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Authors and affiliations:

C L Ebens[1], J A McGrath[2], K Tamai[3], A Hovnanian[4], J E Wagner[1], M J Riddle[1], D R Keene[5], T E DeFor[6], R Tryon[7], M Chen[8], D T Woodley[8], K Hook[9], and J Tolar[1]

[1]Division of Blood and Marrow Transplantation, Department of Pediatrics, Medical School, University of Minnesota, Minneapolis, MN, USA

[2]St. John's Institute of Dermatology, King's College London, London, England

[3]Department of Stem Cell Therapy Science, Graduate School of Medicine, University of Osaka, Osaka, Japan

[4]INSERM UMR1163, Imagine Institute, Department of Genetics, University Paris Descartes, Necker Hospital, Paris, France

[5]Microimaging Center, Shriners Hospital for Children, Portland, OR, USA

[6]Biostatistic Core, Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA

[7]Genetics Division, Department of Medicine, Medical School, University of Minnesota, Minneapolis, MN, USA

[8]Department of Dermatology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

[9]Department of Dermatology, Medical School, University of Minnesota, Minneapolis, MN, USA

Corresponding author:

Jakub Tolar, MD, PhD

University of Minnesota

420 Delaware St SE, MMC 293 Mayo

Minneapolis, MN 55455

USA

Phone: 011-612-626-4949

Email tolar003@umn.edu

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What's already known about this topic?

- Severe, generalized recessive dystrophic epidermolysis bullosa (RDEB) is marked by great morbidity and early death.
- Currently, no cure exists for RDEB.
- Bone marrow transplant (BMT) is the only described systemic therapy for RDEB.

What does this study add?

- First description of post-transplant cyclophosphamide (PTCy) BMT for RDEB.
- The PTCy BMT platform permits identification of a suitable related donor for most patients and for subsequent adoptive transfer of donor non-hematopoietic cells following establishment of immunologic tolerance.

Summary

Background Recessive dystrophic epidermolysis bullosa (RDEB) is a severe systemic genodermatosis lacking therapies beyond supportive care for its extensive, life-limiting manifestations.

Objectives We report the safety and preliminary responses of 10 RDEB patients to bone marrow transplant with post-transplant cyclophosphamide (PTCy BMT) following reduced-intensity conditioning with subsequent infusions of immunomodulatory donor-derived mesenchymal stromal cells (median follow-up of 16 months).

Methods BMT toxicities, donor blood and skin engraftment, skin biopsies, medical photography, and dynamic assessments of RDEB disease activity by providers and parents were obtained at intervals from pre- to 1 year post-BMT.

Results Related donors varied from haploidentical (n=6) to HLA-matched (n=3) with one HLA-matched unrelated donor. Transplant complications included graft failure in 3 patients (2 elected to pursue 2nd PTCy BMT), veno-occlusive disease in 2, posterior reversible encephalopathy in 1, and chronic graft-versus-host disease in 1, the latter deceased. In the 9 ultimately engrafted patients, median donor chimerism at 180 days following transplant was 100% in peripheral blood, 27% in skin. Skin biopsies show stable (7 of 9) to improved (2 of 9) type VII collagen protein expression by immunofluorescence and gain of anchoring fibril components (3 of 9) by transmission electron microscopy. Early signs of clinical response include trends toward reduced body surface area of blisters/erosions from a median of 49.5% to 27.5% at 100 days following BMT (p=0.05), with parental measures indicating stable quality of life.

Conclusions PTCy BMT in RDEB provides a means of attaining immunotolerance for future donor-derived cellular grafts (trial registered as NCT02582775).

Recessive dystrophic epidermolysis bullosa (RDEB) is a clinically heterogeneous genodermatosis characterized by *COL7A1* mutation(s) yielding inadequate type VII collagen (C7) to maintain the integrity of the cutaneous basement and mucosal membranes[1-5]. The impact on an affected individual is skin fragility with cycles of blistering and scarring, acute and chronic pain and pruritus, and progressive functional limitations from pseudosyndactyly and joint contractions. Systemic manifestations often include gastrointestinal strictures, micronutrient deficiencies, anemia of chronic disease, systemic inflammation, and high metabolic demand[6, 7]. Serious infections and development of squamous cell carcinoma (SCC) in adolescence or young adulthood limit life expectancy[8]. The mainstay therapy for RDEB has been supportive care attempting to prevent or respond to disease progression[9, 10].

The pluripotency of bone marrow cells, including the ability to differentiate along the epithelial lineage[11-13] and engraft the skin[14], prompted investigation of bone marrow transplant (BMT) as a cellular source of C7. Following supportive evidence for BMT as a systemic therapy for RDEB in a pre-clinical murine model[15], our group introduced clinical trials of allogeneic BMT for children afflicted with this disease. Initial efforts utilizing fully immunomyeloablative chemotherapy demonstrated increased C7 and donor cells in recipient skin following the procedure as well as decreased blister formation, but enthusiasm was dampened by regimen-related toxicity[16]. Subsequent trials suggested the ability to maintain systemic improvements with less toxic, reduced-intensity conditioning regimens[17], bolstered by 2 cases of reduced-intensity conditioning BMT for RDEB reported by others[18].

The critical C7-producing component of the transplant inoculum remains a topic of active investigation. Non-hematopoietic mesenchymal stromal cells (MSCs) may play a role as they are known to home to sites of tissue damage and inflammation[19, 20], contribute to healing[21-24], and support production of C7[25, 26]. As well as the MSCs infused at the time of BMT, we have incorporated additional MSC infusions into the transplant regimen to capitalize on these intrinsic qualities. Advances in BMT methodology, including the use of post-transplant cyclophosphamide (PTCy), over the past decade now allow for safe use of HLA-haploidentical donors (less-than-perfectly HLA-matched siblings or half-matched parents)[27-30]. While previously limited to short-lived third-party, immunogenic MSCs, the PTCy BMT platform permits infusion of donor-derived non-hematopoietic cells into an immunologically identical recipient at prescribed intervals following BMT. Thus, in principle, the MSCs (derived from the same donor as the original BMT) cannot be rejected. Here we report the dermatologic and transplant outcomes for the initial 10 patients with RDEB treated with reduced intensity conditioning followed by BMT on day 0, PTCy on days +3 and +4, and donor-derived MSCs at days +60, +100, and +180. We show that this coordinated cellular therapy is associated with acceptable safety and stable donor immunotolerance, the latter necessary for success of future donor cellular and tissue graft therapies.

Methods

Patients and Treatment

Pediatric (age <18 years) patients with RDEB undergoing PTCy BMT at the University of Minnesota between April 2016 and July 2017, with a minimum of 6 months of follow-up, were identified from a prospectively recorded Blood and Marrow Transplant Database of

demographic, clinical, and laboratory outcome measures. In accordance with the Declaration of Helsinki, all parents/guardians signed an Institutional Review Board-approved informed consent. As MSCs are not approved for clinical use by the Food and Drug Administration, this regimen is provided as an investigational new drug (IND #14166), registered as study NCT02582775 at ClinicalTrials.gov.

Patients eligible for PTCy BMT had confirmed diagnoses of RDEB by mutation analysis of *COL7A1,* reduced or absent IF staining for C7, lack of normal C7-supported ultrastructural basement membrane components visualized by IEM on skin biopsy, and a clinical history demonstrating generalized severe RDEB. Each case was de-identified and comprehensively assessed by an international external advisory panel composed of three EB experts (External Advisory Panel: JAM, AH, KT) to assess whether disease severity predicted a poor quality of life and threatened survival with the risk of SCC and serious infection, thus warranting therapeutic intervention.

PTCy BMT details including preparation for, eligibility/exclusion criteria, donor selection, conditioning regimen (rabbit anti-thymocyte globulin, cyclophosphamide, fludarabine, and low-dose total body irradiation), GvHD prophylaxis (PTCy, mycophenylate mofetil, ± tacrolimus), and supportive care are described in the Supplemental Materials and **Supplemental Figure 2**. Transplant outcomes include time to engraftment of neutrophils, defined as the first of 3 consecutive days of an absolute neutrophil count $\geq 0.5 \times 10^9$/L, and platelets, defined as the first of 3 consecutive days of a platelet count $\geq 20 \times 10^9$/L independent of platelet transfusion in the prior 7 days. GvHD was scored using standard criteria[31].

