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TITLE: **Translating a Stem Cell-Based Therapy for Epidermolysis Bullosa into the Clinic**

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CONTRACTING ORGANIZATION: **University of Colorado Denver**

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This proposal is developing a stem-cell based therapy for recessive dystrophic epidermolysis bullosa (RDEB), which is one of the most severe forms of epidermolysis bullosa (EB), a group of rare inherited skin blistering diseases. To accomplish this goal, we are utilizing genetic correction of patient-specific induced pluripotent stem cells (iPSCs) followed by the differentiation of these corrected iPSCs into epidermal cells and fibroblasts for the generation of composite full thickness skin grafts for transplantation. During this funding period, we completed our studies on reproducibility of our simultaneous gene editing and reprogramming protocol for the generation of gene corrected RDEB iPSCs. We successfully generated gene corrected RDEB iPSCs from three patients and completed characterization of these iPSC lines on a molecule level. We initiated functional validation of keratinocytes derived from these genetically corrected RDEB iPSCs. In our preliminary analysis, keratinocytes derived from gene corrected RDEB iPSCs show the restoration of the expression of type VII collagen and the formation of a basement membrane in 3D skin equivalents. We also continue working with our cGMP-compliant facility to advance our protocols toward clinical manufacturing. As indicated in the previous report, the initiation of many *in vivo* studies was delayed due to the COVID-19 pandemic and the closure of research laboratories on the University of Colorado Anschutz Medical Campus. While the University allowed research activities to resume on July 1, research personnel are required to work on a staggered basis, with no more than 50% occupancy at any given time to maintain social distancing. These restrictions continue to impact our research progress. The potential delay caused by these restrictions will be mitigated by requesting a no cost extension before the completion of Year 3 of the project.

15. SUBJECT TERMS
Epidermolysis Bullosa (EB), Recessive Dystrophic EB (RDEB), Induced Pluripotent Stem Cells (iPSC)

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TABLE OF CONTENTS

1. INTRODUCTION:

This application is advancing our previously developed induced pluripotent stem cell (iPSC)-based therapy for recessive dystrophic epidermolysis bullosa (RDEB) into the clinic by adapting the production of genetically corrected patient-specific iPSC-derived epidermal sheets and composite full-thickness skin grafts to current Good Manufacturing Practice (cGMP) standards and by generating a set of preclinical data for submission of an Investigational New Drug (IND) application. Therefore, this application directly addresses the FY17 PRMRP Topic Area "**Epidermolysis Bullosa**". This application is also relevant to one of the Areas of Encouragement identified by the Department of Defense (DoD), the Department of Veterans Affairs (VA), and other relevant stakeholders (as indicated in Appendix 2 of the Program Announcement) since, if successful, the study will result in the approval of a Phase I Clinical trial for a product that may enhance wound healing in inherited epidermolysis bullosa (EB). There are significant procedural differences when a cell therapy product is manufactured under general laboratory settings vs. when the same product is manufactured under cGMP-compliant conditions in a cGMP facility. We are currently transferring the technologies that we have developed using our previous awards from the federal government and private foundations to a product development laboratory at a cGMP-compliant facility, the Gates Biomanufacturing Facility (GBF). The GBF will also perform a pilot small-scale cGMP production run of genetically corrected RDEB iPSCs and epidermal progenitors derived from genetically corrected RDEB iPSCs. In addition, we will develop a composite skin graft consisting of both genetically corrected iPSC-derived keratinocytes and fibroblasts as an alternative to genetically corrected iPSC-derived epidermal sheets for the treatment of RDEB. If successful and proven to be safe in a clinical trial for EB, the iPSC-based therapy could then be easily expanded to monogenic diseases affecting internal organs, where the difficulty in monitoring adverse effects of an iPSC-based therapy would make them unlikely first targets. The iPSC-based therapy may potentially be applied to military personnel who develop severe blistering following exposure to vesicants, or who suffer from burns over a large portion of their body. In addition, stem-cell based therapies could also be used to accelerate wound repair in military personnel who experience acute injuries, or in older veterans with chronic wounds.

2. KEYWORDS:

Epidermolysis Bullosa (EB); Recessive Dystrophic EB (RDEB), Induced Pluripotent Stem Cells (iPSC); stem cell-based therapy; current Good Manufacturing Practice (cGMP) standards.

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1: To perform a pilot cGMP-compliant production of genetically corrected RDEB iPSCs.

