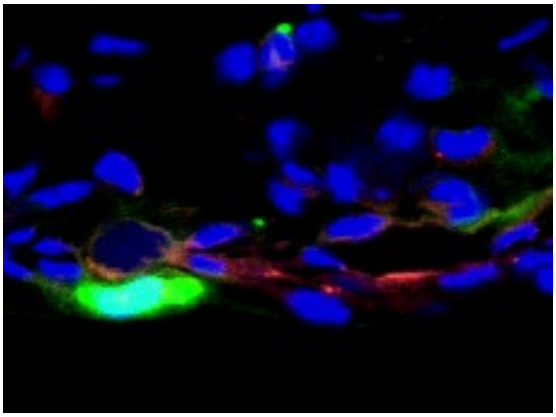
In general, the evidence points out that the *Col7A1* knockout mice show many of the clinical, genetic and ultrastructural features

of the DEB. This animal system allows testing of different therapeutic approaches towards
the cure of the clinical symptoms. A suitable candidate approach is cell replacement therapy using allogeneic hematopoietic cell replacement.

Adoptive Transfer of Col7 after Allogeneic HSCT. As the DEB mutant newborns develop blisters and do not survive after two weeks of life, we have been able to identify the mutant pups readily. Further, rapid lethality provides a robust readout for the cellular interventions. As BM has previously been shown to seed various organs, including skin, we reasoned that a Col7 producing cell may be present in BM compartment. To prove this *in vivo*, we tested multiple BM candidate populations for cellular therapy of DEB in the animal model. Populations tested were: total BM epidermal stem cells, multipotent progenitor cells, MSC, lineage positive and lineage negative BM cell fractions, and transitional epithelial cells. All cell populations tested failed to provide phenotypic correction, even when used at varying time points after birth, varying dosing regimens and varying routes of administration (intravenously or intrahepatically).

Recently identified SLAM positive populations in the BM have been shown to have a significant pluripotentiality[38-40]. Therefore, we tested $CD150^{+}/CD48^{-}$ BM cell populations at multiple dose levels and infused the cells either *in utero* (into preterm pups) or early postnatally into unconditioned DEB mutant mice. $CD150^{+}/CD48^{-}$ BM cells at a dose 8 million cells per mouse administered intravenously on day 3 or 4 after birth resulted in survival in 3 of 13 animals tested. One animal was sacrificed on day 55 of life and two were sacrificed on day 70 of life. Tissues from each animal were evaluated extensively. First, all 3 mice were confirmed to be mutants, not only by phenotype of the blisters postnatally, but also by genotype, using PCR typing. The weights of these animals were lower than that of their normal litter mates and they were weaned two weeks later than usual. The blisters on the paws of these animals healed with contractures, but there was no re-blistering noted. Chimerism (presence

of donor cells) was confirmed in the peripheral blood (mean: 3%) and BM (mean: 12%). Tissue analyses showed that the adoptive transfer of donor SLAM CD150 selected BM resulted in production of VII collagen mRNA, as assessed by reverse transcriptase PCR. BM cells explanted from the 3 surviving recipients showed Col7 producing cells (Figure 2). Remarkably, we were able to show AFs, structures in the skin that require Col7 protein, on electron microscopy, showing conclusively that adoptive transfer of wild-type BM cells resulted in, at least partial, functional correction of collagen synthesizing defect (Figure 3). Collectively, these data demonstrate the proof of principle of BM transfer for correction of DEB defect in the murine model[41] .



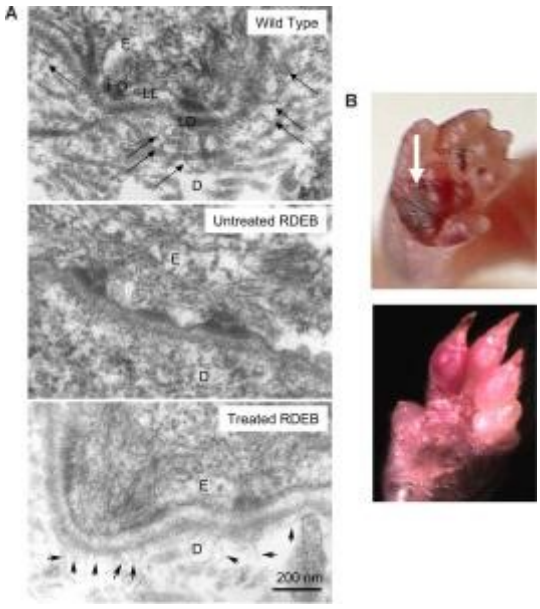


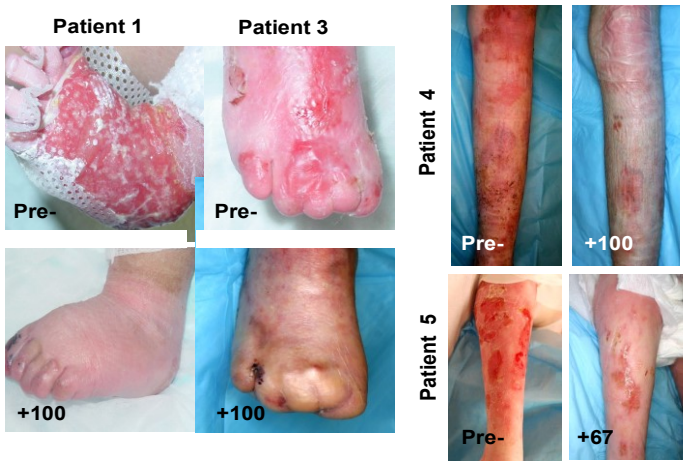
Figure 3

2.3.2 "First in Human" Clinical Trial

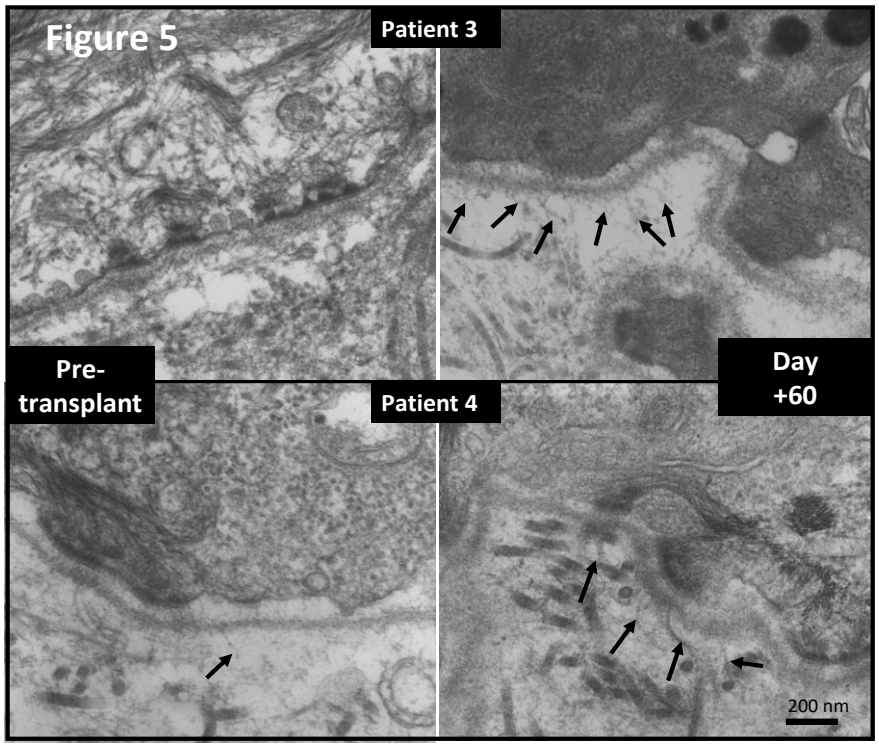
Allogeneic BM transplantation is a standard treatment option for an increasing number of malignant and non-malignant disorders, including metabolic disorders where missing proteins/enzymes that can be replaced by BM-derived cells from healthy related or unrelated donors[36]. To reconstitute hematopoiesis after an intensive myeloablative therapy, the transplantation of pluripotential HSC, such as from BM or UCB, is required.

Between October 2007 and August 28, 2009, 7 children aged 9 months to 14 years were enrolled in a clinical trial to receive BM and/or UCB after high dose chemotherapy (Table 1). All had more than 50% body surface area (BSA) covered with blisters and erosions before transplantation. They were conditioned with busulfan (BU, 12.8-16 mg/kg), fludarabine (FLU, 75 mg/m^2) and CY (200 mg/kg) followed by the infusion of sibling donor BM (n=5) or unrelated donor UCB (n=1) on day 0. Patient 2 who presented with significant renal impairment (glomerular filtration rate 26 mL/min/1.73 m^2) secondary to perinatal cardiac arrest necessitated a 50% FLU dose reduction.

Figure 4



Six of seven patients completed the treatment plan; patient 2 died of cardiomyopathy on day 0 prior to BM infusion. Three of the 6 evaluable patients demonstrated moderate clinical improvement (defined as less than 25% of BSA affected) and the remaining three patients had marked clinical improvement (defined as less than 10% of BSA affected (Figure 4).



Remarkably, results of immunofluorescence (IF) for Col7 and electron microscopic (EM) studies for AFs qualitative and quantitative assessments demonstrated increased quantity of Col7 by IF and gradual increase in numbers and maturity of AFs by EM (Figure 5). Unexpectedly (on the basis of finding in the animal model), a significant proportion of skin cells were of donor origin.

Table 1. Patient Characteristics

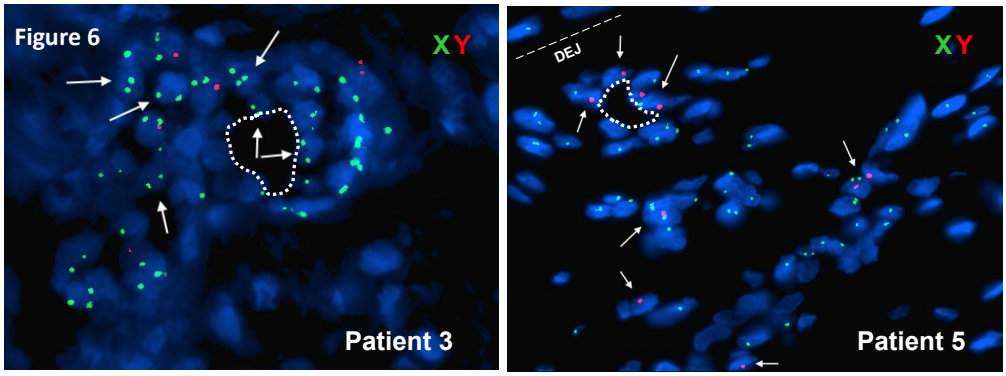
Pt	Pre Treatment Clinical Status and Col7 mutation	Donor (cell dose: NC x 10^8/kg)	Transplant Related Toxicities	C7 Assessment	Anchoring Fibril Assessment	Clinical Outcome and Change in Dressing Use	Skin Chimerism by Molecular Analysis	Survival Days (as of 08/01/09)
1	15 month old male; Mitten deformity; esophageal stricture; FTT, c.3472delC (p.Pro1158fsX3) IVS51+1G>A	HLA 8/8 male Sibling BM (3.04) and UCB (0.66)	Hyper-bilirubinemia	Increased by IF and Western	↑ Rudimentary AFs	Moderate reduction in blistering	11.4%	Alive d1,292
2	9 month old female; renal failure; neonatal cardiac arrest; gastrostomy c.3840delC (p.Thr1280fsX45) c.8780G>A (p.Arg2927His)	HLA 8/8 male Sibling BM (N/D)	Cardiomyopathy	Not evaluable; patient died before BM infusion				
3	5.9 year old male; mitten deformity; gastrostomy, pulmonary artery stenosis, flexion contractures; FTT c.3472delC (p.Pro1158fsX3) IVS51+1G>A	HLA 5/6 female URD UCB (0.55); HLA 5/6 female URD UCB (0.56)	Graft rejection after first transplant; infection and ARDS after second transplant	Increased by IF and Western	↑ Rudimentary AFs	Moderate reduction in blistering	0-46.8%	Died d183
4	6.3 year old male; esophageal stricture; peri-rectal fissures IVS85-2delAG IVS92+5G>A	HLA 8/8 female Sibling BM (3.76)	Transient Hemodialysis; Mechanical Ventilation	No change by IF° but increased by Western	↑ Rudimentary AFs; Normal AFs observed	Marked reduction in blistering; Marked reduction in dressings	0-27.4%	Alive d880
5	6.2 year old female; mitten deformity; flexion contractures; esophageal stricture; FTT, anemia c.6176A>G (p.Glu2059Gly) IVS5+1G>A	HLA 8/8 male Sibling BM (3.07)	Transient Hemodialysis	Increased by IF and Western	↑ Rudimentary AFs	Marked reduction in blistering; Marked reduction in dressings; resolution of anemia	22.1-93%	Alive d761
6	6.9 year old female; mitten deformity; flexion contractures; esophageal stricture; FTT, anemia c.4919delG (p.Gly1640FSX70); c.8254 8255delAG (p.Arg2751fsX20)	HLA 8/8 Female Sibling BM (5.04)	Hyperbilirubinemia	Absent C7	Absent AF	Moderate reduction in blistering	9-33%	Alive d689
7	14.5 year old female; mitten →deformity; esophageal stricture; FTT, c.6781C →T (p.Arg2261X); IVS110-1G→C	HLA 8/8 male Sibling BM (3.04) and UCB (0.66)	Hyperbilirubinemia	Increased by IF and Western	↑ Rudimentary AFs	Marked reduction in blistering; Marked reduction in dressings;	18-51%	Alive d623

FTT=failure to thrive; BM=bone marrow; UCB=umbilical cord blood; URD=unrelated donor; IF=immunofluorescence staining for C7; AF=anchoring fibrils

†HLA typing of the patient and donor was performed at allele level for HLA-A, B, C and DRB1 in marrow recipients; however, in unrelated UCB recipients, HLA typing was performed at antigen level for HLA-A and B and allele level for DRB1.