Disease assessments

At baseline, and at days +100, +180, and +365 post-PTCy BMT, comprehensive medical photography, laboratory assessments of inflammation (C-reactive protein, erythrocyte sedimentation rate), and perilesional skin biopsies of extremities were taken. Full thickness skin biopsies were assessed as previously described[16], including IF for C7 presence [using 6 C7 antibodies: Woodley, Sigma LH7.4, Abnova, Calbio, Abcam, and Clone 185, with controls including fibrillin and collagen IV (Col4)], transmission electron microscopy and IEM for basement membrane ultrastructure, particularly assessment of C7-forming AFs. Whole DNA from a full-thickness skin biopsy was also subjected to variable number of tandem repeats analysis to determine donor contribution. Peripheral blood donor chimerism was assessed concurrently (as well as on day +28).

Both provider/clinician and parent components of the validated Instrument for Scoring Clinical Outcome of Research for Epidermolysis Bullosa (iscorEB)[32, 33] were completed at baseline, and at days +100, +180, and +365 post-PTCy BMT. The iscorEB was preferred to other EB-specific disease scales as it quantifies longitudinal changes with emphasis on dynamic wound healing as opposed to fixed scar disease components. Provider subscores include assessment of BSA of involvement as well as extent (blisters, erosions, scabbing, chronic wounds, and infection); mucosal involvement of mouth, airway, and eye; internal organ involvement as reflected in nutrition, urogenital, or cardiac complications; laboratory abnormalities; and complications including SCC, osteopenia, unscheduled hospital visits, and need for procedures such as esophageal dilatations. Parent subscores highlight quality of life measures including pain,

pruritus, essential functions (ability to eat, drink, and void), sleep disturbance, daily activities (mobility and use of hands), mood, and overall impact of the disease on the ability to participate in school and leisure activities.

Analyses

All analyses are descriptive with formal group comparisons over time by non-parametric paired tests (Wilcoxon signed rank test) using GraphPad Prism 7.0, La Jolla, CA.

Results

Patient characteristics

Ten patients underwent a total of 12 reduced intensity PTCy BMTs between April 2016-July 2017, with a median follow-up of 16 months (range 8-24 months; enrollment data in **Supplemental Figure 1**). Donors included 3 haploidentical siblings, 2 haploidentical mothers, 4 HLA-matched siblings, and one HLA-matched unrelated individual. Characteristics of the patients, donors, and transplant regimens are described **Supplemental Table 1**. Of note, patient 3 had previously undergone an unrelated donor BMT complicated by graft failure (1 year prior to PTCy BMT). Initial hospital discharge occurred at a median of 27 days post-BMT (range 22-61 days). In the first 100 days following all 12 BMTs, 9 patients required readmission for an additional 5 days (median, range 2-27 days).

Outcomes

The dermatologic outcomes of PTCy BMT for RDEB are summarized below, with individual patient results provided in **Table 1.**

Body surface area, blister formation, laboratory measures of inflammation

Disease modulation following PTCy BMT was captured by interval assessments of quantitative measures including body surface area (BSA) involvement (of blisters and erosions), skin fragility as measured by blister time, laboratory markers of inflammation. BSA for donor engrafted patients declined from a median of 49.5% [interquartile range (IQR) =24-65.5] at baseline to 27.5% (IQR=16.3-35.8, n=9, p=0.05) at day +100, though failed to reach statistical significance. Later comparisons were limited by small numbers of patients with median BSA of 35.8% (IQR=14.8-48.5, n=8) at day +180 and 12% (IQR=10-22.5, n=3) at day +365. Conversely, for the 3 patients with graft failure, BSA increased from a median of 37.5% (range 33-67.5%) at baseline to 41% at day +60 (range 14-73.5%, n=3) and 62% at day +180 (range 50-73.5%, n=2, both subsequently re-transplanted). Time to induced blister formation (p=0.94), C-reactive protein (p=0.40), and erythrocyte sedimentation rate (p=0.88) were unchanged on paired comparisons from baseline to day +180.

C7 immunofluorescence and ultrastructural examination of skin

Each patient had skin biopsies at baseline (n=10), day +100 (n=9), +180 (n=9; epidermal loss upon sampling occurred in 2), and 1 year (n=3). Samples were assessed by 6 IF stains against C7 as well as by IEM. **Figure 1** highlights the medical photography, IF, and IEM results of Patient 1, comparing baseline to day+180 following PTCy BMT, with **Supplemental Figure 2** showing IF results over time for each donor-engrafted patient (2nd transplant time points for Patients 2 and 6, Patient 5 excluded as no follow-up biopsies available for comparison). No patient demonstrated persistent loss in the number of positive C7 antibodies, 1 had gain of 1 additional

positive C7 antibody over time, and 1 demonstrated qualitative increase in the brightness of 2 C7 immunostains. Transmission electron microscopy with the addition of IEM revealed improvements in 4 of 9 donor-engrafted patients; 3 with gain of primitive anchoring fibril (AF) components and 4 with increases in C7 immunostaining. While no structurally normal, complete AFs were identified in these early post-PTCy BMT evaluations, the day +180 skin biopsy of Patient 1 showed promising ultra-structural evolution of the cutaneous basement membrane. C7 immunostaining labeled both hemidesmosomes and newly seen primitive AF components.

Quality of life reports

IscorEB (Instrument for Scoring Clinical Outcome of Research for Epidermolysis Bullosa), a validated measure of combined clinical/laboratory domains (medical provider sub-score) and quality of life domains (parent sub-score), was completed for each patient prior to and at intervals following PTCy BMT (d+100, +180, +365), as described in the methods. Provider sub-scale scores are shown for each individual patient in **Table 1**, and parental assessments of quality of life in **Figure 2**. While we detected no statistically significant changes in perceived disease severity over the first year following PTCy BMT, declines from baseline to day +365 were observed in pain (32.5% of maximum severity to 20%), pruritus (75% of maximum severity to 50%), and sleep disturbance (62.5% of maximum severity to 25%) sub-scores.

Donor graft function

Three of the first 6 patients (Patients 2, 5, and 6) had primary, non-neutropenic graft failure with autologous hematopoietic recovery and no evidence of donor cells in peripheral blood or skin, suggestive of inadequate immune suppression from myeloablation. Two had evidence of

immune-mediated graft rejection with panel reactive antibody testing prior to transplant revealing moderate risk [mean fluorescence intensity (MFI) of 1000-3000] HLA class I donor-specific antibodies (two for Patient 2 and one for Patient 5). For subsequent patients, the threshold for clinical relevance of anti-HLA antibody MFI was lowered from 3000 to 1000 and the conditioning regimen was amended to increase total body irradiation (TBI) from 300 cGy to 400 cGy (**Supplemental Figure 3**). Two patients with graft failure were re-transplanted with the same donors after 6-month recovery intervals from initial PTCy BMT; one declined additional therapy. Since increasing the TBI dose and increasing attention to pre-transplant recipient anti-HLA antibody assessments, there has been no graft failure. At the same time, subsequent donors have been HLA-matched related siblings.

Donor chimerism

Donor chimerism in peripheral blood and skin are shown for each patient in **Supplemental Table 1**. Excluding the 3 cases of graft failure, median donor myeloid and lymphoid peripheral blood chimerism are 100% in both compartments at days +180 (n=9, myeloid range 28-100%, lymphoid range 52-100%) and +365 (n=3; myeloid range 90-100%, lymphoid range 76-100%). Donor chimerism in skin for the same patients is a median of 27% (range 7-58%) at day +180 and 11% (range 6-34%) at day +365.

