Pre-Clinical Development of a Genetically-Modified Human Dermal Fibroblast (FCX-007) for the Treatment of Recessive Dystrophic Epidermolysis Bullosa

M.P. Marinkovich[1], MD, N. Ehsani-Chimeh, MD[1], N. Nguyen[1], S. Moncrief, PhD[2], V.K. Dailey[2], M. Chakiath[2], A. Elayadi, PhD[2], S. Krishnan, MS[2] and J. Maslowski, MS[3]

[1]Stanford University School of Medicine, Stanford, CA, United States; [2]Intrexon Corporation, Germantown, MD, United States; [3]Fibrocell Science, Exton, PA, United States

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) **(Figure 2)**. *Ex vivo* transduction will occur through the use of a replication-defective, self-inactivating (SIN) lentiviral vector. 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, 4)**. We present results from a pre-clinical animal model evaluating FCX-007 in RDEB and normal human skin xenografts implanted onto immunodeficient SCID mice **(Figure 5)**. The goals of the study are to confirm persistence, distribution and localization of COL7, and to evaluate any potential for product toxicity or vector biodistribution **(Table 1)**. The data presented were used to support an Investigational New Drug (IND) filing.

We also present the study design for a proposed Phase I/II clinical trial to treat RDEB subjects with FCX-007 **(Table 2)**. The primary endpoints for the study are to evaluate safety and to confirm the formation of anchoring fibrils at the basement membrane zone (BMZ) in biopsies collected from subjects after product administration.

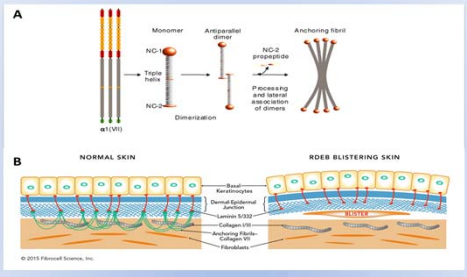

Figure 1

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.

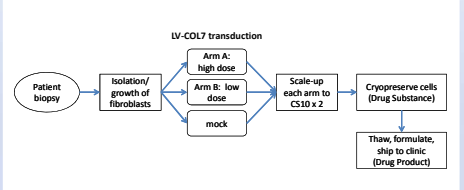

Figure 2

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 **~9 x 10^6 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.

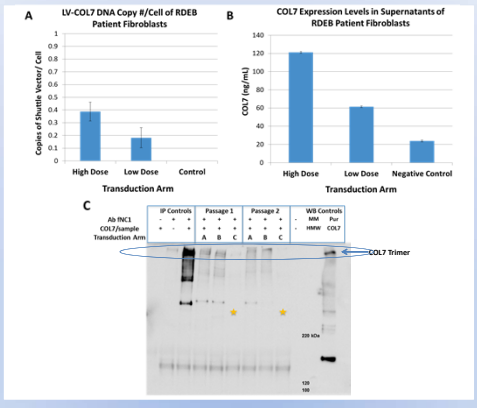

Figure 3

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. 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.

C. 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.

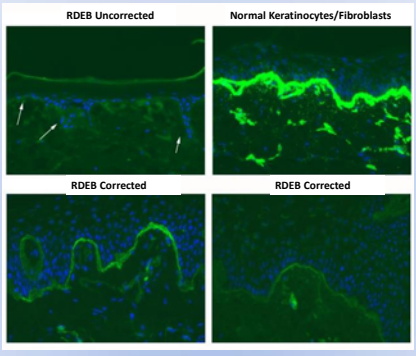

Figure 5

Composite RDEB skin grafts on the dorsum of SCID mice were injected intradermally with 1 x 10^6 GM-HDF and analyzed by immunofluorescent staining with human COL7 specific antibodies. Representative images shown. Localization of COL7 was observed in composite grafts (n=4) generated with RDEB keratinocytes Day 10-post intradermal injection of 1 x 10^6 GM-HDF. Positive control grafts generated from normal keratinocytes and fibroblasts showed intense COL7 staining and negative control grafts did not show COL7 staining at baseline measurements (arrows at DEJ for negative baseline comparison).

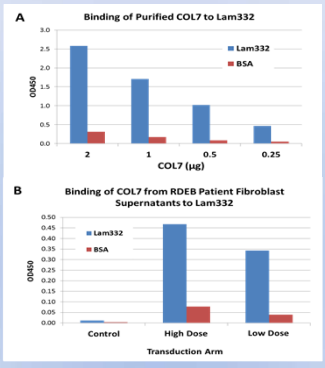

Figure 4

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 Lam332 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 3 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.

Table 1: Toxicology/BioDistribution Results Summary

Title	**Evaluation of Genetically Modified Human Dermal Fibroblasts (GM-HDF) in Normal and RDEB Human Skin Xenograft SCID Mouse Models**
Methods	Grafts of normal human skin on the dorsum of NOD/SCID mice were injected intradermally with low, mid and high doses of GM-HDF and assessed for clinical pathology, vector biodistribution and pathology on Day 15 and Day 43.
Results	No test article-related gross findings were noted. No test article-related microscopic findings were noted. There were no tumors noted in any organs or tissues examined, including skin grafts. There were no statistical differences noted for test article groups in hematology. There was a statistically significant decrease in urea nitrogen for female GM-HDF high dose and RDEB HDF groups vs vehicle control. There were no other statistically significant findings for clinical chemistry. There was no apparent systemic distribution of the vector.
Conclusions	A single intradermal administration of RDEB-HDF or RDEB GM-HDF to SCID mice with skin grafts at cellular concentrations of 0x10^6, 1x10^6, 2.5x10^6, or 5x10^6 cells/injection resulted in no test article-related findings after 15 or 43 days.

Table 2: Study Design for Phase I/II Clinical Trial

Title	**A Phase I/II Trial of FCX-007 (Genetically-Modified Autologous Human Dermal Fibroblasts) for Recessive Dystrophic Epidermolysis Bullosa (RDEB)**
Statement of Purpose	The purpose of this study is threefold: 1) To evaluate the safety of FCX-007 2) To evaluate COL7 expression and the presence of anchoring fibrils resulting from FCX-007 3) To analyze wound healing as a result of FCX-007 administration
Objectives	Primary: 1) The primary objective of this protocol is to evaluate the safety of FCX-007 Secondary: 1) To evaluate mechanism of action of FCX-007 at weeks 4,12, 25, 52, and unscheduled visits through the evaluation of skin biopsies for COL7 expression and the presence of anchoring fibrils 2) To evaluate the efficacy of FCX-007 through an intra-subject paired analysis of target wound area at weeks 4, 8, 12, 25, 52, and unscheduled visits, comparing FCX-007 treated wounds to untreated wounds in Phase I and to wounds administered sterile saline in Phase II through the evaluation of digital imaging of wounds
# of Subjects	Nine (three adults in Phase I followed by six pediatrics in Phase II)

SUMMARY

- Production of LV-COL7 was successful resulting in an infectious titer of ~9 x 10^6 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
- The COL7 expression from the GM-HDF cells was confirmed by ELISA and Western Blot as well as qRT-PCR and immunofluorescence staining (not shown)
- 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 as well as by correction of the hypermotility phenotype of RDEB cells in an *in vitro* migration assay (not shown)
- 2- and 6-week toxicology results using GM-HDF cells in a human skin graft model demonstrated no findings
- GM-HDF cells in a human skin xenograft model expressed COL7 that localized to the BMZ

Acknowledgments

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Potential Conflicts

SM, 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 and NE-C are paid consultants for Intrexon and Fibrocell Science.