Development of a Genetically-Modified Human Dermal Fibroblast for the Treatment of Recessive Dystrophic Epidermolysis Bullosa

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ABSTRACT and INTRODUCTION

Recessive dystrophic epidermolysis bullosa (RDEB) is an autosomal recessive, inherited skin disease caused by null mutations within the type VII collagen gene (*COL7A1*). The mutations cause an absence or reduction of functional collagen VII, which make up anchoring fibrils that maintain binding of the epidermis to the dermis **(Figure 1)**. The disease is characterized by a mechanical fragility and repeated blister formation in the sub-lamina densa, at the level of the structurally defective anchoring fibrils. Currently, there is no effective therapy for this disease, and death is usually the result of aggressive squamous cell carcinoma, sepsis, or malnutrition.

We are developing an autologous, genetically-modified fibroblast cell therapy that is anticipated to improve skin function in RDEB patients through restoration of collagen levels. A patient's fibroblasts will be harvested, genetically modified *ex-vivo* with a functional *COL7A1* gene, and expanded in culture (GM-HDF-COL7 or FCX-007). *Ex vivo* transduction will occur through the use of a replication-defective, self-inactivating (SIN) lentiviral vector **(Figure 2)**. After expansion, the fibroblasts are administered back to the patient as a local intradermal injection into target wound margins. The resulting increase in anchoring fibrils is anticipated to stabilize the connection between skin layers and reduce blistering tendency.

***In vitro* product development data indicates that cGMP scale GM-HDF-COL7 cells express full-length type VII collagen exhibiting the proper trimeric structure, size, and binding functionality (Figures 3-7)**. A hybrid in *vitro/in vivo* pharmacology/toxicology study using an organ culture/SCID mouse model is underway at Stanford University to confirm type VII collagen persistence, distribution, localization and toxicology.

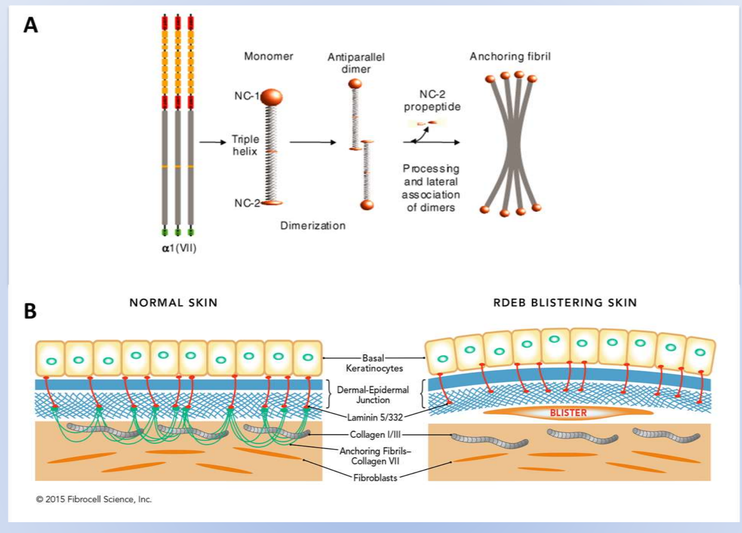

Figure 1
A. COL7 trimers form anchoring fibrils: The COL7A1 gene encodes a 290-kDa alpha chain and three of the chains form a triple helix (trimer). Image from Bruckner-Tuderman L. *Molecular Therapy* (2008) 17:6–7.
B. General structure of normal and RDEB skin: COL7 anchoring fibrils bind to other collagens, extracellular matrix proteins, and Lam332 to mediate attachment of the dermis to the epidermis. Absence of anchoring fibrils can lead to blister formation.