Serious adverse events

Serious adverse events following PTCy BMT were limited, with 2 of 12 transplants complicated by veno-occlusive disease of the liver, both successfully treated with defibrotide, and one case of posterior reversible encephalopathy, which resolved. No acute graft-versus-host disease (GvHD)

was identified. No toxicities have been observed with MSC infusions. Following his second PTCy BMT, Patient 6 never achieved platelet recovery despite trials of thrombopoietin receptor agonists and a CD34+ selected peripheral blood donor stem cell boost. At 7 months post-PTCy BMT, a bone marrow biopsy demonstrated 40-50% cellularity with normal trilineage hematopoiesis. He additionally suffered hyperbilirubinemia and bouts of severe nausea, both persistent despite cholecystectomy and in the absence of etiology from liver and stomach biopsies. Given his constellation of symptoms, he began therapy for chronic GvHD on day +216. On day +356, he died of multi-drug resistant *Pseudomonal* sepsis.

Discussion

Most of what we understand today about BMT for RDEB comes from patient by patient analysis of the complex dynamics of engraftment and persistence of traditional allogeneic BMT[15-18, 34]. However, HLA-matched unrelated and related (typically sibling) donor BMT has been associated with significant physical (from chemotherapy and radiation) and immune (exemplified by GvHD) side effects. The ethical dilemma for clinicians and families considering BMT for their patient or child demands that the near-term morbidity and mortality risks be outweighed by the potential for lifelong benefits of novel therapies in this devastating disorder. Here, we show that PTCy BMT is feasible in individuals with generalized severe RDEB, with acceptable safety to recipients, very low rates of acute (0%) and chronic (10%, n=1) GvHD, with achievement of sustained donor tolerance.

Our present results propose that, in addition to quantitatively expanding the pool of available donors, PTCy BMT can provide yet-unexplored quality to the traditional BMT: adoptive transfer

of non-hematopoietic cells with long-term extra-medullary engraftment potential. In the present study, we have integrated clinical (BSA, skin photographs), histological (IF), ultra-structural (transmission electron microscopy and IEM), laboratory (donor engraftment, inflammatory markers) and quality of life (iscorEB) data to analyze the metrics of clinical efficacy supported by structural tissue correlates. We showed that donor cells engrafted in recipient skin (median of 27% at 6 months after PTCy BMT) and skin involvement decreased (BSA decreased from a median of 45.5% before PTCy BMT to 16.5% at 6 months after PTCy BMT). While all patients undergoing BMT may benefit from excellent hospital-based skin care, periods of decreased mobility-induced trauma, and anti-inflammatory effects of conditioning and immune suppression, two of 3 patients with graft failure demonstrated substantial increases in BSA from baseline pre-PTCy BMT to 60, 100, and 180 days post-PTCy BMT. The safety of HaploBMT has been greatly enhanced by recent addition of PTCy (as in the present study), eliminating the most alloreactive T-cell clones (and thus dramatically reduces the immune complications of BMT, such as GvHD). Of note, we observed no acute and only one case of chronic GvHD in the present study.

We acknowledge several limitations to this study. Outcomes are largely descriptive given the rarity of RDEB and subsequent small study cohort. Only 3 of 10 patients reside in the U.S., none local to our medical facility. Timely follow-up of international patients has both financial and political barriers. Reporting clinical outcomes with a median follow-up of 16 months (range 8-24) may not capture the full response to BMT, the trajectory and stability of which would best be described with greater follow-up. The skin fragility of RDEB makes measurements of clinical response to PTCy BMT challenging. Dressing changes are complex, often with associated pain

and separated over several days, which limits whole-body, high-quality medical photography of the skin. Intrinsic separation at the dermal-epidermal junction limits skin biopsy integrity. Skin donor chimerism is not limited to C7-producing donor cells, but may include donor immune infiltrates responding to cutaneous infections or contributing to local recovery following tissue damage.

While our present results highlight a role for three doses of systemically administered MSCs, our data have several other practical applications. These include the ability of the PTCy BMT platform to accommodate maintenance infusions of MSCs in any combination of intravenous and local (intra-dermal or intra-mucosal) delivery of MSCs, infusion/injection of alternative cells (such as fibroblasts and keratinocytes) and tissue grafts (such as skin, mucosa, and corneal epithelium)—all relevant for treatment of the wounds and systemic manifestations of RDEB.

To our knowledge, this is the first attempt to generate an immunologically synchronous recipient for extramedullary engraftment of non-hematopoietic cells. We acknowledge lack of direct donor hematopoietic C7 production or contribution to new AFs limits the efficacy of isolated BMT. However, our work proposes PTCy BMT as a viable platform for comprehensive, multi-modal systemic therapies aimed at decreasing the morbidity and mortality of RDEB. Future studies will expand upon these findings, extending follow-up to monitor the impact of PTCy BMT on skin integrity (including development of AFs), SCC, and overall survival; incorporating other promising donor cellular therapies and/or epidermal grafting; and exploring safety and effectiveness in PTCy BMT for additional forms of debilitating EB.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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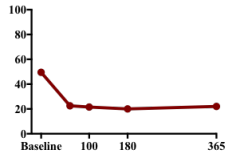
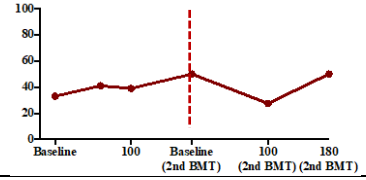
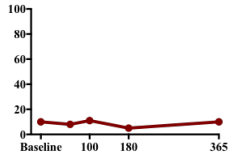
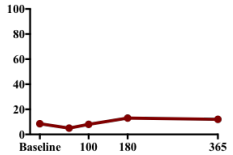
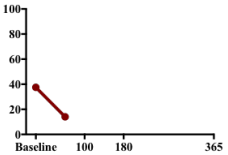
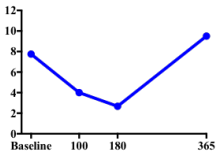
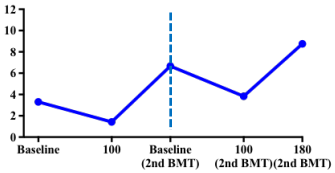
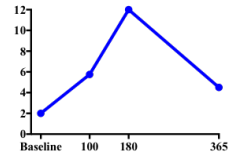
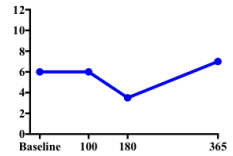
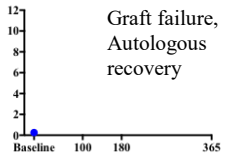
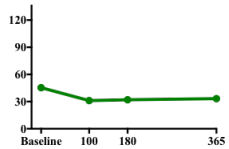
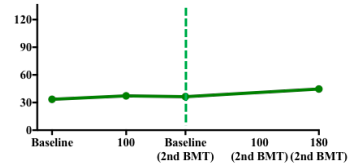
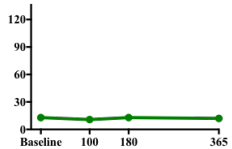
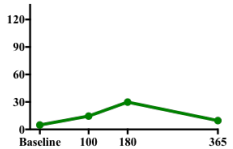
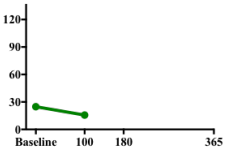
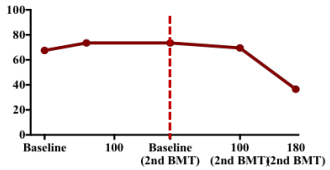
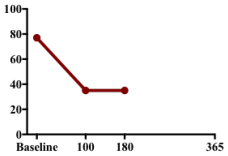
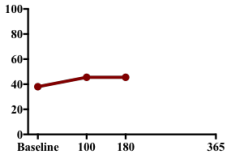
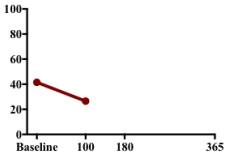
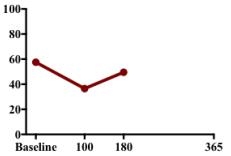
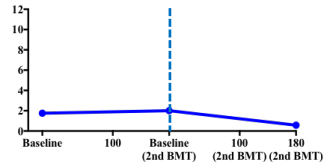
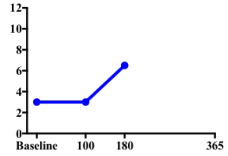
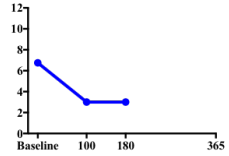
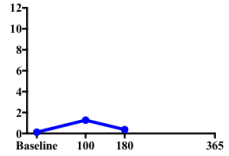
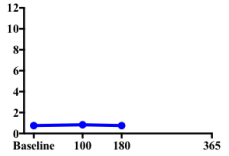
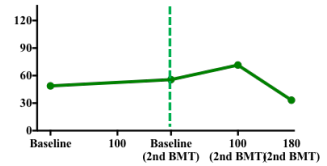
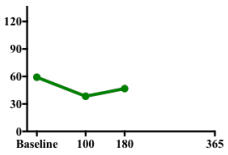
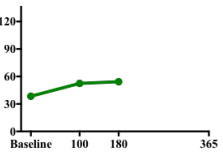
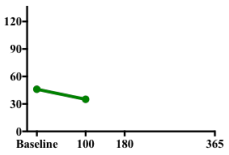
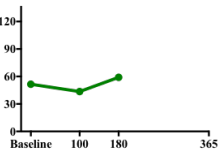
TABLE 1a. PTCy BMT Dermatologic Outcomes

