Correction of Dystrophic Epidermolysis Bullosa by Infusing Bone Marrow Derived Stem Cells after Myeloablation

Prepared for the CIRM Medical and Ethical Standards Working Group

Hypothesis

Bone marrow derived stem cell populations may be capable of reconstituting sufficient collagen type VII that form anchoring fibrils to ameliorate or correct the clinical manifestations of dystrophic epidermolysis bullosa (DEB).

Secondary Hypothesis

Should insufficient levels of collagen type VII be generated to prevent the clinical manifestations of DEB, the achieved state of tolerance after allogeneic hematopoietic stem cell transplantation (HSCT) will permit long term engraftment of epidermal cells from the same donor which itself could improve quality of life and survival.

Background

DEB is a group of heritable mechanobullous skin diseases characterized by skin fragility, blister formation, and scarring. The basement membrane zone (BMZ) is characterized by a paucity or diminutive size of anchoring fibrils (Briggeman, 1975). The most severe form of DEB is 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 half will die from SCC. The prognosis of a DEB patient with SCC is poor with death in most before age 30 (Fine et al 1999; Mallipedi 2002)

In addition, 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 (Fine et al 2004).

Patients with the severe DEB are also affected by profound physical disabilities with social and psychological implications. Daily activities (toileting, feeding, bathing, walking, ect) 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 (Fine et al., 2004).

Type VII collagen is synthesized and secreted by both human keratinocytes and fibroblasts. It is secreted within the BMZ lying between the epidermis and dermis of skin

(Burgeson, 1993). It is the anchoring fibrils which are primarily composed of collagen type VII that are responsible for epidermal-dermal adherence (Briggeman 1975). Genetic defects in the type VII collagen gene, designated COL7A1 result in dystrophic EB (DEB, Uitto and Christiano, 1992, 1994).

Additional information may be found at:

http://www.clinicaltrials.gov/ct2/show/NCT00478244?term=%22Epidermolysis+Bullosa%22&rank=9

Proof of Principle

To assess the beneficial effects of cellular therapy for RDEB, we infused hematopoietic and nonhematopoietic stem cell populations into unconditioned RDEB (Col7a1-/-) mice. The animal was generated by replacing exons 46-69 with antibiotic resistant genes, leading to a truncated messenger with lack of a functional collagen type VII protein in the mice. The null knockout mice show all the hallmarks of the disease--extensive blistering, fusion of digits, sublamina densa detachment from underlying dermis, absent of anchoring fibrils and lack of immunostaining of the collagen VII at the BMZ (Heinonen et al., 1999). Affected mice uniformally die within two weeks of birth.

In utero injections of multipotent adult progenitor cells, mesenchymal stem cells, epidermal stem cells, or unmanipulated bone marrow (BM), failed to prevent disease in more than 200 animals with all dying in less than two weeks. Thirteen mice receiving SLAM family receptor positive ($CD150^+/CD48^-$) BM cells. Three (23%) mice were born with evidence of healing blisters and survived for more than 2 months. Surviving animals had evidence of donor cells in the integument with evidence of collagen type VII production and presence of anchoring fibrils in the BMZ. Collectively, these data demonstrate proof-of-principle of that transfer of marrow could provide functional and clinical correction.

Study Design

This is a open label, single institution, non-randomized phase II study to determine the incidence of detectable donor derived collagen type VII by day 100 after busulfan, cyclophosphamide and fludarabine followed by transplantation of allogeneic HSC from a healthy related donor in patients with EB.

Primary Objective

To determine the incidence of detectable donor derived collagen type VII by day 100 in children with Epidermolysis Bullosa (EB) treated with busulfan (BU), cyclophosphamide (CY) and fludarabine (FLU) and infusion of whole, unfiltered marrow.

Questions and Answers:

1. How long did it take to generate the preclinical data to support the trial and obtain regulatory approvals for opening the study for subject enrollment?

   Three years: 7/2004-7/2007. All aspects of the preclinical and clinical trial work were accomplished by 4 people. Steps included identification of an appropriate animal model and funding source for preclinical studies, identification of experts in DEB, writing the clinical trial, moving trial through the different regulatory agencies, ethics discussions, securing approval from third party payors, identification of care team and scenario planning.

   The principal obstacles were 1) PI time to complete the protocol, 2) identification of care team, 3) identification of funds for preclinical animal studies, and 4) insurance approval.

2. Were there any delays in getting the study approved by the SCRO or IRB? Any issues that we should be alert to in future similar innovative interventions.

   No. Strategy: early discussions with IRB and Ethics Committee.

3. Was this supported with extramural funding? Would funding from an organization like CIRM have facilitated or accelerated the work?

   Family raised funds on small scale. Total: $45,000. Discretionary University funds were used by investigators. Total: ~$150,000 (excluding salary support for faculty and technicians).

   Non project specific support for developmental therapeutics would have greatly enhanced the speed of getting the trial started. Further, it would be enabling the development of the next generation of trials (no additional work has been done since the original proof-of-principle work). There needs to be sufficient flexibility in how funds may be utilized. Here are some observations for consideration:

   - Research grants may not have sufficient flexibility. Skin disease in particular is a challenge for funding.
   - EB itself does not impact a lot of people; frequently funding is directed in a more utilitarian manner towards outcomes impacting more individuals.

     Early-stage trials represent an opportunity to provide proof of concept,eg, use of stem cell populations for treatment of severe skin disorders (not just EB).

4. What are potential necessary next-steps for this research that apply to cellular therapies in general, and are there any regulatory considerations?

   We need to figure out how to enhance safety and efficacy. For example, it would be valuable to identify the specific cell population responsible for the outcome. Also, we need to know how to reduce the allogeneic risks, eg. GVHD, infection. Also, can we

eliminate potentially dangerous cell populations, eg. T cells, ABO mismatched red cells.

One regulatory note, highly manipulated cell populations that are isolated and expanded or populations derived from hESC may trigger SCRO approval or trigger FDA rules. In this trial, no unique FDA issues came up because it involved an approved protocol for bone marrow transplantation.

5. One policy issue is to distinguish this type of rapid translation of basic science to the clinic where patients pay to receive "stem cell transplants" but the protocol is not specified and outcomes are not published. Any suggestions on how to draw the line?

   Trials, results and resulting publications need to be placed in the public domain. There should be a registry requirement with some authority. In CIRMs case, make future funding contingent on updates, publications and attainment of predetermined criteria or benchmarks.