‡Patient 3 received a second transplant 47 days after the first transplant in response to immune graft rejection. Prior to the transplantation of a second HLA 5/6 (DRB1 antigen mismatched) UCB unit, conditioning consisted of rituximab 375 mg/m^2 IV as a single dose (day -5) and anti-thymocyte globulin 15 mg/kg/day IV on three days (days –4 to –2, total dose 45 mg/kg). ARDS=acute respiratory distress syndrome

As shown (Table 1), chimerism in the skin ranged between 0-93% varying between the location of the skin biopsy and time after transplant[42]. Two patients (3 and 5) received sex mismatched BM grafts. Fluorescent in-situ hybridization for XX and XY was performed demonstrated perivascular



clustering of donor cells in the dermis. While the identity of these cells remains to be determined, these results suggest that the application of stem cells from marrow or UCB may not represent homologous use.

2.3.3 Induction of Tolerance after Allogeneic HSCT

Tolerance is routinely achieved after allogeneic HSCT when donor derived immune cells specifically fail to recognize and react to host MHC while maintaining a normal alloreactive response to third party MHC and other pathogeneic antigens. Once tolerance is achieved, the patient is able to come off all immunosuppressive therapy without development of graft rejection or GVHD. In animal models, tolerance is proven when third party skin explants are rejected and donor skin explants are accepted life long[43].

It has previously been shown that MSC are potent immune modulators. The mechanism by which they inhibit various inflammatory reactions include production of immune suppressive cytokines, such as IL-10, induction of tryptophan depletion, and providing signal 1 in absence of costimulation. The immunosuppressive effects of MSCs have been generally ascribed to the inhibitory effect on T cells, but recently it was been shown that MSCs also have inhibitory effects on other immune cells, including dendritic cells[44-46]. In vivo, infusion of MSCs prolong skin allograft survival and may decrease GVHD in animal models[28, 32]. Clinical data also demonstrate that MSC can enhance engraftment and prevent GVHD[24 26, 32, 33]. Together with the potential direct effect of MSC on repair of skin in RDEB, these data provide a rationale for infusing MSC as a strategy for reducing the immunological risks associated with allogeneic HSCT.

2.3.4 Transplantation of HSC and MSC for Severe EB: Preliminary Results

Thus far, 9 children (7 with RDEB and 2 JEB-Herlitz variant) have been treated on this protocol using BU, CY, FLU followed by the infusion of HLA matched or partially matched HSC (4 with a sibling donor, 2 with an unrelated marrow donor and 3 with an unrelated cord blood donor). As of March 31, 2011 7 of 9 are alive 20 to 406 days after transplantation with one child with JEB dying 44 days from infections despite engraftment and one with RDEB dying 101 days after HSCT from multi-organ toxicity. Marrow recovery occurred in all patients with one child with RDEB having autologous recovery. Of the six remaining patients, three had increased levels of the missing protein, two had no change thus far and one has not had the first scheduled evaluation. In all patients with hematopoietic engraftment, chimerism (6-33%) was also present in the skin with 6-33% of skin cells derived from the HSC donor. Notably, one patient had chimerism in the skin from the MSC donor (4%) at day 100. Three patients with increased protein had improved wound healing, and resistance to blister formation. In the one evaluable patient with JEB, blistering markedly decreased.

2.3.5 Rationale for Reduced Intensity Conditioning

The two trials at the University of Minnesota together suggest that the infusion of allogeneic BM can ameliorate the biochemical, structural and clinical manifestations of RDEB and JEB. While allogeneic BMT can be a disease-modifying intervention with clinical benefit for severe EB, the conditioning regimen is associated with considerable toxicity and risk of opportunistic infection. Toxcities in the two prior tirals have included: hepatic veno-occlusive disease (n=3), acute renal failure (n=6) requiring hemodialysis (transiently in most), severe cutaneous toxicity (n=1), respiratory failure (n=8) requiring ventilatory support. Therefore, we propose to evaluate a reduced intensity conditioning regimen, consisting of CY 50 mg/kg, Flu 200 mg/m2, TBI 200 cGy and ATG 90 mg/kg; a regimen used at the University of Minnesota since 2000 in older patients with malignant disease or those with pre-existing comorbidities excluding them from a full myeloablative therapy[47].

2.3.5.1 Clinical Experience with CY50/FLU200/TBI200+/-ATGAM90

In 2000, the reduced intensity conditioning regimen of CY50/FLU200/TBI200 was developed as a strategy for reducing the risk of regimen related toxicity and consequent TRM specifically for patients >45 years of age or younger patients with preexisting co-morbidities or active opportunistic infections, undergoing UCB transplantation. Due to the finding that absence of recent prior chemotherapy (i.e., within 3 months of transplant) was associated

with an increased risk of graft failure, such patients subsequently had ATGAM added to their preparative therapy which successfully increased the probability of engraftment. The following summarizes the outcomes in the first 110 adults aged 17-69 years (median 51 years) treated for malignant diseases.

Engraftment
Compared to historical controls in whom there was no recent chemotherapy, addition of ATGAM improved engraftment (83% versus 64%) in an interim analysis performed in 2007.

In a more recent analysis using this treatment plan where ATGAM is added only for those without recent chemotherapy, engraftment is good whether the graft is composed of a one (90% [95% CI: 80-97%]), n=43, median=16 days) or two UCB units (94% [95% CI: 91-96%]), n=277, median=14 days). Multivariate analysis reveals low UCB graft cell dose (and not absence of recent chemotherapy) as the only risk factor for graft failure, suggesting that the addition of ATGAM has reduced the risk.

GVHD
The cumulative incidences of grades II-IV and grades III and IV acute GVHD at day 100 were 59% (95% CI, 49%-69%) and 22% (95% CI, 14%-30%), respectively.

Twenty one patients had grade III and 3 had grade IV GVHD. In multivariate analysis, patients who received double-unit UCB grafts and those not receiving ATGAM had a higher relative risk of GVHD. The cumulative incidence of chronic GVHD was 23% (95% CI, 15%-31%) at 1 year with no factor identified as predictive of chronic GVHD.

EBV Reactivation
Among 95 patients who received an NMA preparative regimen, the incidence of EBV viremia or PTLD was 7% (95% CI, 2%-14%). However, there was a significantly higher risk among patients who received ATG (21% vs 2%, p=.01). Among 30 patients who received ATG, 5 developed EBV PTLD and 1 developed EBV viremia. Patients who received an NMA preparative regimen with ATG were more likely to be older, male, weigh more, and be a recipient of 2 UCB units.

Rituximab was administered in 9 of 15 patients who developed EBV-related complications, with 5 responding and all 5 alive and EBV disease free beyond 1 year. Among the 4 patients who failed

rituximab, 3 received the drug alone and 1 received the drug in combination with vincristine.[48]

2.3.6 Impact of Reduced Intensity Conditioning in Patients with EB

Due to the toxicity profile with a myeloablative regimen, a new conditioning regimen was developed that was non-myeloablative. It has been better tolerated with less renal and hepatic complications.

2.3.6.1 Clinical Experience with the Nonmyeloablative Regimen In Patients With Severe EB

Engraftment
Thus far 12 patients were treated on with the non-myeloablative regimen. Engraftment was good. By donor type:3 of 4 with a sibling marrow donor engrafted (one graft failure); 6 of 6 with a matched unrelated marrow donor engrafted (no graft failure) and 1 of 2 with a mismatched unrelated UCB donor engrafted (one graft failure). However, one patient (RDEB6) had primary engraftment but experienced a late graft failure after a viral like illness and underwent second transplant successfully.

Importantly, while engraftment was documented in all, 3 patients (RDEB9, RDEB12 and RDEB15) required donor lymphocyte infusions to enhance the proportion of donor cells. Two of the three responded; the other died from infectious complications prior complete evaluation.

GVHD
GVHD was uncommon occurring in one patient thus far. Whether this is related to the immunosuppressive properties of the MSC infusion, differences in GVHD targets in the mucosa and skin of children with EB or some other factor, is as yet not known.

EBV Reactivation
Among 12 patients who received the non-myeloablative regimen, no patient demonstrated EBV reactivation or PTLD.

2.3.7 Rationale for TBI 300 cGy

Delaney et al [49] recently reported on the efficacy of TBI 300 cGy in reducing the risk of graft failure in patients considered to be high risk for graft failure. In a study where all patients received an identical treatment except for TBI dose, ie fludarabine and cyclophosphamide as part of the conditioning and CsA and MMF for GVHD prophylaxis (exactly as in

patients with EB), TBI 300 cGy resulted in high engraftment rates with no increase in toxicity.

Specifically, in this study, 48 patients median age 59 years (range 25 - 73) and comorbidity index = 3 (range 0 - 8) with high risk hematologic malignancies received TBI 200 cGy if there was no risk factor for graft failure and TBI 300 cGy if the patient was considered high risk for graft failure. High risk for graft failure was defined as receiving < 2 cycles of multiagent chemotherapy or no multiagent chemotherapy within the 3 months prior to UCB transplant. Forty-seven of 48 patients received 2 UCB units (one patient received a single UCB) with a median cell dose of 4.05 x 10^7 nucleated cells per kg and a post-thaw median $CD34^+$cells/kg of 1.75 x 10^5. UCB grafts (98%) were 1-2 HLA antigen mismatched with the recipient. Twenty-six patients were assigned to Arm 1 (low risk for graft failure) and 22 patients were assigned to Arm 2 (high risk for graft failure).

All evaluable patients engrafted (n = 46). Patients on Arm 1 achieved neutrophil recovery (ANC > 500 x 3 days) at a median of 12 days (range 6 - 38) versus 23 days (range 7-46) in patients on Arm 2 (p = 0.01). Day +28 chimerism values were evaluable for 23 patients on Arm 1 and 20 patients on Arm 2. Median chimerism in the $CD3^+$ compartment was 100% (57 - 100) and 100% (0-100) on Arm 1 and Arm 2 respectively (p = 0.58). Median chimerism in the $CD33^+$ compartment was 98% (15-100) and 100% (44-100) on Arm 1 and Arm 2 respectively (p =0.26). Median chimerism in the bone marrow was 98% (15-100) and 100% (4-100) on Arm 1 and Arm 2 respectively (p = 0.84). This was maintained at Day +80. Acute graft versus host disease (GVHD) grades II - IV was seen in 84% (n = 21) and 68% (n = 15) of patients on Arm 1 and 2 respectively with 5% and 12% grade III-IV GVHD in the respective arms. Chronic GVHD was seen in 40% of patients on Arm 1 (8 mild, 2 moderate) and 23% on Arm 2 (3 mild, 1 moderate, 1 severe). Overall survival at 1 year in Arm 1 was 43% (95% CI: 22-62%) and Arm 2 was 46% (95% CI: 20-69%).