	Patient 1	Patient 2 1st HaploBMT 2nd HaploBMT	Patient 3	Patient 4	Patient 5
Patient: Sex, *Col7A1* mutation(s), Age at BMT	Female c.8201G>A, c.8528-1G>A 8.2 years	Male c.425A>G, c.5797C>T 3.1 years → 3.6 years	Female c.6501+1G>C, c.7787del 22 months	Female c.6527dupC, c.6527dupC 22 months	Male c.611T>C, delexon25-52 11.5 years
Clinical features[1]	Esophageal narrowing, pseudosyndactyly, Constipation	Oral blisters, corneal ulcers/abrasions, beginning fusion of toes	Oral blistering	Mucosal blistering & erosions	Oral lesions, dysphagia, constipation, corneal abrasions, dental caries
IF interpretation	6/6 anti-C7 stains positive at baseline, d+100, 180, 365; 2 anti-C7 stains with increased brightness and extension from the basement membrane to the dermis from d+180 onward	2/6 anti-C7 stains positive at baseline until d+100 following 2nd HaploBMT; Increased to 3/6 with additional 1 anti-C7 stain with increased brightness and extension from the basement membrane to the dermis at d+180	3/6 anti-C7 stains positive at baseline, d+100, 180, 365; No change over time	6/6 anti-C7 stains positive at baseline, d+100, 180, 365; No change over time	6/6 anti-C7 stains positive at baseline, no subsequent skin biopsies for comparison
EM interpretation	AFs: filamentous/wispy, slight banding/arching (d+180); C7 immunolabeling: very light (d0) → moderate, associated with hemidesmosomes and AFs (d+180)	No AFs or C7 immunolabeling detected (d+180)	AFs: none (d0) → occasional linear projections (d+180); C7 immunostaining from none (d0) → very light (d+180)	AFs: none (d0) → occasional linear projections (d+365); C7 immunostaining: very light with 1 antibody (d0) → 2 antibodies (d+365)	N/A
BSA involvement (%) × BMT day					
Blister time (minutes) × BMT day					 Graft failure, Autologous recovery
Provider iscorEB (max 137) × BMT day					
Current status, Time since BMT	Alive, 24 months	Alive, 17 months from 2nd HaploBMT	Alive, 20 months	Alive, 20 months	Alive, 19 months

[1]All patients: skin surface covered with erosions and blisters; history of pruritus and pain.

PTCy, post-transplant cyclophosphamide; haplo, haploidentical; BMT, bone marrow transplant; IF, immunofluorescence; C7, collagen VII; EM, electron microscopy; AFs, anchoring fibrils; BSA, body surface area; iscorEB, Instrument for Scoring Clinical Outcome of Research for Epidermolysis Bullosa.

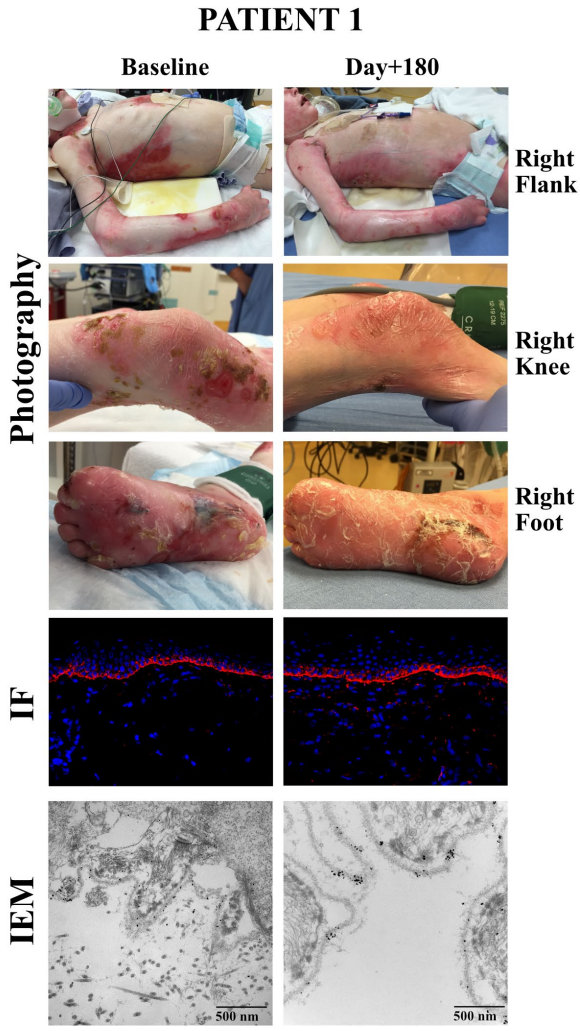
TABLE 1b. PTCy BMT Dermatologic Outcomes

	Patient 6 1st HaploBMT 2nd HaploBMT	Patient 7	Patient 8	Patient 9	Patient 10
Patient: Sex, *Col7A1* mutation(s), Age at BMT	Male c.7723G>A, c.6236G>T 16.3 years→16.9 years	Male c.3140-1G>A, c.3140-1G>A 6.4 years	Female c.3140-1G>A, c.3140-1G>A 22.1 years	Male c.4738_4740delinsA, c.425A>G 12.2 years	Female c.5888G>A, c.5888G>A 12.8 years
Clinical features[1]	Mucosal erosions, contractures, corneal abrasion, dental carries, osteoporosis, FTT	Esophageal strictures, dental carries, microstomia & ankyloglossia, nail loss, anemia, mild contractures	Esophageal strictures, dental carries, microstomia & ankyloglossia, nail loss, anemia, moderate contractures	Esophageal strictures, constipation, anemia, contractures, nail loss	Corneal abrasions, esophageal stenosis, dental carries, anemia, FTT, contractures, nail loss, depression
IF interpretation	6/6 anti-C7 stains positive at baseline, d+100, 180 following 2nd HaploBMT; No change over time	3/6 anti-C7 stains positive at baseline, d+100 (d+180, epidermis lost in procedure); No change over time	3/6 anti-C7 stains positive at baseline, d+100 (d+180, epidermis lost in procedure); 2 anti-C7 stains less bright at d+100	3/6 anti-C7 stains positive at baseline, 2 lost at d+100 but regained for again 3/6 anti-C7 stains positive at d+180	3/6 anti-C7 stains positive at baseline, d+100, 180; No change over time
EM interpretation	No AFs or C7 Immunolabeling detected (d+180)	No AFs or C7 Immunolabeling detected (d+180)	No AFs (d0, d+180); C7 immunostaining from none (d0) → very light (d+180)	No AFs or C7 Immunolabeling detected (d+180)	Possible AFs (d0, d+180), C7 immunolabeling absent
BSA involvement (%) × BMT day					
Blister time (minutes) × BMT day					
Provider iscorEB (max 137) × BMT day					
Current status, Time since BMT	Died, 11 months from 2nd HaploBMT	Alive, 14 months	Alive, 13 months	Alive, 8 months	Alive, 8 months

[1]All patients: skin surface covered with erosions and blisters; history of pruritus and pain.

