Reduced Toxicity Conditioning and Allogeneic Hematopoietic Progenitor Cell Transplantation for Recessive Dystrophic Epidermolysis Bullosa

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Recessive dystrophic epidermolysis bullosa is a severe, incurable, inherited blistering disease caused by *COL7A1* mutations. Emerging evidence suggests hematopoietic progenitor cells (HPCs) can be reprogrammed into skin; HPC-derived cells can restore COL7 expression in COL7-deficient mice. We report two children with recessive dystrophic epidermolysis bullosa treated with reduced-toxicity conditioning and HLA-matched HPC transplantation. *(J Pediatr 2015;167:765-9).*

Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited blistering disorder caused by type-VII collagen gene (*COL7A1*) mutations. It is characterized by severely reduced or absent functional COL7, which comprises anchoring fibrils (AFs) connecting the cutaneous basement membrane zone (BMZ) to dermis.[1,2] Patients with RDEB exhibit functional defects of AFs, resulting in impaired dermal-epidermal cohesion, producing tense blisters and erosions healing with extensive, mutilating scarring.[3]

Observations of the capacity of hematopoietic progenitor cells (HPCs) to differentiate into other tissues, including skin, prompted us to consider HPC therapy for treating severe RDEB.[4-9] We hypothesized skin injury in RDEB generates a microenvironment promoting homing of HPC-derived multipotent cells, which can then differentiate into skin cells, produce COL7, and restore functionally deficient AFs. Systemic delivery of HPCs enables targeting of affected skin and internal organs. Wagner et al[10] demonstrated decreased blistering, mixed dermal chimerism, and increased COL7 deposition at the dermal-epidermal junction in several children with RDEB following myeloablative conditioning (MAC) and allogeneic HPCs (AlloHPCs). However, 2 of 7 patients died of regimen-related mortality, likely because of MAC-associated toxicity and opportunistic infection. RDEB-associated morbidity, including blistering/erosions, mucositis, and malnutrition/poor feeding, further limit the use of MAC. Reduced toxicity conditioning prior to AlloHPC results in sustained donor chimerism with lower transplantation-related morbidity.[11,12]

An 18-month-old boy (patient 1) and 6-year-old boy (patient 2) with RDEB underwent reduced toxicity conditioning with busulfan (2 mg/kg/d intravenous [IV] twice daily, days −8, −7, −6, and −5), fludarabine (30 mg/m^2 IV, days −8, −7, −6, −5, and −4), and alemtuzumab (total 54 mg/m^2 IV, days −6, −5, −4, −3, and −2), followed by HLA-matched unaffected sibling-donor unmanipulated bone marrow AlloHPC on a multicenter institutional review board-approved study (NCT00881556).[13] Graft-versus-host disease prophylaxis consisted of tacrolimus (starting day −8) and mycophenolate mofetil (starting day +1).[14] Mucositis prophylaxis consisted of palifermin and Caphosol. Herpes simplex virus, cytomegalovirus, antibacterial, antifungal, and anti-*Pneumocystis* prophylaxis were as we have previously reported.[15,16] Donor chimerism was assessed as previously described.[11,13] Direct immunofluorescence (DIF) and transmission electron microscopy (TEM) were performed

AF	Anchoring fibril
AlloHPC	Allogeneic HPC
BMZ	Basement membrane zone
DIF	Direct immunofluorescence
HPC	Hematopoietic progenitor cell
IV	Intravenous
MAC	Myeloablative conditioning
mRNA	Messenger RNA
RDEB	Recessive dystrophic epidermolysis bullosa
TEM	Transmission electron microscopy

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centrally on skin biopsy specimens prior to and following AlloHPC.[17,18] Objective structured clinical skin assessments were performed prior to and following AlloHPC (**Table**; available at www.jpeds.com).

Patient 1 was born to consanguineous Yemeni parents, with pre-AlloHPC history notable for persistent methicillin-resistant *Staphylococcus aureus*-colonized wounds, severe pruritus, bloody emesis, and hospitalizations for failure to thrive, requiring gastrostomy tube placement. He carries a homozygous mutation in COL7A1 (c.2035_14del10Ins2) encompassing the splice junction of exon 17 and intron 17, resulting in deletion of 5 and insertion of 2 amino acids, leading to protein instability, consistent with a diagnosis of generalized severe RDEB.[19] He is >4.5 years post-AlloHPC and has achieved >90% stable donor chimerism in whole blood, 19% skin donor chimerism, and *COL7A1* messenger RNA (mRNA) levels of 2.5 × pre-AlloHPC levels at day +30, and 4.75 × pre-AlloHPC levels at day +365 (**Figure 1**, A, C, and D). DIF demonstrated COL7 positivity in scattered dermal hair follicle epithelial cells on day +181, though skin has not expressed detectable COL7 at the BMZ. TEM analysis disclosed no normal AFs prior to transplantation; possible interval development of rudimentary AFs was observed at day +365 (**Figure 2**, A and B). Pre-AlloHPC, diffuse open blisters were noted on his neck, tongue, back, diaper