2.4 The goal of this study was to assess engraftment rates by neutrophil recovery and Day +28 chimerism in patients at low versus high risk of graft rejection. Although time to neutrophil recovery was significantly faster in patients with low risk of graft failure treated with TBI 200 cGy, patients at high risk of graft failure treated with TBI 300 cGy achieved a similar incidence of completer chimerism at Day +28 as those at low risk.

Summary

We believe that engraftment after TBI 200 cGy, while good, is still not optimal in patients with EB. On the basis of the recent report by Delaney et al, we plan to make modest increase in the TBI dose from 200 cGy to 300 cGy in an attempt to enhance the proportion of patients with complete chimerism without the need for ATG or donor lymphocyte infusions. While

engraftment will be slowest in recipients of UCB, the majority of patients will receive sibling or unrelated marrow in whom the rate of hematopoietic recovery will likely be faster.

3 STUDY DESIGN

This is an open label, single institution, phase II study.

The primary objective is to estimate the event-free survival rate by 1 year post-transplant with an event defined as death or failure to have a demonstrable increase in collagen, laminin, intergrin, keratin or plakin deposition or other biochemical, structural or physical measure of improvement.

4 ELIGIBILITY CRITERIA

4.1 Age and Disease Criteria

4.1.1 Patients aged 0-25 years

4.1.2 Diagnosis of severe form of EB characterized by collagen, laminin, integrin, keratin or plakin deficiency. Assessment criteria for severe EB:

a. Documented collagen, laminin, integrin, keratin or plakin deficiency (by immunofluorescence staining with protein specific antibodies or Western blotting and by mutation analysis)

4.2 Adequate Organ Function Criteria

4.2.1 Renal: glomerular filtration rate within normal range for age

4.2.2 Hepatic: bilirubin, AST/ALT, ALP < 5 x upper limit of normal

4.2.3 Pulmonary: adequate pulmonary function in the opinion of the enrolling investigator

4.2.4 Cardiac: left ventricular ejection fraction ≥ 45%, normal EKG or approved by Cardiology for transplant.

4.3 Available Healthy HSC Donor (order of preference)

4.3.1 Related Donor (marrow or UCB)

4.3.1.1 HLA-A, B, C, DRB1 genotypic identical (sibling) donor

4.3.1.2 HLA-A, B, C, DRB1 phenotypic identical donor

4.3.1.3 7/8 HLA matched donor at HLA-A, B, C, DRB1

4.3.2 Unrelated Donor

4.3.2.1 Marrow

4.3.2.1.1 HLA-A, B, C, DRB1 phenotypic identical donor

4.3.2.1.2 7/8 HLA matched donor at HLA-A, B, C, DRB1

4.3.2.2 UCB

4.3.2.2.1 HLA-A, B (antigen level) and DRB1 (allele level) matched donor

4.3.2.2.2 5/6 HLA matched donor at HLA-A, B, DRB1

4.3.2.2.3 4/6 HLA matched donor at HLA-A, B, DRB1

Considerations when using UCB as a cell source

The use of a FDA-licensed unit is preferred; however an unlicensed UCB unit that meets the required cell dose and HLA matching criteria (as defined in section 4.3) may be used if an 'equivalent', licensed UCB unit is not available. UCB units will be selected using the current University of Minnesota UCB Graft Selection Algorithm.

4.4 Available 'off-the-shelf' MSC

MSC product with a dose of 2 x 10^6/kg.

4.5 Absence of Exclusion Criteria

4.5.1 Active systemic infection at time of transplantation (including active infection with Aspergillus or other mold within 30 days).

4.5.2 History of HIV infection

4.5.3 Evidence of squamous cell carcinoma

4.5.4 Donor has EB

4.5.5 Pregnancy Females of child-bearing age must have a documented negative pregnancy test and agree to use contraception as a condition for enrollment.

4.6 Consent

Voluntary written consent

Refer to appendix 2 regarding review of patient disease status by members of an external advisory panel at the time of study work-up and appendix 3 for the work-up process flow.

5 SUBJECT REGISTRATION

5.1 Registration Procedure

Parental or subject informed consent must be signed prior to the performance of any study related procedures or assessments.

To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist found in ONCORE based on the eligibility assessment documented in the patient's medical record.

Upon completion of the screening evaluation, eligibility confirmation and obtaining informed consent, a designated study site staff person will enroll the patient into ONCORE and contact the BMT Biostatistical Support Group at the University of Minnesota by fax to register the subject.

5.2 Staggering of Enrollment

On February 8, 2012 permission was received from the FDA to remove the staggering of enrollment effective immediately. The 4 week waiting period between patients within each stratum is no longer required.

5.3 Updating ONCORE for Sibling Donor MSC's Infusions

ONCORE will be updated to reflect the 6 month post-transplant infusion of sibling donor MSCs to release the associated electronic case report forms.

5.4 Subject Withdrawal Prior to Treatment Start

If a patient is registered to the study, and is later found not able to begin the planned study treatment, the patient will be removed from study. The BMT Registrar will be notified of the patient's non-treatment status and ONCORE will be updated. Study data will be collected until the time the patient is off study. The reason for removal from study will be clearly indicated on the case report forms. The registration number cannot be reassigned.

6 TREATMENT PLAN

Day	Drug	Dose
-6	Cyclophosphamide Fludarabine	50mg/kg IV over 120 min 40mg/ m^2 IV over 60 min
-5	Fludarabine	40 mg/ m^2 IV over 60 min
-4	Fludarabine ATG	40 mg/ m^2 IV over 60 min 30 mg/kg IV over 6-8 hours
-3	Fludarabine ATG	40 mg/ m^2 IV over 60 min 30 mg/kg IV over 6-8 hours
-2	Fludarabine ATG	40 mg/ m^2 IV over 60 min 30 mg/kg IV over 6-8 hours
-1	TBI 300cGy	
0	HSC/MSC infusion	

6.1 Conditioning Regimen

Cyclophosphamide
CY 50 mg/kg/day IV over 2 hours x 1 day, total dose 50 mg/kg (days –6). CY will be administered with a high volume fluid flush starting >4 hours prior to CY and MESNA (MT[S]1996-02) on day -6 with CY according to institutional practices.

Fludarabine
Fludarabine 40 mg/m^2/day IV over 1 hour x 5 days, total dose 200 mg/m^2 (days –6 to –2). Flu will be administered IV over one hour at a constant rate. Flu dose will be modified based if there is renal dysfunction according to institutional practices.

Anti-thymocyte Globulin (ATG)
ATGAM 30 mg/kg IV over 6-8 hours x 3 days, total dose 90 mg/kg (days -4 to -2). ATGAM premedication will include diphenhydramine and methylprednisolone 1 mg/kg (or equivalent steroid) IV 30 minutes prior to the start of ATGAM and it may be repeated during the infusion for treatment of hives or pruritis.

Radiation Therapy (total body irradiation, (TBI))
The TBI dose will be 300 cGy administered in a single fraction at a dose rate of 10-19 cGy/minute prescribed to the midplane of the patient at the level of the umbilicus. The TBI will be delivered with right and left lateral

fields, with the patient supine on a specially designed couch. TBI will be delivered with a linear accelerator using 6, 18, or 24 MV X-rays. Based on measurements of transverse thicknesses, aluminum compensators may be used to ensure that the dose homogeneity across the field is within 10% of the prescribed dose. Usually head/neck, leg and lung compensators are used (although based on calculated midmediastinal doses, lung compensators are often not needed). A beam "spoiler" will be used to ensure a full skin dose. Half value layer lung and kidney blocks will not be utilized. Testicular boosts are not needed.

6.2 Immunosuppressive Therapies

Patients will not be eligible for any GVHD prophylaxis studies.
All patients will receive GVHD prophylaxis with 2 drugs as follows:

Cyclosporine (CSA*)*
Patients will receive cyclosporine (CSA) therapy beginning on day -3 maintaining a level of > 200 ng/mL. For children < 40 kg the initial dose will be 2.5 mg/kg IV over 2 hours every 8 hours. For children > 40 kg, the dosing is 2.5 mg/kg/dose IV q 12 hours.

Dose adjustments will be made on the basis of toxicity and CSA levels with a targeted trough level of 200-400 mg/L (Supportive Care, Appendix 5). Once the patient can tolerate oral medications, CSA will be converted to an oral form suggested at 5 mg/kg PO twice daily. CSA dosing will be monitored and altered as clinically appropriate by Pharm D or physician.

Patients with an HLA matched sibling donor will receive CSA until day +100. If no GVHD, the dose will be tapered 10% per week beginning on day 101, to discontinue 10 weeks later. Patients with an alternative (non HLA matched sibling) donor will receive CSA until day +180. If no GVHD, the dose will be tapered 10% per week beginning on day 181, to discontinue 10 weeks later.

Mycophenolate Mofetil (MMF)
All patients will begin mycophenolate mofetil (MMF) on day -3. Patients <45 kilograms will receive MMF at the dose of 15 mg/kg/dose every 8 hours (max dose 1 gm/dose) (PO or IV). IV route will be used between days –3 and +5, then, if tolerated, may change to PO between days +6 and +30. Patients >45 kg will receive MMF 1.5gm IV/po every 12 hours. Levels will be modified if clinically indicated.

Stop MMF at day +30 *or* 7 days after engraftment or 7 days after control of acute GVHD, whichever is later. (Definition of engraftment is 1st day of 3 consecutive days of absolute neutrophil count [ANC] $\geq$ 0.5 x 10^9 /L).

6.3 Processing and Infusion of HSC

6.3.1 HLA identical related donor BM

- No T cell depletion
- Infuse fresh, unirradiated with filtration of bone fragments
- May be supplemented with cryopreserved UCB if available as source of HSC to supplement marrow if available
- 30 mL of BM will be removed for potential future manufacture of MSC should residual focal areas of blistering be identified.

6.3.2 HLA 8/8 allele matched unrelated donor BM

- No T cell depletion
- Infuse fresh, unirradiated with filtration of bone fragments
- 30 mL removed for potential future manufacture of MSC

6.3.3 HLA matched or mismatched related or unrelated UCB

- Thaw, wash and infuse without further manipulation

6.3.4 HSC infusions (regardless of ABO match) will be infused with:

- Twice maintenance fluids initiated 12 hours prior to scheduled transplant and maintained for 24 hours.
- Premedication with acetaminophen, diphenhydramine, anti-emetic (if cryopreserved product is infused).
- The product is infused via IV drip directly into the central line without a needle or pump.
- UCB products will be infused as soon as the product arrives on the PCU and within 30 minutes.
- After the product bag empties (regardless of HSC source or prior processing), 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.

6.4 Processing and Infusion of MSC

6.4.1 MSC Donors

To be eligible as a MSC donor, the donor must have tested negative for HIV-I, HIV-II, HTLV-I/II, hepatitis B and C, CMV, syphilis, CJD and tuberculosis, and free of active infection at the time of BM harvest, in good health. For unmatched, 'off-the-shelf' MSC, 25-35 mL BM will be harvested from an unrelated donor using local anesthesia only.

Because of the potential future need for additional MSC therapy to focal areas of persistent disease after transplant, 25-35 mL BM will be obtained at the time of marrow harvest for HSC collection. BM from the HSC donor will be cryopreserved and stored until the time it is needed.

6.4.2 MSC Manufacture and Storage

BM MNC's are cultured under standardized conditions. After expansion culture, cells are removed for testing with the remainder cryopreserved in aliquots for future use. MSC's will be banked prior to use and will not be released to patient until 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 MSC lots meeting lot release will be available for clinical use.

All patients who are enrolled onto this trial are eligible to receive "off-the-shelf" MSC.