PTCy, post-transplant cyclophosphamide; haplo, haploidentical; BMT, bone marrow transplant; FTT, failure to thrive; IF, immunofluorescence; C7, collagen VII; EM, electron microscopy; AFs, anchoring fibrils; BSA, body surface area; iscorEB, Instrument for Scoring Clinical Outcome of Research for Epidermolysis Bullosa.

FIGURE LEGENDS



IF, Immunofluorescence; IEM, immunoelectron microscopy

Figure 1. Dermatologic outcomes. Select photographs, demonstrating dermatologic involvement of EB, and skin biopsy results, including immunofluorescence [40× magnification merged dapi (blue) and C7 collagen antibody (red)] and immunoelectron microscopy [transmission electron microscopy with C7 collagen-directed immunostain (black)], shown for Patient 1 at baseline and day +180 after transplantation.

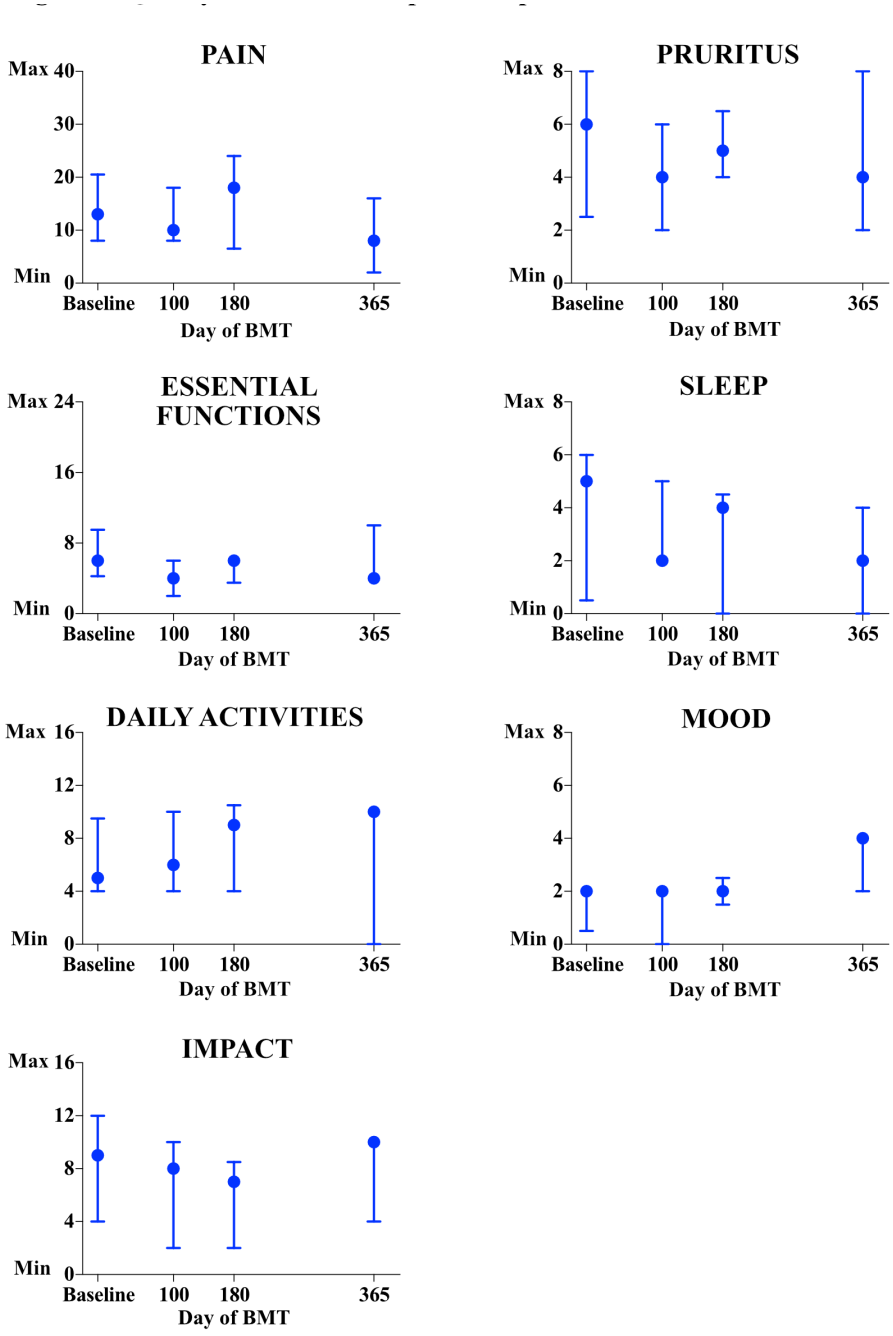


Figure 2. Quality of life: iscorEB parent reports. The median and interquartile ranges are of parent iscorEB sub-scores are displayed at PTCy BMT time points as labeled (baseline, n=12; day +100, n=11; day 180, n=10; and day +365, n=3).

SUPPLEMENTAL MATERIALS: PTCy BMT details

Prior to PTCy BMT, patients underwent gastrostomy tube placement to optimize nutrition, provide a mechanism to reduce nausea/vomiting during transplant that may contribute to esophageal stricture formation, and allow for enteral administration of medications. Eligibility to proceed to BMT included adequate major organ function—including renal glomerular filtration rate within normal range for age, liver function tests and bilirubin <5× the upper limit of normal, adequate pulmonary function (by pulmonary function testing if age appropriate, or by history and physical exam), normal electrocardiogram and echocardiogram showing left ventricular ejection fraction ≥45%. Sexually active patients must agree to use adequate birth control from the time of conditioning until 1 year following transplant, and pregnant or breast-feeding patients were excluded. Finally, evidence of squamous cell carcinoma, history of HIV infection, or active, untreated systemic infection at the time of transplant (or any mold infection in the prior 30 days) rendered a patient ineligible for BMT.

PTCy BMT required identification of a stem cell donor, typically related, matching the patient on at least one of two alleles at any given HLA locus. In the absence of an 8/8 HLA-matched sibling donor, patients were also tested for the presence of donor-specific antibodies (DSA, circulating anti-HLA antibodies specific to the donor HLA). When more than one donor was considered, selection was informed by donor-specific antigen negativity, as well as preference for younger age, nulliparous, ABO-compatible, and cytomegalovirus (CMV) serostatus consistent with that of the patient.

The initial 6 patients undergoing PTCy BMT received the following conditioning regimen (**Supplemental Figure 2**): rabbit anti-thymocyte globulin (rATG), pre-medicated with methylprednisolone, acetaminophen, and diphenhydramine, infused at a dose of 0.5 mg/kg recipient weight intravenously (IV) on day -9 prior to BMT, then 2 mg/kg/dose IV on days -8 and -7; fludarabine 30 mg/m^2 recipient body-surface-area/day IV on days -6 to -2; cyclophosphamide 14.5 mg/kg/dose IV on days -6 and -5 (with mesna for uroprotection); and a single fraction of low-dose total body irradiation (TBI) of 300 cGy on day -1. With graft failure and autologous recovery of hematopoiesis noted in 3 of the first 6 patients receiving this regimen, immunomyeloablation was escalated by increasing the dose of TBI to two fractions of 200 cGy (total of 400 cGy) on day -1.

On day 0, all patients were infused fresh, un-irradiated, donor bone marrow, filtered to remove any bone fragments. Donor-recipient blood type mismatch, when present, prompted red blood cell depletion of the bone marrow. Methylprednisolone, acetaminophen, and diphenhydramine premedication was provided. For each patient, the goal total nucleated cell count was 2-10 × 10^8/kg of recipient's ideal body weight. In the situation where a cryopreserved donor umbilical cord was available to supplement, it was thawed and infused.