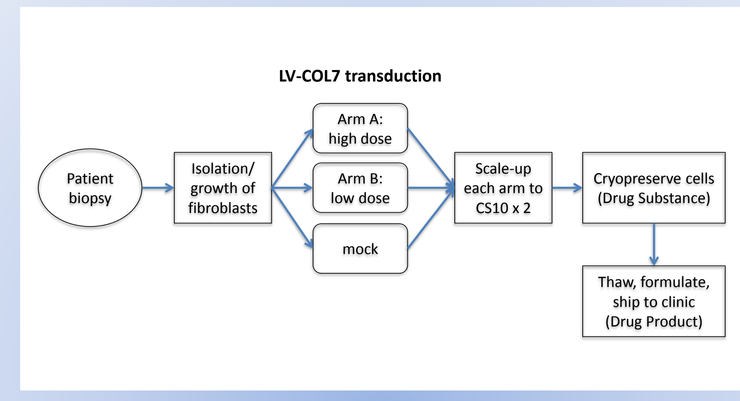

Figure 2
cGMP-scale GM-HDF production process: A COL7 expression cassette was cloned into a self-inactivating lentivirus backbone. A pilot-scale production of LV-COL7 with a titer of **~1E+07 IU/mL** was generated for use in the cGMP-scale production process. Fibroblasts were isolated from RDEB patient biopsies, grown, then split into three arms for mock, high-, and low-dose LV-COL7 transduction. Each arm of fibroblasts was grown to 2 x CS10 scale then cryopreserved (Drug Substance). For patient treatment, Drug Substance vials are thawed and formulated (Drug Product), then shipped back to the clinic for wound-site injection of the originating patient.

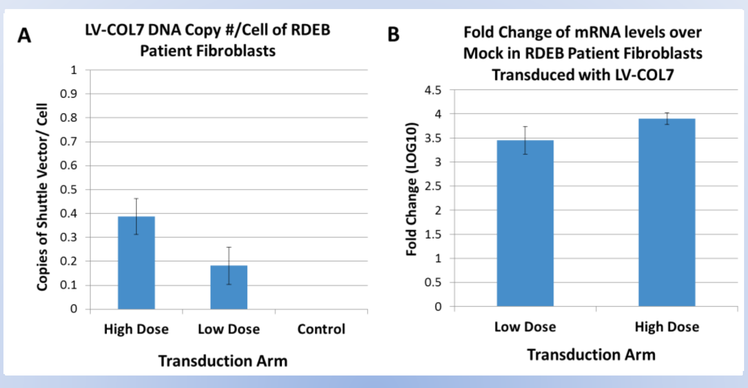

Figure 3
A. LV-COL7 copy number per cell: Drug Substance vials were thawed and assayed for LV-COL7 DNA copies per cell using qPCR. Primers were specific for the LV shuttle vector. Results demonstrate dose-dependent levels of copies per cell with an average of 0.38 and 0.18 copies from the High and Low Dose arms, respectively.
B. Cellular LV-COL7 mRNA levels compared to mock-transduced fibroblasts: Cells from Drug Substance vials were also assayed for COL7 transgene mRNA levels. Fold-change in mRNA expression compared to mock-transduced cells in Log10 scale is shown. Results demonstrate dose-dependent levels of COL7 mRNA with ~3000 and ~7000-fold more COL7 mRNA than mock-transduced cells in the low and high dose conditions, respectively.

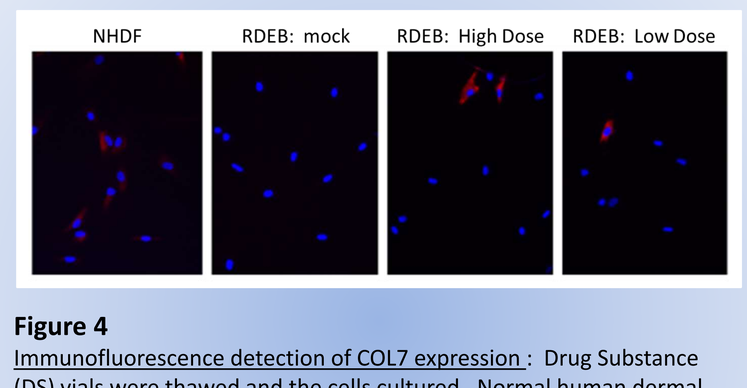

Figure 4
Immunofluorescence detection of COL7 expression : Drug Substance (DS) vials were thawed and the cells cultured. Normal human dermal fibroblasts (NHDFs) were also cultured. NHDFs and RDEB cells from each DS transduction group were fixed, permeabilized, and stained with DAPI to visualize nuclei (blue) and fNC1 antibody to COL7 (Stanford, 1.25 ug/mL) plus AlexaFluor 555-congugated goat anti-rabbit IgG to visualize COL7 expression (red). Images were acquired at 20X magnification using an exposure time of 290 ms. COL7 is detected on NHDFs, and High and Low Dose LV-COL7-transduced RDEB cell groups, but not mock-transduced RDEB cells.