6.4.3 Administration of Unmatched "Off-the-Shelf" MSC (dose of 2 x 10^6/kg)

1. Infuse 4 hours after BM or UCB infusion.
2. Premedication with acetaminophen, diphenhydramine, anti-emetic; if infused as scheduled additional hydration is not required beyond that described for HSC infusion in section 6.4.1.
3. MSC product will be infused within 30 minutes of arrival on the PCU.
4. The product is infused via IV drip directly into the central line without a needle or pump at 4-6 mL/minute.
5. While adverse events are rare based on substantial past experiences with MSC, infusional toxicity will be evaluated by continuous monitoring of the subject's vital signs and O2 saturation by pulse oximetry during the MSC administration and for two hours afterward. Infusional toxicity will be based on a decrease in oxygen saturation during the infusion period. If there are signs of respiratory distress (oxygen saturation below 90% off oxygen or shortness of breath), the infusion will be discontinued. Such event will count toward the study stopping rules (refer to section 10.4.1).
6. After the product bag empties (regardless of HSC source or prior processing), 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.
7. Post infusion assessments will be done per section 8.2

6.5 Supportive Care

Supportive care is not specific to patients with EB but to transplant patients in general. For this study, patients will receive Neupogen (G-CSF) 5 mcg/kg/day IV based on the actual body weight IV beginning on day +1 after HSC infusion regardless of HSC source. Neupogen will be administered daily until the ANC exceeds 2.5 x 10^9/L for three consecutive days and then discontinued. If the

ANC decreases to <1.0 x 10^9/L, G-CSF will be reinstituted. Supportive care guidelines, including the management of graft failure and EBV reactivation with or without post-transplant lymphoproliferative disease (PTLD) are detailed in institutional SOPs and summarized in Appendix 5.

However, in the event that focal blistering persists, patients may be eligible to receive HSC donor derived MSC infusions in an attempt to reduce further blistering. Eligibility and treatment plan are shown below:

6.5.1 Eligibility for 'Directed-Donor' MSC Infusions

6.5.1.1 .Patient >6 months from HSC transplant with complete chimerism (i.e., ≥ 95% donor cells) in the blood and marrow

6.5.1.2 Persistent mucocutaneous blistering

6.5.1.3 Adequate organ function (as in 4.2)

6.5.1.4 Available donor specific MSC containing a dose of 2 x 10^6/kg

6.5.1.5 Absence of exclusion criteria (as in 4.5)

6.5.1.6 Voluntary written consent (separate consent)

6.5.2 Treatment Plan

Infusions will be given in the outpatient clinic.

6.5.2.1 Premedication with acetaminophen, diphenhydramine, anti-emetic; additional hydration is not required.

6.5.2.2 MSC product will be infused within 30 minutes of product arrival in clinic.

6.5.2.3 The product is infused via IV drip directly into the central line without a needle or pump at 4-6 mL/minute.

6.5.2.4 While adverse events are rare based on substantial past experiences with MSC, infusional toxicity will be evaluated by continuous monitoring of the subject's vital signs and O2 saturation by pulse oximetry during the MSC administration and for two hours afterward. Infusional toxicity will be based on a decrease in oxygen saturation during the infusion period. If there are signs of respiratory distress (oxygen saturation below 90% off oxygen or shortness of breath), the infusion will be discontinued. Such event will count toward the study stopping rules (refer to section 10.4.1).

6.5.2.5 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.

6.5.2.6 Post infusion assessments will be done per section 8.2

6.6 Treatment Related Toxicities

6.6.1 Potential Toxicities Associated With Preparative Therapies

Cyclophosphamide

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
hypotension nausea and vomiting diarrhea hemorrhagic cystitis leukopenia amenorrhea		syndrome of inappropriate ADH hemorrhagic myocardopathy Stevens-Johnson syndrome toxic epidermal necrolysis azoospermia oligozoospermia interstitial pneumonia

Fludarabine

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
severe suppression of blood counts diarrhea anorexia mucositis nausea/vomiting stomatitis osteoporosis dysuria	chills fever GI bleeding peripheral edema	neurotoxicity agitation and confusion blurred vision peripheral neuropathy hearing loss headache cerebellar syndrome blindness coma weakness depression insomnia hemorrhagic cystitis (except in FA) abnormal renal function test autoimmune hemolytic anemia deep venous thrombosis aneurysms pruritic skin rash abnormal liver function/liver failure constipation transient ischemic attack dysphagia myalgia arthralgia renal failure

ATGAM

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
malaise hives/rash fever/chills decreased white blood cells	joint aches and pains infection low blood pressure	seizure difficulty breathing

TBI 300 cGy

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
nausea decreased white blood cells mucositis	diarrhea vomiting	dysphagia parotiditis

Other effects associated with TBI but unknown at this low dose include cataracts, increased risk of secondary neoplasms, sterility/infertility, endocrinopathies, interstitial pneumonitis.

6.6.2 Potential Toxicities Associated With Immunosuppressive Therapies

Cyclosporine (CSA)	**Mycophenolate Mofetil (MMF)**
nephrotoxcity	pancytopenia
seizures	headache
hypertension	insomnia
hirsutism	electrolyte imbalances
increased risk of relapse	leg cramps/bone pain
thrombotic thrombocytopenic purpura	hypertension
electrolyte imbalances	dizziness
paresthesias/neuropathy	hyperglycemia
gingival hyperplasia	rash
increased risk of opportunistic infection	nausea/diarrhea

6.6.3 Potential Toxicities Associated With Neupogen (G-CSF)

bone pain	insomnia
headaches	dyspnea
body aches	rash
fatigue	edema
nausea/vomiting	

6.6.4 Potential Toxicities Associated With MSC Infusions

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

7 STUDY PARAMETERS

7.1 Assessments and Procedures

7.1.1 Patient Clinical Assessments and Procedures (Clinical Costs)

	Screen	Day 0 to 30			Day 31 to 100			Follow-up
		Daily	Weekly	Day +28 (+/- 3 days)	Weekly	Day +60 (+/- 7 days)	Day +100 (+/- 7 days)	6 months, 1 and 2 years (+/- 30 days)
Patient's General Assessments								
Informed consent	X							
Medical history	X	X			X			X
Physical exam	X	X			X			X
Quality of Life Questionnaire or iscorEB[3]	X						X	X
Performance status	X			X			X	X
Height/Weight	X						X	X
GVHD evaluation			X		X			X
Adverse event notation		X			X			X
Patient's Laboratory Assessments[2]								
CBC, diff	X	X			X			X
Platelet	X	X			X			X
PT/PTT	X						X	
Comprehensive Metabolic Panel	X		X		X			X
Basic Metabolic Panel		X[4]						
Iron studies	X						X	X
Zinc, Selenium, Vitamin D3	X							6 month, 1 year only
Viral serology	X							X
GFR	X							
Blood DNA to Molecular Diagnostics Lab for Chimerism Assessment	X			X		X	X	X
Skin DNA to Molecular Diagnostics Lab for Chimerism Assessment							X	X
Immune Panel and Ig levels	X			X		X	X	X
Patient's Procedures								
EKG	X							
ECHO	X							
Chest Xray or CT	X							
Oxygen saturation	X							
Renal Function	X							
Ophthalmology consult[1]							X	X
Skin biopsy and GIendoscopy and biopsy*	X						X	X

*Skin and gastrointestinal evaluations will be performed at scheduled intervals in order to document severity of disease pre-transplant and response to therapy after transplant. GI mucosal biopsy material will only be obtained if there is a need for clinical care. Additional skin biopsies maybe completed as clinically indicated. Standard procedures for these evaluations are briefly described below:

Skin Biopsy

- Alcohol swab is applied to the skin.
- Lidocaine with epinephrine [1-3 mL] is injected SQ for anesthesia.

- 3-4 mm punch biopsies are performed around skin erosions.
- Each of them is closed with a 4-0 Prolene suture; alternatively 5 mm x 5mm gelfoam "plugs" are used for hemostasis.
- Dressings are applied in a manner usual for each patient.

Gastrointestinal Endoscopy and Mucosal Biopsy

- Antibiotics are given before procedure.
- General anesthesia is introduced.
- Endoscopic evaluation of stomach and esophagus is performed up to the superior esophageal sphincter via a previously placed gastric tube; alternatively oral route may be used.
- 1-2 mm mucosal biopsies are collected from esophagus and stomach (if clinically required).
- Dilatation of esophageal strictures is done using a balloon technique (if indicated).

1 **If clinically indicated**

2 If lab draw outside of window due to blood volume limitations, this will not be considered a deviation from protocol

3 If patient began protocol prior to incorporation of iscorEB, quality of life survey will continue to be collected; if patient began protocol after iscorEB added to protocol, this will be collected in place of the quality of life survey (iscorEB is considered standard of care, quality of life questionnaire is research; iscorEB will be given to patient/parent any time a skin biopsy is done as standard of care as well)

4 If patient undergoes a comprehensive metabolic panel, do not also draw basic metabolic panel

7.1.2 Patient Research Assessments (Research Costs)

	Screen	Day +28 (+/- 3 days)	Day +60 (+/- 7 days)	Day +100 (+/- 7 days)	6 months, 1 and 2 years (+/- 30 days)
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
Skin biopsy specimens -protein expression -structural assessment -blister roofs	X			X	X
Skin fragility testing (Appendix 6)	X			X	X
Photographs of skin	X			X	X
Quality of Life Questionnaire (not required for children < 2 years) (Appendix 7, 11) or iscorEB[3]	X			X	X (1 and 2 years)

3 If patient began protocol prior to incorporation of iscorEB, quality of life survey will continue to be collected; if patient began protocol after iscorEB added to protocol, this will be collected in place of the quality of life survey (iscorEB is considered standard of care, quality of life questionnaire is research)

7.1.3 HSC Donor Clinical Assessments and Procedures (Clinical Costs)

	Screen	Day 0
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	
Serum chemistries	X	
Mutation analysis	X	
Viral serology	X	
Urinalysis	X	
Blood DNA to Molecular Diagnostics Lab for future chimerism Assessment	X	
Donor's Procedures		
BM Harvest		X
EKG	X	
Chest X-ray	X	
Skin biopsy		X

7.1.4 HSC Donor Research Assessments (Research Costs)

Donor's Procedures	
	after consent
Skin fragility test (including parents) (Appendix 6)	X
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
Skin biopsy specimens -protein expression -structural assessment -blister roofs	X

7.1.5 MSC Donor Clinical Assessments (Research Costs)

	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	
MSC DNA to Molecular Diagnostics Lab for future chimerism Assessment		X (after MSC manufacture)
Donor's Procedures		
BM Aspirate		X

7.2 Patient Pre-Study Screening Procedure

- Medical history of allergies, previous chemotherapy, prior radiotherapy and response to treatment
- Performance status (Appendix 1)
- Physical examination
- Complete blood count with leukocyte differential

- Comprehensive Metabolic Panel
- PTT, CH50, C3 and C4
- Ferritin, TIBC, serum iron zinc, selenium, vitamin D3
- Assessment of immune function (complete phenotype panel; immunoglobulin levels), ANA
- Viral tests (HSV, CMV, EBV, HIV, HBsAg, HBcAb, HC, HTLV1/2)
- Electrocardiogram and echocardiography with measurement of the left ventricular ejection fraction (LVEF)
- Oxygen saturation
- Chest CT without contrast to exclude occult infection prior to transplant for patients
- GFR
- Blood
    - DNA for chimerism assessments (Molecular Diagnostic Laboratory)
    - 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)
- Skin evaluations
    - DNA for chimerism assessment
    - Photography (skin)
    - IF or Western to assess protein expression (collagen, laminin, integrin, keratin or plakin)
    - EM to assess structural changes
    - Skin fragility testing (time to blister formation) to assess physical changes (Appendix 6)
- GI mucosa evaluations
    - Photography (oral mucosa)
    - Endoscopy via G tube
- Quality of Life questionnaire (Appendix 7- not required for children ≤ 2 years of age, Appendix 11) or iscorEB

7.3 Patient Evaluations: During Therapy to Day 28

- Medical history interval history daily (while hospitalized)
- Assessment of MSC infusional adverse events (during infusion and for 4 hours thereafter)
- Performance status at day 28
- Physical examination daily (while hospitalized)
- Complete blood count (with leukocyte differential when count is >500/uL) daily (while hospitalized)
- BMP daily, CMP weekly while hospitalized
- Blood submitted to Molecular Diagnostic Laboratory for chimerism studies on day 28 +/- 3 days

- Blood on day 28 +/- 3 days
  - DNA for chimerism assessments (Molecular Diagnostic Laboratory).
  - 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
- GI mucosa evaluations on day 28 +/- 3 days (if clinically indicated)
- Photography (oral mucosa) (if clinically indicated)
- Endoscopy via G tube (if clinically indicated)
- Assessment of acute GVHD weekly in hospital (Appendix 8)