With donor consent, an additional 30 mL of bone marrow was collected for expansion of donor-derived MSCs. Once expanded in adequate numbers, an aliquot of the MSCs were tested for sterility, epitope analysis, and differentiation potential, while the remainder was cryopreserved in doses of approximately 2 × 10^6/kg recipient weight. At days +60, +100, and +180 following

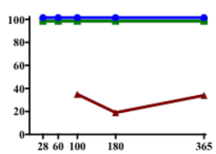
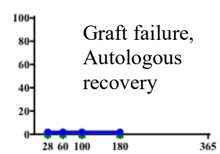
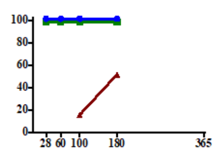
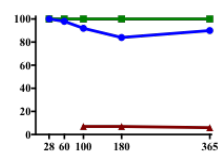
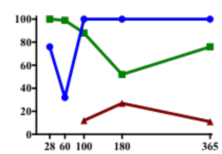
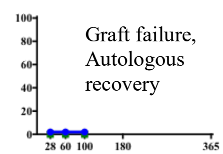
PTCy BMT, MSC doses meeting lot release criteria were infused intravenously, and patients were monitored for any infusion reactions.

Graft-versus-host disease (GvHD) prophylaxis included infusion of PTCy 50 mg/kg recipient weight on days +3 and +4 following BMT, with hyperhydration and mesna for uroprotection. In the absence of acute GvHD, all patients received mycophenylate mofetil 15 mg/kg/dose IV every 8 hours from day +5 to +35 or 7 days following neutrophil recovery (defined as an absolute neutrophil count of $>0.5 \times 10^9$/L for 3 consecutive days), whichever occurred later. Tacrolimus was provided from day +5 to +100, initially as a continuous infusion, then transitioned to twice or thrice daily oral dosing to achieve a target trough of 5-10 μg/L. In the absence of acute GvHD, tacrolimus was tapered off over 10 weeks starting at day +101. Planned tacrolimus was omitted from GvHD prophylaxis for HLA-identical sibling donor recipients; however, it was added in cases of engraftment syndrome.

Supportive care for PTCy BMT included hospitalization in single occupancy, high-efficiency particulate air-filtered rooms with use of antibiotic prophylaxis through neutrophil engraftment. Anti-fungal and anti-viral (targeting CMV and HSV as appropriate) prophylaxis was provided until at least day +100, with prophylaxis against *Pneumocystis jiroveci* pneumonia following engraftment until one year post-BMT. Antibiotic coverage was broadened empirically with fever and adjusted based on infection surveillance. Blood product transfusions were CMV-safe. Granulocyte colony-stimulating factor has been provided in cases of slow blood cell count recovery. Patients were screened by blood CMV PCRs weekly until day +100. Intensive skin care including continuous assessment, lancing of blisters with sterile needles, and dressing

changes/bathing 2-3×/week, was tailored to each patient based on history and response to transplant-associated fluid shifts and skin infections. Central line dressings were changed at a minimum of weekly, often more frequently depending on visual inspection. Pain management for baseline EB wound-associated pain and pruritus, as well as for BMT mucositis, was also patient-dependent and provided with the assistance of a Pediatric Advanced and Complex Care Team.

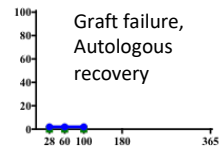
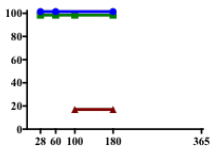
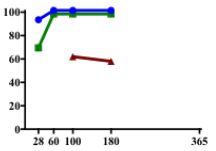
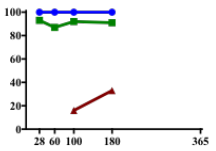
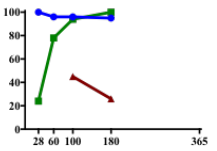
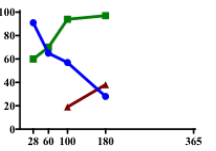
Supplemental Table 1a. PTCy BMT characteristics and outcomes

	Patient 1	Patient 2		Patient 3	Patient 4	Patient 5
		1st HaploBMT	2nd HaploBMT			
Patient: Sex, *Col7A1* mutation(s), Age at BMT	Female c.8201G>A, c.8528-1G>A 8.2 years	Male c.425A>G, c.5797C>T 3.1 years → 3.6 years		Female c.6501+1G>C, c.7787del 22 months	Female c.6527dupC, c.6527dupC 22 months	Male c.611T>C, delexon25-52 11.5 years
Donor: Stem cell source, HLA match, DSA status	Sister BM, 5/10, no DSA	Mother BM, 5/10, no DSA		Sister BM, 6/10, no DSA	Unrelated BM, 8/8, no DSA	Sister BM, 5/10, no DSA
Conditioning regimen	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg TBI 200 cGy	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy
GvHD prophylaxis	PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF
BMT dose: **TNC** **CD34+**	 7.05×10^8 30.38×10^6	 13.25×10^8 11×10^6	 7.25×10^8 6.26×10^6	 7.08×10^8 19.95×10^6	 7.25×10^8 7.39×10^6	 8.19×10^8 21.3×10^6
MSC doses[2]	Days +60, +100, +180	Days +60, +100 (to 2nd BMT)	Days +60, +100, +180	Days +60, +100, +180	Days +60, +100, +180	Days +60, +100 (home prior to d+180)
Neutrophil, Platelet recovery	Day +19, Day +47	Day +26, Day +31	Day +15, Day +19	Day +16, Day +22	Day +17, Day +22	Day +19, Day +24
Severe adverse events[3]	VOD, PRES	None	None	None	None	None
GvHD	None	None	None	None	None	None
% Donor Chimerism × BMT day Myeloid PB Lymphoid PB Skin						

[1]All patients had: skin surface covered with erosions and blisters; history of pruritus and pain. [2]intravenous doses of 2×10^6 donor-derived MSCs/kg recipient weight. [3]Serious adverse events restricted to non-hematologic, non-infectious, non-dermatologic adverse events through engraftment, unexpected adverse events, and MSC-targeted toxicities.

PTCy, post-transplant cyclophosphamide; haplo, haploidentical; BMT, bone marrow transplant; HLA, human leukocyte antigen; DSA, donor specific antibodies; BM, bone marrow; N/A, not applicable; GvHD, graft-versus-host disease; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; MMF, mycophenylate mofetil; TNC, total nucleated cells/kg recipient weight; CD34+, CD34+ cells/kg recipient weight; MSC, mesenchymal stromal cell; VOD, veno-occlusive disease; PRES, posterior-reversible encephalopathy syndrome; PB, peripheral blood.