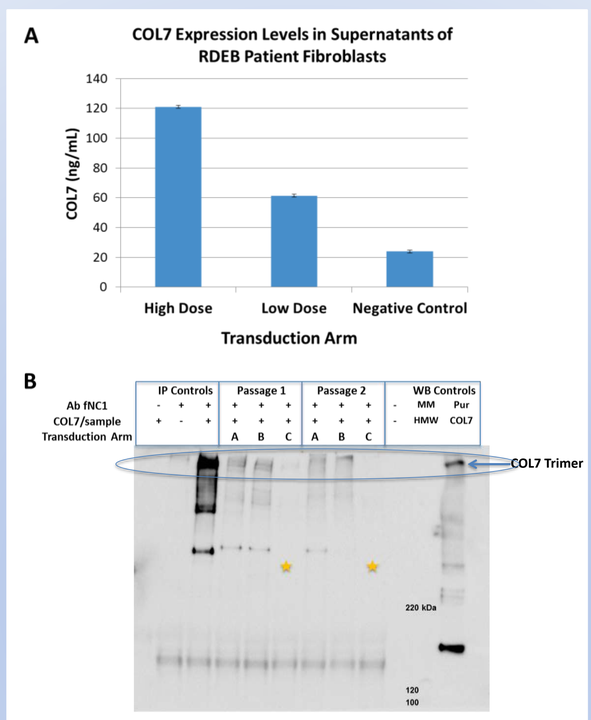

Figure 5
A. COL7 expression levels produced by RDEB patient fibroblasts transduced with LV-COL7 : Drug Substance vials were thawed and cultured for 3 days. Conditioned cell culture supernatants were collected and assayed for COL7 levels by ELISA. Results show virus dose-dependent protein expression that ranges from 60 to 120 ng/mL COL7 in LV-COL7-transduced cells.
B. Trimeric form of COL7 produced by RDEB patient fibroblasts transduced with LV-COL7: Conditioned cell culture supernatants were also used in an immunoprecipitation assay. Immunoprecipitated COL7 was separated on non-denaturing SDS-PAGE and visualized by western blot. The COL7 produced by RDEB fibroblasts was predominantly trimeric with LV-COL7-transduced cells expressing more COL7 than mock-transduced cells (Arm C ⭐) . Some lower molecular weight species showing immunoreactivity, were also observed.