7.4 Subject Evaluations: Day 31 to day 100

- Medical interval history weekly
- Performance status at day 100 +/- 7 days
- Physical examination weekly
- Complete blood count with leukocyte differential weekly
- CMP weekly
- PTT, CH50, C3 and C4day 100 +/- 7 days
- Ferritin, TIBC, and serum Fe day 100 +/- 7 days
- Assessment of immune function (complete phenotype panel; immunoglobulin levels) on day 60 +/- 3 days and day 100 +/- 7 days
- Blood on day 60 +/- 3 days and day 100 +/- 7 days
  - DNA for chimerism assessments (Molecular Diagnostic Laboratory)
  - 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
- Skin evaluations on day 100 +/- 7 days
  - DNA for chimerism assessment
  - Photography (skin)
  - IF or Western to assess protein expression (collagen, laminin, integrin, keratin or plakin)
  - EM to assess structural changes
  - Skin fragility testing (time to blister formation) to assess physical changes (Appendix 6)
- GI mucosa evaluations on day100 +/- 7 days (if clinically indicated)
  - Photography (oral mucosa)
  - Endoscopy via G tube
- Ophthalmology consult day 100 +/- 7 days (if clinically indicated)

- Quality of Life questionnaire or iscorEB on day 100 +/- 7 days (appendix 7- quality of life questionnaire not required for children ≤ 2 years of age, appendix 11)
- Assessment of acute GVHD on day 60 +/- 3 days and day 100 +/- 7 days

7.5 Subject Evaluations: 6 Months, 1 Year and 2 year

- Medical interval history
- Performance status
- Physical examination
- Complete blood count with leukocyte differential
- CMP
- Zinc, Selenium and Vitamin D3 on day +180 and 1 year
- Assessment of immune function (complete phenotype panel; immunoglobulin levels), ANA
- Ferritin, TIBC, serum iron
- Viral tests (CMV, EBV, HBsAg, HBcAb, HC)
- Blood
  - DNA for chimerism assessments (Molecular Diagnostic Laboratory)
  - 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)
- Skin evaluations
  - DNA for chimerism assessment
  - Photography (skin)
  - IF or Western to assess protein expression (collagen, laminin, integrin, keratin or plakin)
  - EM to assess structural changes
  - Skin fragility testing (time to blister formation) to assess physical changes (Appendix 6)
- GI mucosa evaluations (if clinically indicated)
  - Photography (oral mucosa)
  - Endoscopy via G tube
- Ophthalmology consult (if clinically indicated)
- Quality of Life questionnaire (appendix 7- not required for children ≤2 years of age, appendix 11) or iscorEB
- Assessment of acute GVHD and chronic GVHD (Appendix 8 and 9)

7.6 Skin Evaluations

Prior to transplant, on days 100, and at 6 months, 1 and 2 years after transplant, the following skin evaluations will be performed in an attempt to objectively demonstrate change in skin structure and function over time.

7.6.1 Assessment of Chimerism (also day +28 and +60)

Skin biopsies will be sent to the Molecular Diagnostics Laboratory and the proportion of cells derived from the HSC and MSC donors will be determined. Standard methods will be used to distinguish DNA polymorphisms unique to the two stem cell donors and host. In cases where there is a sex mismatched HSC or MSC donor, a histological section will be further evaluated for the presence of XX and XY by fluorescent in situ hybridization.

7.6.2 Biochemical Assessment

Protein expression (collagen, laminin, integrin, keratin or plakin) will be assessed using standard IF and Western blot assays on biopsy sections or cell preparations.

7.6.3 Structural Assessments

Skin architecture will be determined using standard transmission electron microscopy.

7.6.4 Physical Assessments

- Assessment of Skin Fragility. Using negative pressure, time to blister formation before and after transplant will be determined. As shown in Appendix 6, the device permits an objective assessment of blister formation resistance.
- Photographs. Total body photographs will be obtained to assess changes in degree of blistering and surface area involved. In addition photographs of mouth, stomach and esophagus will be obtained at the time of endoscopy via the G tube if possible.

7.7 Hematopoietic Engraftment Evaluation

Chimerism studies will be performed on the blood on days 28, 60, and 100, and at 6 months, 1 and 2 years. In cases of slow engraftment, a BM aspirate and biopsy will be performed at any time.

7.8 GVHD Evaluation

Patients will be staged weekly between days 0 and 100 after transplantation using standard criteria (acute GVHD, Appendix 8). Patients will be assigned an overall acute GVHD score based on extent of skin rash, volume of diarrhea and maximum bilirubin level. Patients will be rescored at 6 months, and at 1 and 2 years with additional scores as events occur once discharged to home. Chronic GVHD is graded using standard criteria (Appendix 9).

7.9 Sample Storage

Residual skin biopsy and blood specimens will be cryopreseved for future molecular analyses. Skin biopsy samples and blood samples will be stored at

the University of Minnesota (Laboratory of Jakub Tolar). Samples will be stored indefinitely. Samples will only be used for additional analyses to verify correction of the EB and engraftment of donor cells, presence of antibody to fetal calf serum, MSC HLA antigens, new protein (collagen, laminin, integrin, plakin), and further studies of skin architecture.

8 ADVERSE EVENT DOCUMENTATION AND REPORTING

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

Note: throughout this section the generic term “drug” refers to the study treatment.

8.1 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.

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.

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.

If either the IND sponsor or the investigator believes the event is life-threatening or serious, the event must be evaluated by the sponsor for expedited reporting (21CRF 312.32(a)).

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, adverse events that occur as part of the disease process or underlying medical conditions are considered *unexpected;* however, they will not be reportable per section 8.3.

Unanticipated (unexpected) problems/events as defined by the University Of Minnesota IRB are those that are *not* already described as potential risks in the consent form, *not* listed in the Investigator's Brochure or *not* part of an underlying disease.

Note: The major discord between the FDA and IRB definitions is whether or not the underlying disease is included when considering expectedness.

FOR UNLICENSED UCB UNITS ONLY: Selected expected adverse reactions determined to be caused by or probably caused by the UCB unit based on objective evidence will be reported in an expedited manner to the FDA under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD – sponsor/investigator). Included are the following:

- The unit is mislabeled or failure to pass local lot release
- Serious infusion reaction within first 24 hours after infusion
- Recipient bacteremia with clinical signs and symptoms related to a contaminated UCB within 24 hours after infusion

UPIRTSO: Federal regulations [45CFR46.103(b)(5) and 21CFR56.108(b)(1)] require the IRB to ensure that researchers promptly report "any unanticipated problems involving risk to subjects or others" (UPIRTSOs). The University of Minnesota IRB defines a UPIRTSO as <u>any problem or event which in the opinion of the local researcher was *unanticipated, reflects new or increased risk to the subjects and at least possibly related* to the research procedures</u>.

In addition, the IRB has defined the following problems/events as reportable within 5 working days using the UPRITSO form found on the IRB website:

- Any accidental or unintentional change to the IRB-approved protocol that increases risk or has the potential to recur
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject
- Any publication in the literature, safety monitoring report (including Data and Safety Monitoring Reports), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research
- Any breach in confidentiality that may involve risk to the subject or others
- Any complaint of a subject that cannot be resolved by the research staff
- Any other possibly related event which in the opinion of the investigator constitutes an unanticipated risk

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in section 8.3. For the IRB this is 5 working days. For studies under an IND, it is 7 or 15 calendar days.

8.2 Adverse Event Documentation

Due to the intentional clearing of the marrow with chemotherapy as preparation for the HSCT and MSC infusion, it is expected that all patients will experience severe depression of their blood counts and other related toxicities as detailed in section 6.6. Therefore, adverse event collection for the purposes of this study will focus on **targeted adverse events and unexpected adverse events at specific time points** in relation to the MSC infusion. A list of targeted adverse events is found in appendix 10.

The targeted toxicity form (appendix 10) will be completed at the following time points:

Day 0: pre HSCT
Day 0: pre MSC infusion
Day 0: 4 hours post MSC infusion

The pre-MSC infusion assessment will record the worse grade of the targeted toxicity observed in the previous 24 hours.

The 4 hour post infusion assessment will record the worse grade of any targeted toxicity since the MSC infusion, in addition to any unexpected toxicity observed. After 4 hours, targeted adverse event data collection will not be done however; standard of care post-transplant adverse event assessments will continue per 7.1.1. From these assessments through day 100, events deemed unexpected, regardless of grade, and/or meet the definition of serious will be recorded on the study's case report forms.

Patients also will be closely monitored for excessive toxicity as measured by inability to complete the MSC infusion, graft failure and treatment related mortality by day 100. While these events may not necessarily constitute an

adverse event; such events count toward early study stopping rules as detailed in section 10.4.

For patients receiving donor MSC infusions > 6 months from HSCT the targeted toxicity form will be completed at the following time points:

- pre MSC infusion
- 4 hours post MSC infusion

Since these patients will be more than 100 days from transplant, event documentation will end with the 4 hour post MSC infusion assessment, unless an event meets the definition of requiring expedited reporting per section 8.3. Transplant related outcomes will continue to be collected as routine by the BMT Database.

8.3 Required Reporting: IRB, FDA, and MCC SAE Coordinator

Monitoring and documenting of adverse events will end at day 100; however after this time point the investigator is obligated, upon knowledge of, to report any adverse event requiring expedited reporting as defined in section 8.1. An exception to this is the short time frame around the MSC infusion given more than 6 months after transplant.

<table>
<tr><th>Agency</th><th>Criteria for reporting</th><th>Timeframe</th><th>Form to Use</th><th>Submission address/ fax numbers</th><th>Copy AE to:</th></tr>
<tr><td rowspan="2">U of MN IRB</td><td><u>UPIRTSO</u>: any event which is unanticipated, involved new or increased risk to subjects, and was at least possibly related to study procedures</td><td rowspan="2">5 Working Days</td><td>UMCC SAE and IRB Report form</td><td rowspan="2">University Of Minnesota IRB
MMC 820</td><td rowspan="4">Masonic Cancer Center SAE Coordinator
mcc-saes@umn.edu</td></tr>
<tr><td><u>Other Problems or Events</u> – refer to the IRB website for complete details</td><td>IRB Report Form</td></tr>
<tr><td rowspan="3">FDA</td><td>Unexpected <u>and</u> fatal <u>or</u> life threatening suspected adverse reaction</td><td>7 Calendar-Day</td><td rowspan="2">FDA prefers MedWatch 3500a Form but alternative formats are acceptable (e.g. summary letter)</td><td rowspan="2">Submit to as an amendment to IND</td></tr>
<tr><td>1) Serious <u>and</u> unexpected suspected adverse reaction <u>or</u>
2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure <u>or</u>
3) findings from other sources (other studies, animal or in vitro testing)</td><td>15 Calendar-Day</td></tr>
<tr><td>All other events per CRF 312.33</td><td>At time of IND annual report</td><td>Summary format</td><td>Submit as part of the IND annual report</td><td>Not applicable</td></tr>
</table>

	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				
MCC SAE Coordinator	Early study stopping rule event	upon reporting	Early study stopping rule	SAE Coordinator mcc-saes@umn.edu	Not applicable

In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of the previous, similar reports or other relevant information.

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

9 DATA AND SAFETY MONITORING

9.1 External Data and Safety Monitoring Board

The DSMB is composed of the three External Advisory Panel members (appendix 2) who are considered experts in EB, in addition to an expert in pediatric blood and marrow transplantation. The Board members will review the clinical and histopathological data at the following specified time points:

- weeks after first 5 subjects have been treated
- weeks after first 5 subjects have been treated within a specific stratum (ie., sibling donor, unrelated marrow donor, unrelated UCB donor)
- Annually (all strata)

The Board will be asked to assess the safety and efficacy endpoints. The DSMB will make a recommendation at each time point regarding the appropriateness of study continuation and whether enrollment should be staggered as defined in section 5.2 for the first 5 patients in each stratum.

The DSMB reports will be submitted to the IRB and FDA along with the scheduled annual reports to the various University and external agencies.

9.2 Data and Safety Monitoring Plan

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://www.cancer.umn.edu/for-researchers/investigator-resources/cancer-protocol-review-commitee.

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

- The PI will complete and submit a quarterly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC) with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project 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 8.3 to the Masonic Cancer Center's SAE Coordinator and the University of Minnesota IRB.

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

9.3 IND Annual Reports

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

Additional reporting on unlicensed UCB units will be done under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD - sponsor/investigator).