Task 1.1 To verify the reproducibility of simultaneous reprogramming and gene editing on cells isolated from three independent patients sharing the *COL7A1*$^{c.7485+G>A}$ mutation (3 human subjects)	**100% completed**
Task 1.2 To validate the absence of off-target events post-correction	**50% completed**
Task 1.3 To validate custom-made antibodies to quantify the level of WT Col 7 post-*COL7A1*$^{c.7485+G>A}$ correction	**100% completed**
Task 1.4 To examine the functionality of corrected Col7 in RDEB iPSCs (18 mice for teratoma and 48 mice for xenotransplantation)	**40% completed**
Task 1.5 To transfer the protocols to a product development laboratory at a cGMP-compliant facility, perform optimizations and prepare documentation/batch record	**10% completed**
Task 1.6 To implement a pilot small-scale cGMP-compliant run of the protocol for the generation of a MCB of genetically corrected iPSCs at a cGMP-compliant facility	Not initiated
Milestone(s) Achieved: Successful pilot cGMP production of genetically corrected RDEB iPSCs	Expected: 20 months after project initiation
Local IRB/IACUC Approval	**Completed**
Milestone Achieved: HRPO/ACURO Approval	**Completed**

Aim 2: To perform a pilot cGMP-compliant production of epidermal progenitors from genetically corrected iPSCs.

Task 2.1 To confirm reproducibility of keratinocyte differentiation protocol on iPSCs with the corrected *COL7A1*$^{c.7485+G>A}$ mutation (3 human subjects)	**70% completed**
Task 2.2 To transfer the protocol for the generation of epidermal progenitors to a product development laboratory at a cGMP-compliant facility, perform optimizations and prepare documentation/batch record	**10% completed**
Task 2.3 To implement a pilot small-scale cGMP-compliant run of the protocol for the generation and characterization of genetically corrected iPSC-derived epidermal progenitors at a cGMP-compliant facility	Not initiated
Milestone(s) Achieved: Successful pilot cGMP production of genetically corrected iPSC-derived epidermal progenitors	Expected: 19 months after project initiation

Aim 3: To develop a cGMP-compatible protocol for the generation of a composite skin graft and to generate IND-enabling safety and efficacy data for the FDA

Task 3.1 To optimize a protocol for the differentiation of iPSCs into a fibroblast lineage	**100% completed**
Task 3.2 To examine the functionality of iPSC-derived fibroblasts (12 mice for xenotransplantation)	**20% completed**
Task 3.3 To develop a cGMP-compatible protocol for the differentiation of *COL7A1*$^{c.7485+G>A}$ corrected iPSCs into fibroblasts	**30% completed**
Task 3.4 To generate a composite graft using genetically corrected iPSC-derived keratinocytes and fibroblasts in organotypic cultures and verify type VII collagen deposition	**20% completed**
Task 3.5 To assess wound closure by iPSC-derived composite grafts in immunocompromised mice (180 mice per site)	**20% completed**
Task 3.6 To assess tumorigenecity and the presence of residual iPSCs in the composite grafts (288 mice per site)	Not initiated
Milestone(s) Achieved: Generation of Pre-IND-enabling safety and efficacy data for composite grafts using cGMP-compatible protocols	Expected: 36 months after project initiation

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Task 1.1 To verify the reproducibility of simultaneous reprogramming and gene editing on cells isolated from three independent patients sharing the COL7A1$^{c.7485+G>A}$ *mutation (3 human subjects).*

During this funding period, we completed characterization of gene corrected RDEB iPSC clones from three patient fibroblast lines carrying the c.7485+5G>A mutation. Please, see previous semi-annual technical progress report for the description of our combined Cas9-mediated gene correction and reprogramming procedure. **Table 1** summarizes our results, including the status of gene correction, genomic stability (karyotyping) and pluripotency. The efficiency of correction varied between 2 and 5% across experiments without any significant differences between unmodified ssODN and ssODN carrying silent SNPs.