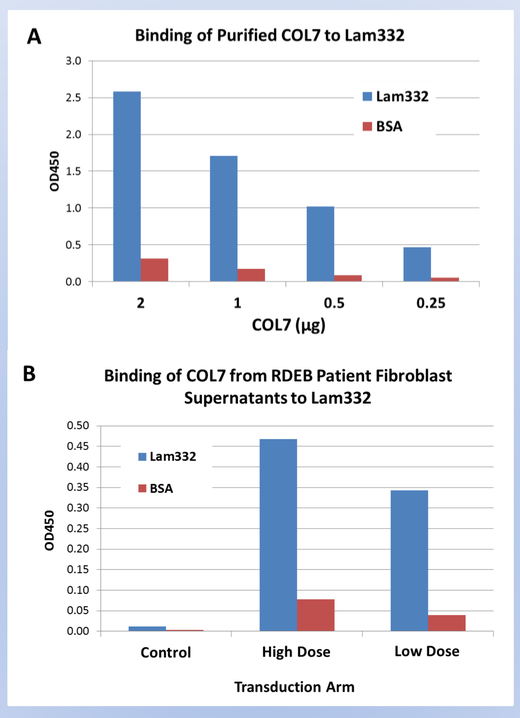

Figure 6
A. Binding of purified COL7 to Lam332: COL7 was expressed in CHO-DG44 cells, was purified by size exclusion chromatography, and was assayed for preferential binding to Laminin332 as compared to BSA to establish the assay (increase in OD450 corresponds to increase in COL7 binding to Laminin332).
B. Lam332 binding of COL7 in LV-COL7-transduced fibroblasts culture supernatants: Drug Substance vials were thawed and cultured for 2 days. Conditioned cell culture supernatants were collected and assayed for binding to Lam332 compared with BSA control. Results show virus dose-dependent binding to Lam332.

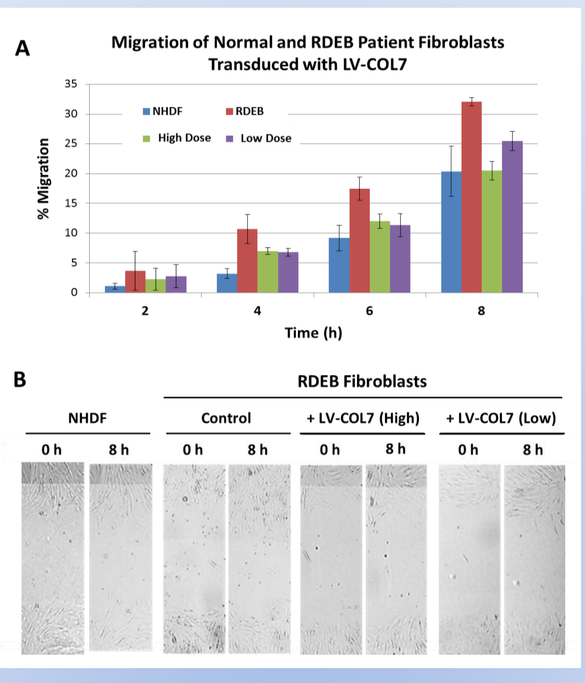

Figure 7
Migration of LV-COL7 transduced RDEB Patient fibroblasts: NHDFs and cells from Drug Substance vials were thawed and cultured. Cells were seeded into culture dishes with an insert to prevent cell adherence in a small strip of the culture dish. The strip was then removed and the rate at which the cells migrated into the open area was monitored by microscopy and quantified using the irregular shape delineation plugin for ImageJ software. Percent migration (**A**) and images of migration (**B**) are shown. The results shows that the mock-transduced RDEB patient fibroblasts migrate into the open area faster than NHDF, and transduction with LV-COL7 reverts the patient cells to a rate of migration similar to NHDFs. These results are consistent with those described by Chen, M. *et al*, *J. Biol. Chem.* (2000) 275:24429-35.

SUMMARY

- Production of LV-COL7 was successful resulting in an infectious titer of 9E+06 IU/mL
- GM-HDF cells were successfully produced by expanding LV-COL7-transduced RDEB patient fibroblasts from a biopsy sample
- The integrated transgene copy number per cell was dependent on the virus dose averaging 0.38 and 0.18 transgene copies per cell for high and low dose, respectively
- The COL7 expression from the GM-HDF cells was confirmed by qRT-PCR, immunofluorescence staining, and ELISA
- The structure of the COL7 expressed by the GM-HDF cells was confirmed to be predominantly trimeric by immunoprecipitation/SDS-PAGE/Western blot analysis
- The COL7 produced from the GM-HDF cells was demonstrated to be functional by binding to Laminin332 in an *in vitro* binding assay and by correction of the hypermotility phenotype of RDEB cells in an *in vitro* migration assay
- 2- and 6-week toxicology results using GM-HDF cells in a human skin graft model demonstrated no findings

Acknowledgments

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Potential Conflicts

VKD, MC, AE, and SK have financial interest in Intrexon stock. JM is the Vice President of Scientific Affairs has financial interest in Fibrocell Science stock and owns XON stock. SK is Senior Vice President with Intrexon. MPM is a paid consultant for Intrexon and Fibrocell Science.