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Endpoints

The underlying hypothesis is that the infusion of BM or UCB from a healthy unaffected donor will correct the underlying collagen, laminin, integrin, keratin or plakin deficiency and reduce the skin fragility characteristic of severe EB. In addition, it is hypothesized that MSCs from a healthy donor will enhance the safety and effectiveness of the transplant procedure as demonstrated by engraftment in the skin on day 28 after MSC administration.

10.1.1 Primary Endpoint

To estimate the event-free survival rate by 1 year post-transplant with an event defined as death or failure to have a demonstrable increase in collagen, laminin, intergrin, keratin or plakin deposition by 1 year post-transplant or other biochemical, structural or physical measure of improvement.

10.1.2 Secondary Endpoints

- Incidence of transplant-related mortality (TRM) at 180 days.
- Incidence of biochemical improvement as measured by increased collagen, laminin, intergrin, keratin or plakin deposition.

- Describe health quality of life at day 365 and 730 as compared to pretreatment results.
- Incidence of HSC and third party MSC donor engraftment in the skin at day 100
- Incidence of acute GVHD at 100 days
- Probability of survival at 1 year

10.2 Statistical Analysis

Kaplan-Meier curves will be used to estimate event-free survival and survival. Cumulative incidence will be used to estimate the probability of biochemical improvement, engraftment of HSC and third party MSC donor cells in the skin and acute GVHD, treating non-event deaths as a competing risk. Ninety-five percent confidence intervals will be calculated for all estimates. Descriptive statistics will be applied to the quality of life assessments to determine whether there has been any positive or negative change compared to the pretransplant assessment. Endpoints will be estimated separately for the three different donor types.

10.3 Sample-Size Justification

A minimum sample size of 25 patients per group (n=75 total) is sufficient to maintain an overall type I error of 5% while providing 80% statistical power. The sample size calculation is based on the Simon method using an optimum two-stage (44). The two-stage design uses a two-step enrollment procedure with early stopping for low observed success as defined by the primary endpoint. Using 50% event-free survival as unacceptably low and 75% as a rate worthy of further study, the first stage of enrollment will include 11 patients with an early stop if 6 or fewer patients fail to have event-free survival by 1 year. If 7 or more respond and are alive at 1 year after transplant, we would enroll an additional 16 patients. Significant clinical activity would require at least 17 patients with event-free survival by 1 year with an event defined as death or failure to have a demonstrable increase in collagen, laminin, intergrin, keratin or plakin deposition or other biochemical, structural or physical measure of improvement. Enrollment will continue during the potential 1 year waiting period after the 11^{th} patient has been enrolled if no stopping rules have been met.

Initially, we expect to enroll 5-15 patients per year but with current demand among these patients, we expect this rate to increase significantly.

10.4 Toxicity Monitoring and Stopping Rules

Monitoring guidelines will be set up for MSC infusional toxicity, graft failure by day 42 and TRM by day 100. Monitoring guidelines will be applied separately to each stratum (donor type).

10.4.1 MSC Infusional Toxicity and Late Effects

While adverse events are rare based on substantial past experiences with MSC, infusional toxicity will be evaluated by continuous monitoring of the subject's vital signs and O2 saturation by pulse oximetry during the MSC administration and for two hours afterward. Infusional toxicity will be based on a decrease in oxygen saturation during the infusion period. If there are signs of respiratory distress (oxygen saturation below 90% off oxygen or shortness of breath), the infusion will be discontinued. If 3 patients cannot obtain the complete MSC infusion, the study will be stopped.

10.4.2 Graft Failure

Given a hypothesized graft failure (defined as failing to achieve an ANC >500/uL of donor origin by day 42) rate of 10%, a maximum tolerated rate of 25% and a maximum sample size of 10 patients, the trial will be stopped if: 4/8, 5/14, 6/20 or 7 patients fail to engraft. This has a type I error rate of 5% and a power of 80%.

10.4.3 Treatment Related Morbidity by Day 100

Given a hypothesized treatment related mortality rate of 10%, a maximum tolerated rate of 25% and a sample size of 10 patients, the trial will be stopped if: 4/8, 5/14, 6/20 or 7 patients die by day 100 post-transplant. This has a type I error rate of 5% and a power of 80%.

10.5 Directed MSC Donor Subset Analysis

Response to directed MSCs will be descriptive. Toxicities will be assessed as with 'off the shelf' MSCs.

10.6 Gender and Ethnicities Statement

This study is open to both males and females and to all racial/ethnic groups. The patient enrollment pattern is expected to be similar to that of other hematological malignancy studies. It is not anticipated that the outcome will be affected by either race or gender. The study will not have separate accrual targets for different subgroups.

10.7 Data Collection

Routine peri- and post-transplant data will be collected by the principal investigator, biostatistics and clinical management team and stored in the Blood and Marrow Transplant database in Oracle 8i.

11 CONDUCT OF THE STUDY

11.1 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, informed 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.

11.2 Auditing and Monitoring

The investigator will permit study-related monitoring, audits, IRB, government regulatory bodies, and University of Minnesota compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities.

11.3 Record Retention

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. The investigator will retain study records, including source data, copies of case report form, and all study correspondence in a secured facility until at least 6 years after the IRB/IND study file closure. In addition, the Masonic Cancer Center Clinical Trials Office will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records.

Please contact the Masonic Cancer Center Clinical Trials Office prior to destroying of any study related records.

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Appendix 1 – Performance Status Scales

Lansky Play Scale

Percentage	
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 activities
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; 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	Unresponsive
0	Dead

Karnofsky Scale

Percentage	
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 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 necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

Appendix 2 – External Advisory Board (EAP)

The EAP is composed of three members who are considered expert in EB. The panel members will review the clinical and histopathological data for each prospective patient and assess the severity of the EB. Patients considered to have severe EB, based on biochemical and clinical data as well as self- or parental-assessment of quality of life, will be offered therapy.

In addition, panel members will review the clinical and histopathological data for all patients annually at the time of the annual IRB review. Patient demographics and identifiers will be removed.

Panel Members:

John A. McGrath (john.mcgrath@kcl.ac.uk) advisory panel chair
Alain Hovnanian (alain.hovnanian@inserm.fr)
Katsuto Tamai (tamai@gts.med.osaka-u.ac.jp)

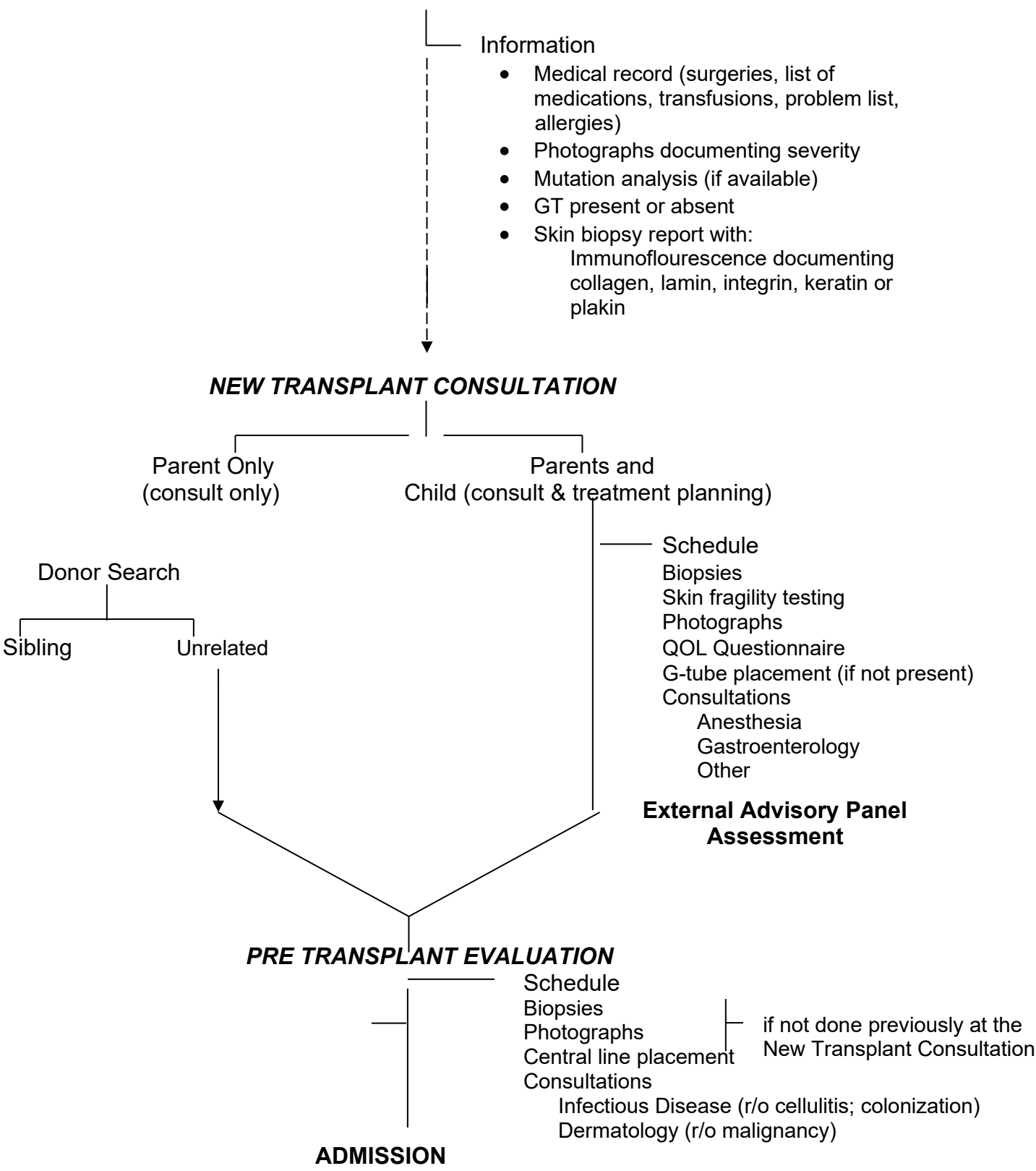
Severity Assessment

EAP members will assess the severity of the patient's disease on the basis of

- Initial history and physical at the transplant center
- Photographs of the affected skin and mucosa
- Examination of the biochemical and structural assessments and/or mutation analysis
- Quality of life survey

In order to be eligible for the proposed treatment, the patient must be considered to have a severe form of EB. The EAP will receive patient data without identifiers via a password protected secure website.

Appendix 3 – Process Flow



Appendix 4 - Data and Safety Monitoring Board

The DSMB is composed of the three EAP members who are considered expert in EB in addition to an expert in pediatric blood and marrow transplantation. The Board members will review the clinical and histopathological data at specified time points defined in the protocol. The Board will be asked to assess the safety and efficacy endpoints. The DSMB will make a recommendation at each time point regarding the appropriateness of study continuation and whether enrollment should be staggered as defined in the protocol for the first 5 patients in each stratum.

Board Members:

- Colleen Delaney, M.D., Blood and Marrow Transplant Program, Fred Hutchinson Cancer Research Center, Seattle, Washington—DSMB Chair
- John A. McGrath (john.mcgrath@kcl.ac.uk)
- Alain Hovnanian (alain.hovnanian@inserm.fr)
- Katsuto Tamai (tamai@gts.med.osaka-u.ac.jp)

Assessments:

Safety Assessments
DSMB members will assess
- MSC infusional toxicities
- All SAEs and unexpected AEs

Efficacy Assessments
DSMB members will assess
- Hematopoietic engraftment
- Evidence of biochemical, structural and clinical correction
- Chimerism in the skin (from the marrow and MSC donors)

The DSMB will receive patient data via a password protected secure website.

DSMB Reporting Requirements:
- 4 weeks after first 5 subjects have been treated
- 4 weeks after first 5 subjects have been treated within a specific stratum (ie., sibling donor, unrelated marrow donor, unrelated UCB donor)
- Annually (all strata)

Appendix 5 – Supportive Care Guidelines

1. Anemia

Transfusions of packed red blood cells are indicated for symptomatic management of anemia. An attempt will be made to maintain the hematocrit >24% and hemoglobin >8 g/dl. Irradiated (1500-3000 cCy) blood products will be used. If CMV negative patient, CMV negative blood products or leukocyte poor blood products will be administered.