Several tests were performed to validate the successful correction of iPSCs and characterize pluripotency. These tests will also be implemented during the cGMP-compatible run. Specifically,

(1) Upon appearance of iPSC colonies during reprogramming, about ~100 colonies are picked into 48 wells and all re-grown colonies are characterized by droplet digital PCR to quantify COL7A1 correction of the patient mutation via a reference probe that displays a biallelic locus. In our ddPCR assay, the hydrolysis probe indicated in green detects the c.7485+5G>A mutation, while the probe indicated in blue detects the corrected sequence. **Fig. 1** shows an example of the ddPCR analysis of multiple iPSC clones picked at the end of our combined gene editing and reprogramming procedure performed on homozygous c.7485+5G>A RDEB fibroblasts. In this example, among 96 analyzed clones, 6 clones show the correction of one allele, as indicated by the appearance of a blue signal at approx. 50% intensity of the green (mutant) probe.

Table 1: Successful generation of gene corrected RDEB iPSC clones from three patient fibroblast lines carrying the c.7485+5G>A mutation

iPSC line	COL7A1 status	Allele 1	Allele 2	Karyotyping	Pluripotency
CO1-8	RDEB (control)	c.7485+5G>A	c.7485+5G>A		
CO1-60	corrected	corrected	corrected	normal	+
CO2-18	RDEB (control)	c.7485+5G>A	c.7485+5G>A		
CO2-6	corrected	corrected	c.7485+5G>A	normal	+
CO4-219	RDEB (control)	c.7485+5G>A	c.4621delG		
CO4-194	corrected	corrected	c.4621delG	normal	+

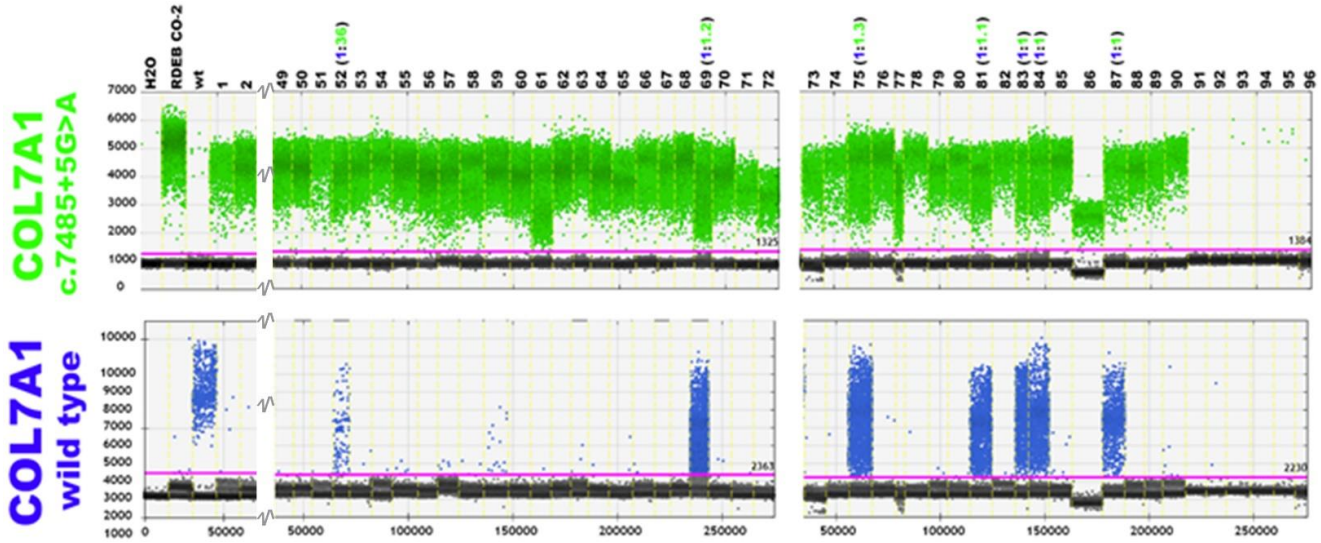

Fig. 1: Analysis of iPSC colonies derived during combined gene editing and reprogramming of homozygous c.7485+5G>A RDEB-CO2 fibroblasts. After combined reprogramming and gene editing, colonies were manually isolated, clonally expanded, and screened by ddPCR. The hydrolysis probe indicated in green detects the c.7485+5G>A mutation, while the probe indicated in blue detects the corrected sequence. Among 96 analyzed clones, 6 show the correction of one allele based on the appearance of a signal from the blue wt probe at 50% intensity of the signal from the green mutant probe.