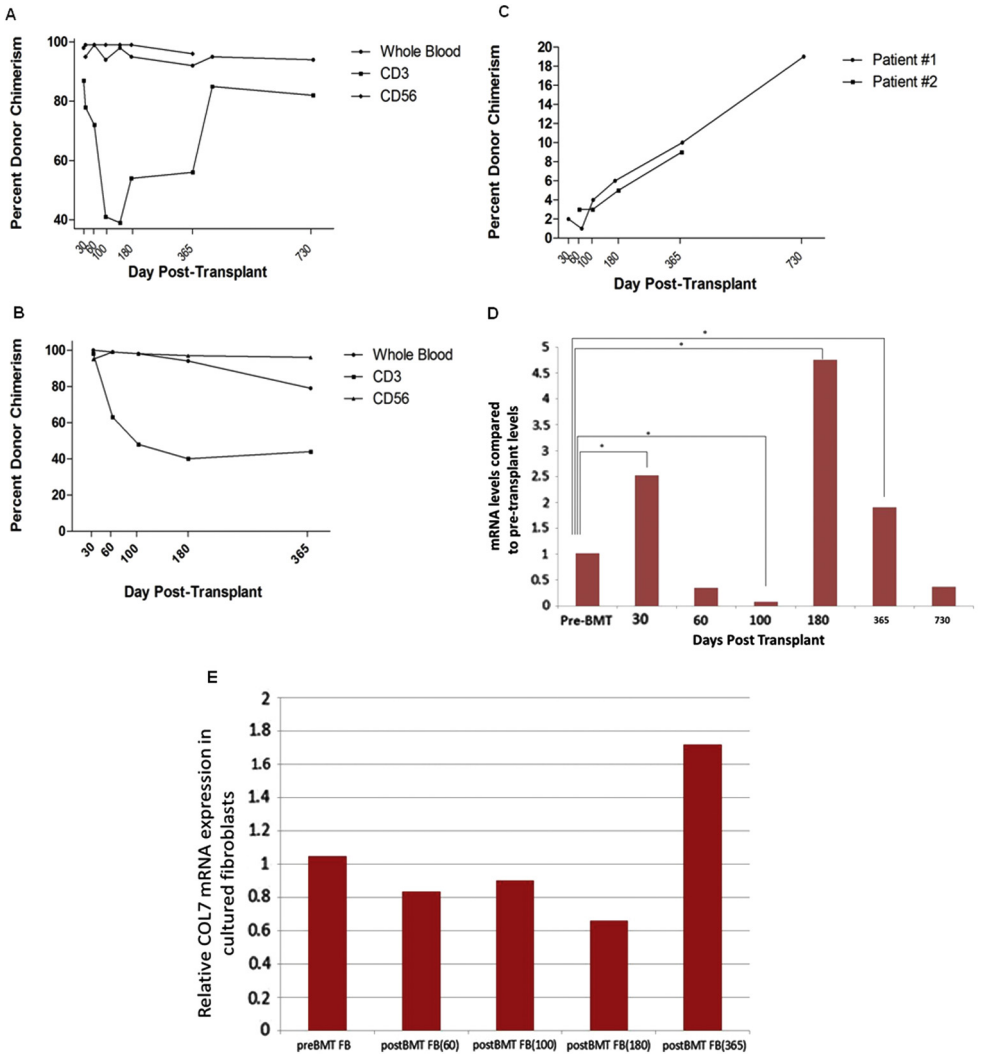

Figure 1. Donor chimerism in whole blood and immunophenotypic subsets post-AlloHPC in **A**, patient 1, and **B**, patient 2. **C**, Donor chimerism in skin (determined via DNA isolation from fluorescence-activated cell sorting-purified keratinocyte and fibroblasts cultured from skin biopsy specimens) in each patient post-AlloHPC. **D**, *COL7A1* mRNA expression in skin samples from patient 1 pre- and post-transplant, expressed as a proportion of expression observed at baseline. Differences significant statistically by two-way ANOVA differences between groups of replicates are marked with an asterisk (*). **E**, *COL7A1* mRNA expression in cultured fibroblasts from patient 2 post-bone marrow transplant, expressed as a proportion of expression observed in normal control cells.