2. Thrombocytopenia

Prophylactic platelet transfusions should be given to maintain the platelet count >10,000 ml or above the level at which signs of bleeding are known to occur, whichever is greater. When available, autologous stored platelets may be used. All aspirin-containing drugs are to be avoided. The patients should receive no IM nor SQ injections. Irradiated (1500-3000 cCy) blood products will be used. If CMV negative patient, CMV negative blood products or leukocyte poor blood products will be administered.

3. Fever

Aggressive diagnostic and therapeutic management of fever in the neutropenic patient is mandatory. At the onset of fever, the patient will be thoroughly examined and cultured, after which empiric broad-spectrum antibiotics will be initiated. Anti-mold antibiotics should be considered for patients failing to respond to antibiotics. Granulocyte transfusions may be given at the investigator's discretion. Irradiation (1500-3000 cCy) of blood products is required.

4. Nutrition

All patients will be candidates for total parenteral nutrition

5. CSA dose modifications

To minimize the risk of CSA toxicity, the serum creatinine/BUN will be determined daily for the first 30 days after transplantation. Subsequently, the frequency will be determined by the patient's clinical status. Trough CSA levels will be determined twice weekly for all inpatients with levels obtained on the basis of the patients clinical status thereafter. CSA dose adjustment on the basis of toxicity or low trough level. The goal is to achieve a CSA trough level of at least 200-400 mg/L without significant renal toxicity.

Adjustment of CSA should be based on CSA levels in conjunction with clinical observations of the biological effects of the drug, i.e., renal and neurologic toxicity, and the physician's assessment of the patient's need for immunosuppression. As a guideline, if the serum creatinine exceeds 2x baseline or is >2.0 mg/dL, then the CSA dose should be decreased by 50%; if the serum creatinine exceeds 3x baseline or the patient requires hemodialysis, then the CSA dose should be held. As a guideline, if the patient becomes disoriented or develops visual disturbance, paresis, aphasia or seizure, the CSA dose should be held during the evaluation of the neurological disturbance. CSA should be reinstituted as soon as possible.

6. G-CSF

All patients will receive G-CSF 5 ug/kg/day IV based on the actual body weight IV beginning on day +1 after HSC infusion. G-CSF will be administered daily until the ANC exceeds 2.5 x 10^9/L for three consecutive days and then discontinued. If the ANC decreases to <1.0 x 10^9/L, G-CSF will be reinstituted.

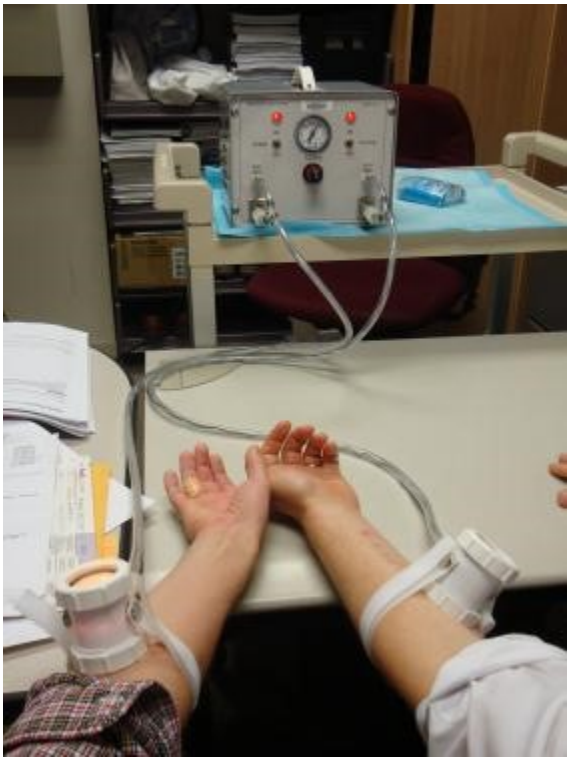
7. Monitoring and Treatment of EBV-Reactivation and PTLD

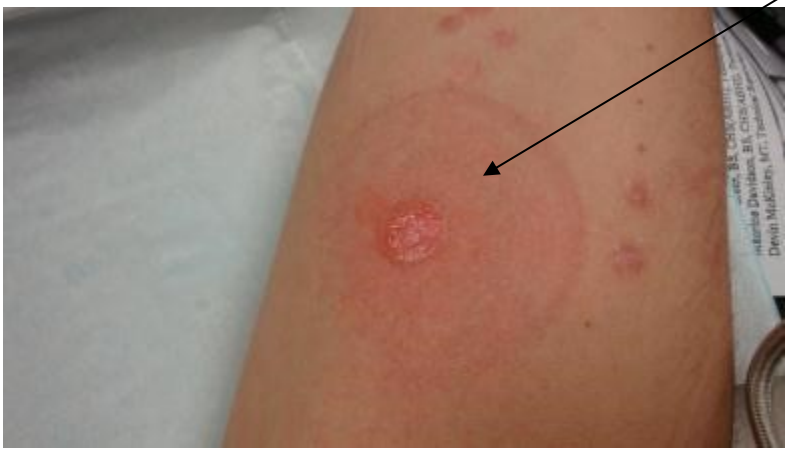
EBV-reactivation and PTLD are relatively rare complications after allogeneic HSCT, unless the graft is T cell depleted. However, we have previously observed an increased incidence specifically in adult recipients of unrelated UCB who receive ATGAM in combination with CY50/FLU200/TBI200[48]. In response to this observation, we monitor patients for EBV reactivation with planned early intervention using Rituximab, anti-CD20 antibody, to eradicate the infected B cell population. While the incidence of this complication has been lower than in this initial report[48], Rituximab is a generally accepted treatment strategy when rising titers of EBV are detected or PTLD has been diagnosed.

Monitoring. EBV PCR is assessed every 2 weeks between days 30 and 180 after transplant. If PCR is positive (> 1000 copies of EBV DNA per milliliter of whole blood) the patient will be evaluated for adenopathy by physicial examination and CT scans of the chest, abdomen and pelvis.

Treatment. If the diagnosis is EBV reactivation only with PTLD, the patient will receive Rituximab with frequent monitoring thereafter according to institutional practice. If there is a adenopathy, the patient will receive Rituximab with or without chemotherapy depending upon the extent of disease, results of the lymph node biopsy, and other active problems related to the transplant.

Appendix 6 – Measurement of Skin Fragility





To assess skin fragility after transplant, gentle negative pressure is applied to the skin. In normal individuals, blister formation takes up to 1 hour. Notably, individuals with one mutation in Col7A1 will form a blister after approximately 30 minutes in comparison to 1-3 minutes in patients with RDEB who have mutations in both alleles. It is hypothesized that biochemical correction of severe EB will result in increasing resistance to the negative pressure and a prolongation in time before blister formation occurs.

Appendix 7 – Quality of Life Questionnaire

Please answer these questions about how EB affects your life. Please choose an option from the right hand column that most closely matches your situation. Completion not required for children ≤ 2 years of age

1) Does your EB affect your ability to move around at home?	• Not at all • A little • A lot • Severely
2) Does your EB affect your ability to bath or shower?	• No, No impact • Yes, I sometimes need assistance • Yes, need assistance most of the time • Yes, I need assistance every time I bath/shower
3) Does your EB cause you physical pain?	• No pain • Occasional pain • Frequent pain • Constant pain
4) How does your EB affect your ability to write?	• It does not interfere with writing • I find it difficult to grip the pen • I find it easier to type than write • I cannot write due to my EB
5) Does your EB affect your ability to eat?	• No, I eat normally • A little • A lot • I rely on my gastrostomy tube for nutrition
6) Does your EB affect your ability to go shopping?	• No, not at all • A little • A lot • I need assistance all the time.
7) How does EB affect your involvement in sports?	• No impact. • I need to be cautious in sports • I need to avoid some sports • I need to avoid all sports
8) How frustrated do you feel about your EB?	• No frustration • A little • A lot • So frustrated that I am angry most of the time

9) Does your EB affect your ability to move around outside of your home?	• Not at all • A little • A lot • Severely
10) How does your EB affect your relationships with family members?	• No impact at all • A small impact • A large impact • A very large impact
11) How embarrassed do people make you feel about your EB?	• No embarrassment • A little • A lot • Extremely
12) Have you needed to, or do you need to modify your home (installing ramps etc) due to your EB?	• No, not at all • A few • A lot • Extensive
13) Does your EB affect your relationships with friends?	• No, Not at all • A little • A lot • It severely restricts my social interaction
14) How worried or anxious do you feel because of your EB?	• Not anxious at all • A little • A lot • Extremely
15) How are you or your family affected financially by your EB?	• No financial impact • Slightly Affected • Greatly Affected • Severely Affected
16) How depressed do you feel because of your EB?	• Not depressed at all • A little • A lot • Constantly very depressed
17) How uncomfortable are you made to feel by others (eg- teasing or staring) because of your EB?	• Not at all • A little • A lot • So much that I don ‘t go out socially

How long did it take you to complete this questionnaire? …….. Minutes

Appendix 8 – Diagnosis and Treatment of Acute GVHD

Patients will be considered evaluable acute GVHD if they demonstrate donor cell engraftment and survive to day 28. 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.

Patients progressing during initial therapy or not improving sufficiently after 2 courses of therapy are to be treated as severe GVHD.

Acute GVHD treatment may be by institutional guidelines. The recommended treatment for patients demonstrating moderate or severe GVHD (Grade II-IV) is methylprednisolone at 48 mg/m2/day or prednisone 60 mg/m2/day followed by a taper which starts a day 8 of acute GVHD therapy. If there is no response in 7 days or there is progression of the disease, ATG 15 mg/kg every 12 hours for 5 days will be added.

Appendix 9 – Classification of Chronic GVHD

Limited CGVHD
Localized skin involvement (<50% body surface area) and/or
Limited hepatic involvement (abnormal LFTS; bilirubin < 3 mg/dl)

Extensive CGVHD
The presence of one or more of the following criteria may be used for the diagnosis of extensive CGVHD:

- Generalized skin involvement (≥ 50% body surface area)
- Liver histology consistent with involvement by CGVHD with bilirubin ≥ 3 mg/dl
- Positive Schirmer's test (< 5 mm wetting)
- Histologically-proven involvement by CGVHD of oral mucosa or salivary glands
- Lung dysfunction with bronchiolitis obliterans with no evidence of viral causation on histology.
- Gastrointestinal involvement: malabsorption and/or weight loss due to anorexia without explanation other than CGVHD

Appendix 10 – Targeted Toxicity Form

Refer to next page

MT2009-09 - Biochemical Correction of Severe Epidermolysis Bullosa by Allogeneic Stem Cell Transplantation

Targeted Toxicity Form

Patient Initials: ☐☐☐☐ **Study Day** ☐☐

Date of assessment: ☐☐-☐☐-☐☐☐☐
Month Day Year

☐ **baseline assessment** ☐ **4 hours post MSC infusion**

Toxicity	Grade 0	1	2	3	4
Allergy reaction / hypersensitivity (including drug fever)	0 ☐ None	1 ☐ Transient rash, drug fever <38° C (<100.4° F)	2 ☐ Urticaria, drug fever 38° C (100.4° F) and/or asymptomatic bronchospasm	3 ☐ Systematic bronchospasm requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	4 ☐ Anaphylaxis
Sinus bradycardia	0 ☐ None	1 ☐ Asymptomatic , not requiring treatment	2 ☐ Symptomatic, but not requiring treatment	3 ☐ Symptomatic and requiring treatment	4 ☐ Life-threatening (e.g.) arrhythmia associated with CHF,hypotension, syncope, shock)
Bleeding- Hemorrhage	0 ☐ None	1 ☐ Mild without a transfusion	2 ☐	3 ☐ Requiring transfusion	4 ☐ Catastrophic bleeding requiring major non- elective intervention
Sinus tachycardia	0 ☐ None	1 ☐ Asymptomatic, not requiring treatment	2 ☐ Symptomatic, but not requiring treatment	3 ☐ Symptomatic and requiring treatment of underlying cause	4 ☐
Hypertension	0 ☐ None	1 ☐ asymptomatic, transcient increase by >20 mmHg (diastolic) or to >150/100* if previously WNL; not requiring treatment	2 ☐ Recurrent or persistent symptomatic increase by >20 mmHg (diastolic) or to >150/100* if previously WNL; not requiring treatment	3 ☐ Requiring therapy or more intensive therapy than previously	4 ☐ Hypertensive crisis
Hypotension	0 ☐ None	1 ☐ Changes, but not requiring therapy (including transient orthostatic hypotension)	2 ☐ Requiring brief fluid replacement or other therapy; no physiologic consequences	3 ☐ Requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	4 ☐ Shock (associated with academia and impairing vital organ function due to tissue hypoperfusion)
Fever	0 ☐ None	1 ☐ 38.0 – 39.0 °C (100.4 – 102.2 °F)	2 ☐ 39.1 – 40.0 °C (102.3 – 104.0 °F)	3 ☐ >40.0 °C (>104.0 °F) for <24 hrs	4 ☐ >40.0 °C (>104.0 °F) for >24 hrs
			Note: The temperature measurements listed above are oral or tympanic.		
Rigors, chills	0 ☐ None	1 ☐ Mild, requiring symptomatic treatment (e.g., blanket) or non-narcotic medication	2 ☐ Severe and/or prolonged, requiring narcotic medication	3 ☐ Not responsive to narcotic medication	
Rash	0 ☐ None	1 ☐ Erythema <25% of surface	2 ☐ Erythema 25% to 50% of skin surface	3 ☐ Erythema >50% of surface requires intervention	4 ☐ Blistering skin