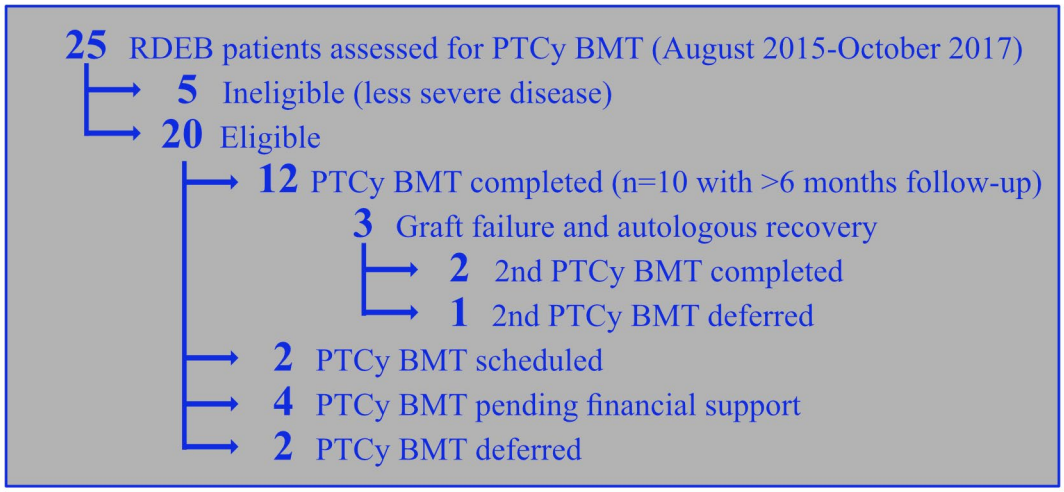
Supplemental Table 1b. PTCy BMT characteristics and outcomes

	Patient 6 1st HaploBMT	Patient 6 2nd HaploBMT	Patient 7	Patient 8	Patient 9	Patient 10
Patient: Sex, *Col7A1* mutation(s), Age at BMT	Male c.7723G>A, c.6236G>T 16.3 years→16.9 years		Male c.3140-1G>A, c.3140-1G>A 6.4 years	Female c.3140-1G>A, c.3140-1G>A 22.1 years	Male c.4738_4740delinsA, c.425A>G 12.2 years	Female c.5888G>A, c.5888G>A 12.8 years
Donor: Stem cell source, HLA match, DSA status	Mother BM, 5/10 Moderate risk DSA positive → no DSA		Brother BM, 10/10, DSA N/A	Sister BM, 10/10, DSA N/A	Sister BM, 10/10, DSA N/A	Sister BM, 10/10, DSA N/A
Conditioning regimen **GvHD prophylaxis**	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 300 cGy PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 400 cGy PTCy 50 mg/kg/d on d+3, +4 MMF	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 400 cGy PTCy 50 mg/kg/d on d+3, +4 Tacrolimus, MMF	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 400 cGy PTCy 50 mg/kg/d on d+3, +4 MMF	Fludarabine 150 mg/m^2 Cyclophosphamide 29 mg/kg rATG 4.5 mg/kg TBI 400 cGy PTCy 50 mg/kg/d on d+3, +4 MMF
BMT dose: **TNC** **CD34+**	5.56×10^8 3.11×10^6	4.10×10^8 1.8×10^6 (& Day+123: CD34+ PB boost)	4.83×10^8 11.94×10^6	6.05×10^8 5.33×10^6	3.85×10^8 4.78×10^6	4.62×10^8 6.52×10^6
MSC doses[2]	Days +60, +100 (to 2nd BMT)	Days +60, +100 (additional MSCs failed to meet release criteria)	Days +60, +100, +180	Days +60, +100, +180	Days +60, +100, +180	Days +60, +100, +180
Neutrophil, Platelet Recovery	Day +31, Day +49	Day +29, Not achieved	Day +15, Day +32	Day +15, Day +22	Day +17, Day +28	Day +25, Day +63
Severe adverse events[3]	None	None	None	None	None	VOD
GvHD	None	Chronic GvHD (severe, dx day+216 on tx until death) Died day+356 of multi-drug resistant *Pseudomonas* sepsis	None	None	None	None
% Donor Chimerism × BMT day Myeloid PB Lymphoid PB Skin						

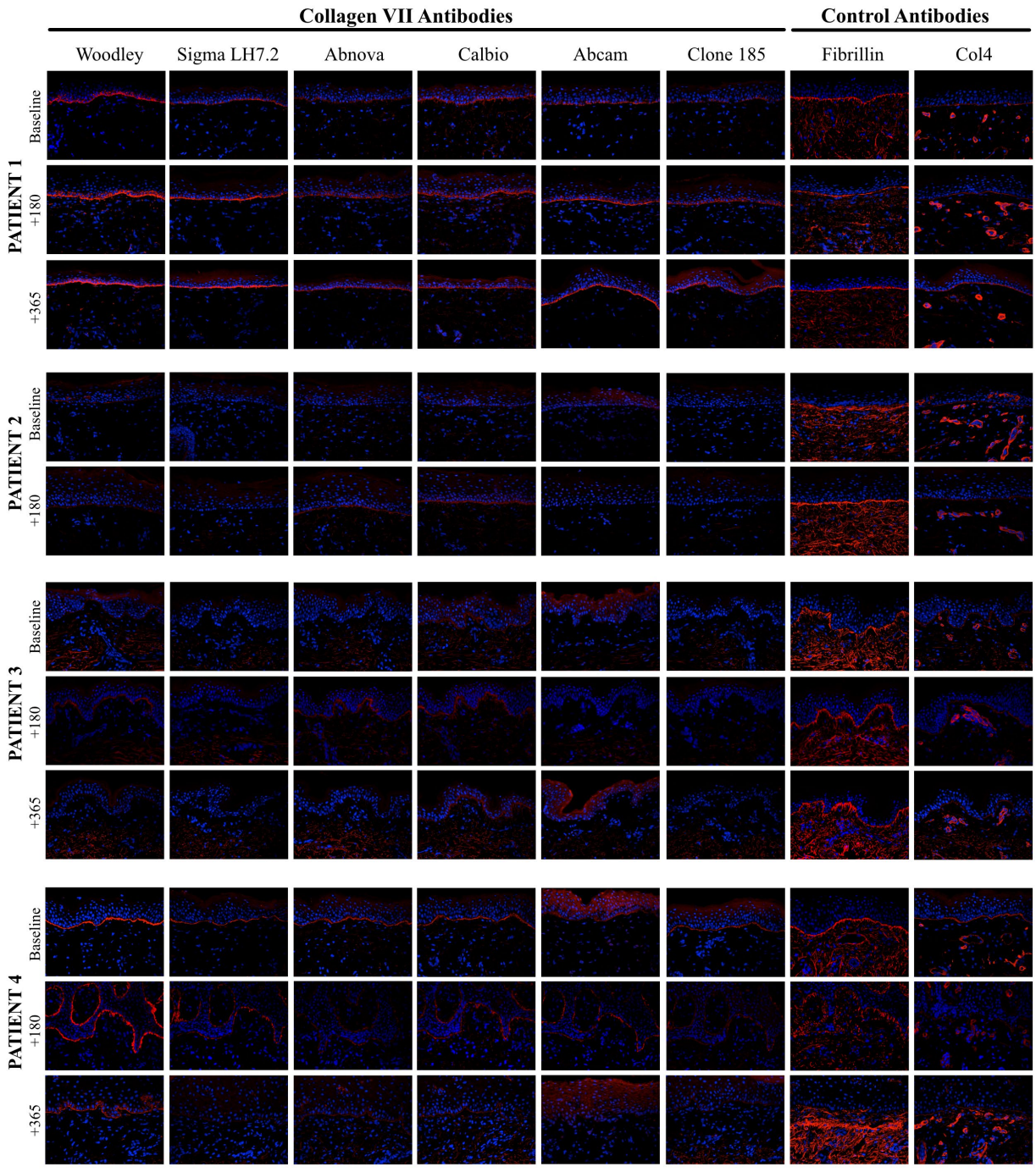
[1]All patients had: skin surface covered with erosions and blisters; history of pruritus and pain. [2]Intravenous doses of 2×10^6 donor-derived MSCs/kg recipient weight. [3]Serious adverse events restricted to non-hematologic, non-infectious, non-dermatologic adverse events through engraftment, unexpected adverse events, and MSC targeted toxicities.