Patient Initials: ☐☐☐☐ **Study Day** ☐☐

Date of assessment: ☐☐-☐☐-☐☐☐☐
Month Day Year

☐ **baseline assessment** ☐ **4 hours post MSC infusion**

page 2

Toxicity	Grade 0	1	2	3	4
Dyspnea (shortness of breath)	0 ☐ None	n/a	2 ☐ Dyspnea on exertion	3 ☐ Dyspnea at normal level or activity	4 ☐ Dyspnea at rest
Hypoxia	0 ☐ Normal	n/a	2 ☐ Decreased oxygen saturation with exercise	3 ☐ Decreased oxygen saturation at rest, requiring supplemental oxygen	4 ☐ Decreased oxygen saturation, requiring pressure support (CPAP) or assisted ventilation
Neurologic: **Specify:_____**	0 ☐ Norne	1 ☐ Mild	2 ☐ Moderate	3 ☐ Severe	4 ☐ Life-threatening; disabling
Other: **Specify:_____** **Expected or unexpected**	0 ☐ Norne	1 ☐ Mild	2 ☐ Moderate	3 ☐ Severe	4 ☐ Life-threatening; disabling

Comments:

All are treatment related except the following:

☐☐-☐☐-☐☐☐☐
Month Day Year

Completed by: _____

Physician signature: _____

Appendix 11 - iscorEB (Instrument for Scoring Clinical Outcomes for Research of Epidermolysis Bullosa) – insert iscorEB survey of 2015-20

An app is now available free of charge from the iTunes Store.

iscorEB: CLINICIAN SUBSCORE (max 114)

ID:	Age (years):	Male Female	Date (mm/dd/yy):

A. SKIN INVOLVEMENT

Rating: 0=absent, 1=present, unless otherwise specified

Skin characteristics	Head and neck	Upper extremities	Trunk	Lower extremities
☐ **Intact Blisters =1)**				
☐ **Erosions (denuded skin) =1)**				
☐ **Crusting/scabbing=1**				
☐ **Chronic wounds (> 6 weeks)=4**				
☐ **Infection=3** (at least one wound with ≥3 of expanding /raised borders, local pain, increased local temperature, purulent exudate, foul odour)				
A Score				
weighing factor (wf)	*0.1*	*0*	*0.3*	*0.4*
B score (A x wf)				
C Score % of regional surface area affected: 0=none; 1=1-9%; 2=10-29%; 3=30-49%; 4=50-69%; 5=70-89%; 6=>90%				
REGIONAL SKIN SCORE (BXC)				

A: TOTAL SKIN SCORE added for each body part max 60	A=

B. MUCOSAL INVOLVEMENT

(present at exam and/or in the last 4 weeks)

Rating: 0=absent, 1=present, unless otherwise specified

B1.Mouth	☐ **Erosions**	☐**Mouth Opening** (distance between the upper and lower incisors at maximal mouth opening)		Mouth Score
	Absent=0 1-2 days/month=1 1-2 days/week=2 ≥3 days/week=3	More than 5 cm=0 Between 3.1-4.9cm=1 Less ≤3cm=2		
Subscore				B1=
B2. Airway	☐ **Stridor**	☐ **Hoarseness**	☐ **EB-related use of steroid nebulizers**	Airway Score
	Absent=0 1-2/month=1 1-2 days/week=2 ≥3 days/week=3	Absent=0 1-2/month=1 1-2 days/week=2 ≥3 days/week=3	None=0 1-2/month=1 1-2 days/week=2 ≥3 days/week=3	
Subscore				B2=
B3.Eye	☐ **Eye redness**	☐ **Palpebral closure** (patient supine with eyes		Eye Score
	Absent=0 1-2days/month=1 1-2 days/week=2 ≥3 days/week=3	Full closure=0 White to inferior conjunctiva=1 White to cornea=2 White to pupil=3		
Subscore				B3=

B. TOTAL MUCOSAL SCORE (mouth+ airway + eye score) max 20	B=

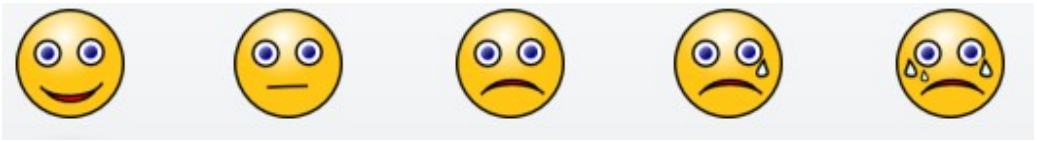
iscorEB PATIENT SUBSCORE (max 120)

The following questions attempt to assess how much of your (your child's) life is affected by having epidermolysis bullosa (EB). All responses range from 0 to 8; where 0 means no impact (for example if the question is about pain, 0 =no pain) to a maximum of 8 (for example if the question is about pain, 8 = the worst possible pain).
There are 15 questions grouped in 7 separate categories (domains). Please provide an answer to each question by circling the number that corresponds to the best descriptor of your (your child's) status in the **past 4 weeks**.

ID:	Age (years):	Male Female	Date (mm/dd/yy): / /

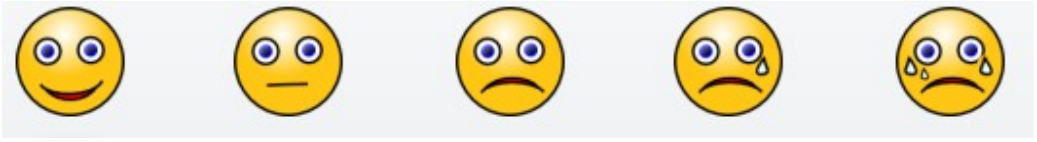
F. PAIN DOMAIN

F1. Please rate how much **OVERALL PAIN** you (your child) typically had ***in the last 4 weeks*** by circling one of the options below.



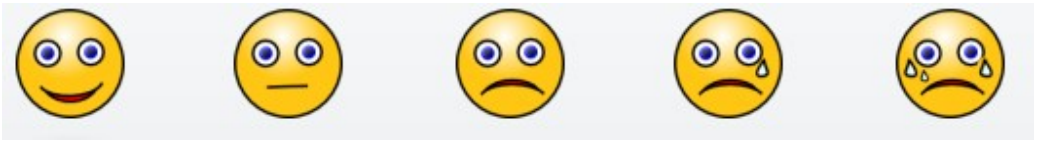
SCORES					
	None	*Mild*	*Moderate*	*Severe*	*Worst possible*
F1=	0	2	4	6	8

F2. Please rate how much **SKIN PAIN** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.



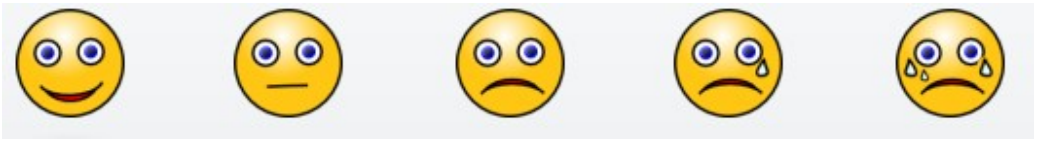
	None	*Mild*	*Moderate*	*Severe*	*Worst possible*
F2=	0	2	4	6	8

F3. Please rate how much **MOUTH PAIN** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.



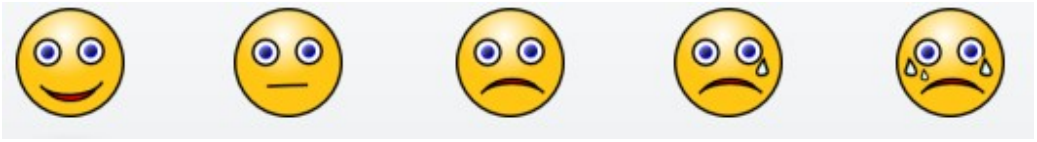
	None	*Mild*	*Moderate*	*Severe*	*Worst possible*
F3=	0	2	4	6	8

F4. Please rate how much **EYE PAIN** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.



None	*Mild*	*Moderate*	*Severe*	*Worst possible*	F4=
0	2	4	6	8	

F5. Please rate how much **BONE/JOINT PAIN** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.



None	*Mild*	*Moderate*	*Severe*	*Worst possible*	F5=
0	2	4	6	8	

G. ITCHING DOMAIN

G. Please rate how much **ITCH** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.

None	*Mild*	*Moderate*	*Severe*	*Worst possible*	G=
0	2	4	6	8	

H. ESSENTIAL FUNCTIONS DOMAIN

H1. Please rate how much **DIFFICULTY WITH EATING/DRINKING** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.

None	*Mild*	*Moderate*	*Severe*	*Unable to drink*	H1=
0	2	4	6	8	

H2. Please rate how much **DIFFICULTY HAVING A REGULAR BOWEL MOVEMENT (BM)** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.

None	*Mild*	*Moderate*	*Severe*	*Unable to have a BM*	H2=
0	2	4	6	8	

H3. Please rate how much **URINATING/VOIDING** you (your child) **typically** experienced in ***the last 4 weeks*** by circling one of the options below.

None	*Mild*	*Moderate*	*Severe*	*Unable to urinate*	H3=
0	2	4	6	8	

I. SLEEPING DOMAIN					
I. Please rate how much **SLEEP DISTURBANCE (difficulty falling or staying asleep)** you (your child) **typically** had in ***the last 4 weeks*** by circling one of the options below.					
None	*Mild*	*Moderate*	*Severe*	*Unable to sleep*	
0	2	4	6	8	I=
J. DAILY ACTIVITIES DOMAIN					
J1. Please rate how much **DIFFICULTY MOVING AROUND** you (your child) **typically** experienced in ***the last 4 weeks*** by circling one of the options below.					
None	*Mild*	*Moderate*	*Severe*	*Unable to move*	
0	2	4	6	8	J1=
J2. Please rate how much **DIFFICULTY USING HANDS** you (your child) **typically** experienced in ***the last 4 weeks*** by circling one of the options below.					
None	*Mild*	*Moderate*	*Severe*	*Unable to use hands*	
0	2	4	6	8	J2=
K. MOOD DOMAIN					
K. Please rate how you/your child **typically FELT** in ***the last 4 weeks*** by circling one of the options below.					
Happy	*Mostly happy*	*Somewhat unhappy*	*Unhappy*	*Very Unhappy*	
0	2	4	6	8	K=
L. IMPACT DOMAIN					
L1. Please rate how much **IMPACT ON LEISURELY ACTIVITIES** (play, relaxation, etc) your (your child's) disease **typically** had on your activities in ***the last 4 weeks*** by circling one of the options below.					
None	*Mild*	*Moderate*	*Severe*	*Unable to do anything*	
0	2	4	6	8	L1=
L2. Please rate how much **IMPACT ON WORK/SCHOOL/LEARNING** your (your child's) disease **typically** had on your activities in ***the last 4 weeks*** by circling one of the options below.					
None	*Mild*	*Moderate*	*Severe*	*Unable to go to work/school/learn*	
0	2	4	6	8	L2=
PATIENT SUBSCORE (sum of all domains)				**max 120**	

☐ Filled by patient ☐ Parent/Caregiver

Clinician Subtotal Score	
Patient Subtotal Score	
TOTAL iscorEB	