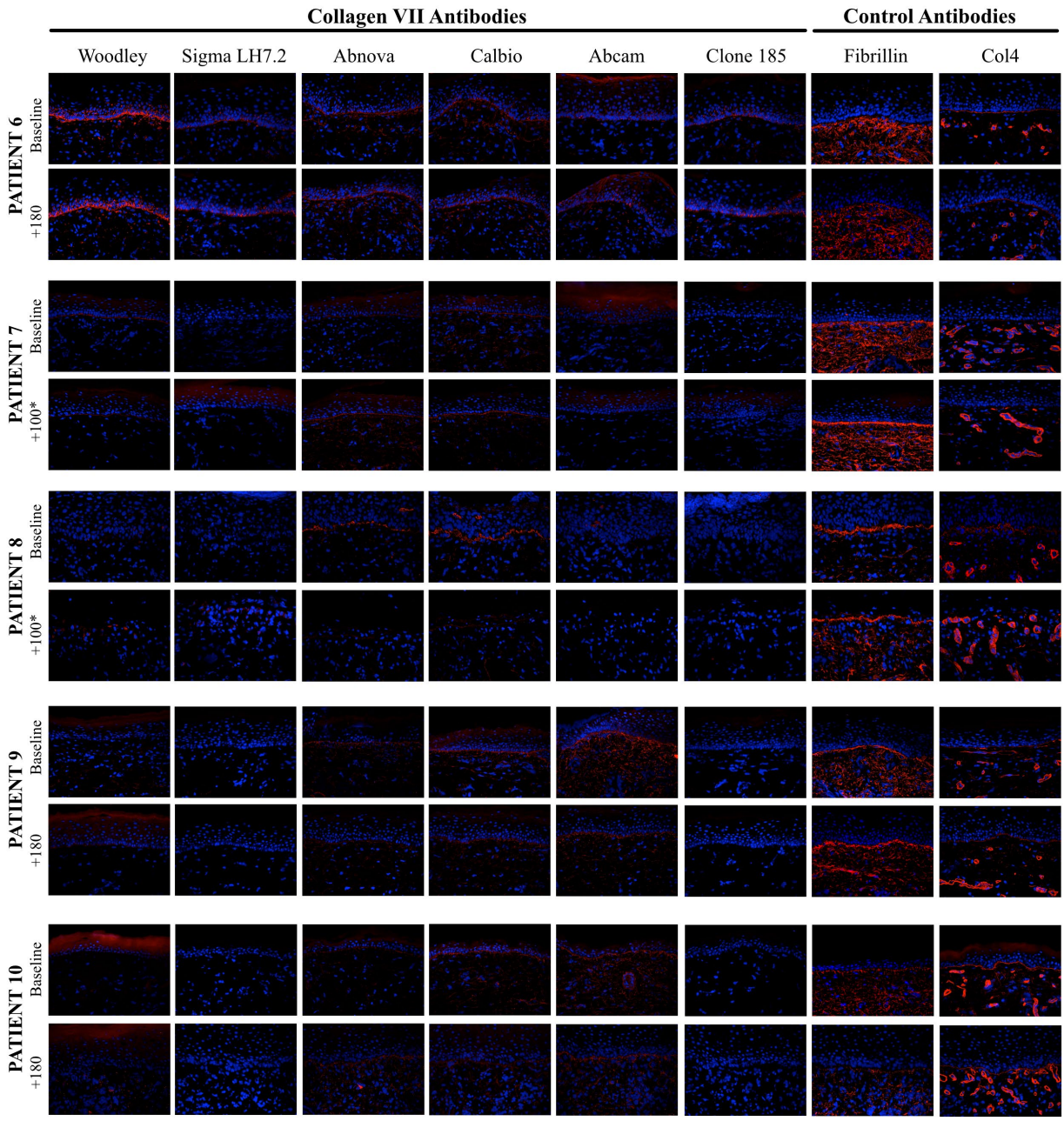
PTCy, post-transplant cyclophosphamide; haplo, haploidentical; BMT, bone marrow transplant; FTT, failure to thrive; HLA, human leukocyte antigen; DSA, donor specific antibodies; BM, bone marrow; N/A, not applicable; GvHD, graft-versus-host disease; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; MMF, mycophenylate mofetil; TNC, total nucleated cells/kg recipient weight; CD34+, CD34+ cells/kg recipient weight; PB, peripheral blood; MSC, mesenchymal stromal cell; VOD, veno-occlusive disease; dx, diagnosed; tx; treatment.

SUPPLEMENTAL FIGURE LEGENDS



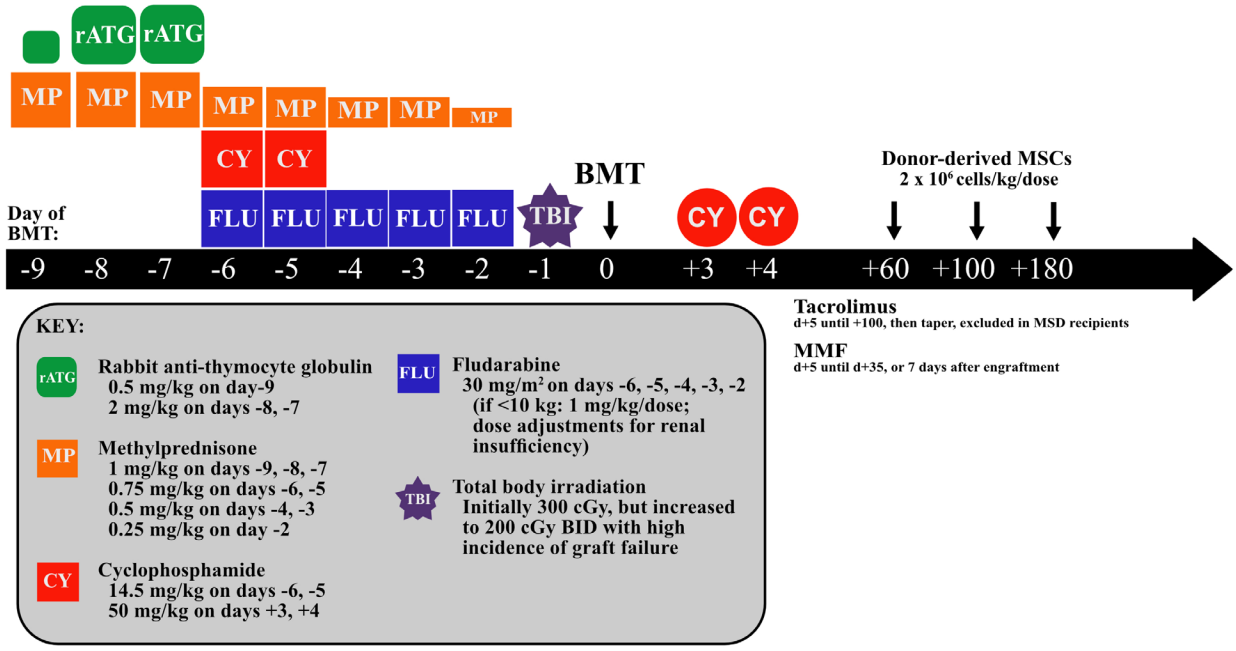
Supplemental Figure 1. Protocol enrollment. Flow diagram of RDEB patients from eligibility assessment to enrollment and PTCy BMT.


Collagen VII Antibodies
Control Antibodies
Woodley
Sigma LH7.2
Abnova
Calbio
Abcam
Clone 185
Fibrillin
Col4
PATIENT 1
PATIENT 2
PATIENT 3
PATIENT 4
Baseline
+180
+365



*Day+180 biopsies missing epidermis, day+100 results shown here

Supplemental Figure 2. Collagen VII immunofluorescence of the dermal-epidermal junction. For each RDEB patient with available skin biopsies over time (**a**, patients 1-4; **b**, patients 6-10; patient 5 had graft failure and is not shown), baseline, day +180 (or day +100 if day +180 biopsies lacked epidermis), and day +365 (where available) are shown [40× magnification merged dapi (blue) and C7 collagen antibody (red)]. C7 antibodies include Woodley, Sigma LH7.4, Abnova, Calbio, Abcam, and Clone 185, with controls including fibrillin and collagen IV (Col4).



PTCy BMT, Post-transplant cyclophosphamide bone marrow transplant; TBI, total body irradiation; cGy, centigray; MSCs, mesenchymal stromal cells; MSD, matched sibling donor; MMF, mycophenylate mofetil

Supplemental Figure 3. PTCy BMT for RDEB Regimen. Timeline provided for pre-BMT conditioning chemotherapy and radiation (days and doses), BMT, GvHD prophylaxis, and MSC infusions.