(2) Colonies positive by ddPCR (i.e. range of corrected allele frequency 0.4-0.6 in comparison with the biallelic reference probe) are further expanded and the remaining colonies are discarded. Expanded ddPCR+ colonies are subject to in-depth characterization of the COL7A1 locus by PCR amplification, Sanger sequencing of the PCR product, Topo-cloning and Sanger sequencing of 100 individual PCR amplified loci. Those iPSC lines whose genome yields the expected size PCR product of the amplified edited COL7A1 locus, and whose Sanger sequencing of the edited locus and the unedited locus (i.e. the other COL7A1 allele) shows the expected sequence in 95 out of 100 isolated alleles (i.e. Topo clones) will be used for differentiation into keratinocytes and fibroblasts.

(3) The generated iPSCs are then characterized by immunostaining for TRA 1-60, Oct 4 and Nanog and by G-band karyotyping (see the previous report for representative images). The later analysis is especially critical due to known instability of iPSCs.

One corrected clone from our experiment (CO2-6) was shared with the groups at Stanford and Columbia Universities for characterization and differentiation studies as outlined in our original application in Aim 2.

Task 1.2 *To validate the absence of off-target events post-correction.* During our previous reporting period, using the CRISPOR tool: http://crispor.tefor.net/crispor.py?batchId=ds81mrGkbMYHHDMdtiFY, we identified a set of genomic loci that can potentially be targeted by our Cas9-mediated strategy due to minimal mismatches between our gRNA sequence and the potential target sequences in these loci. During current reporting period, we characterized all three corrected clones (CO1-60, CO2-6 and CO4-194) and did not find any mutations in any of the top 5 predicted off-target sites using Sanger Sequencing (see example in **Fig. 2**).

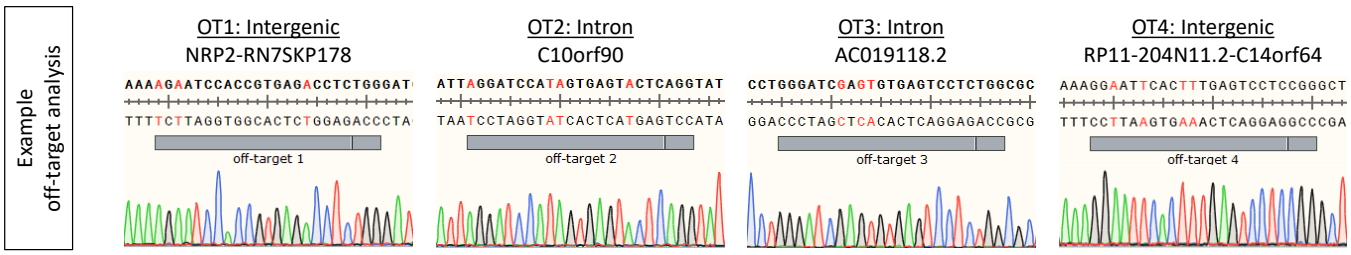

Fig. 2. No off-target events were identified in gene corrected RDEB iPSCs. Cas9 off-target sites were identified using the CRISPOR design tool. Primers were designed to amplify the top 4 off-target sites for interrogation by Sanger sequencing. No mutations were detected at any off-target sites. Sequencing results from the top 4 sites of the corrected CO2-6 are shown.

We have also initiated an *in vitro* strategy to further analyze potential off-target events of our Cas9-mediated gene correction strategy using CIRCLE Sequencing (CIRCLE-Seq) as described in the previous report. We are also growing cells to perform Whole-Genome Sequencing to further validate the absence of any off-target events resulting from gene editing and reprogramming.

Task 1.3 *To validate custom-made antibodies to quantify the level of WT Col 7 post-COL7A1$^{c.7485+G>A}$ correction.* During this funding period, we further validated the antibody on keratinocytes isolated from three RDEB patients with the *COL7A1$^{c.7485+G>A}$* mutation. We confirmed that we can use a previously identified commercial antibody to detect the restoration of Col7 expression in our genetically corrected RDEB cells.

Task 1.4 *To examine the functionality of corrected Col7 in RDEB iPSCs (18 mice for teratoma and 48 mice for xenotransplantation)*

We have injected gene corrected iPSCs into immunocompromised mice for teratoma analysis as described in the original application. These results are pending. Several grafts with differentiated iPSCs have also been performed. The results are also pending. The initiation of these *in vivo* studies was delayed due to the COVID-19 pandemic and the closure of research laboratories on the University of Colorado Anschutz Medical Campus.

Task 1.5 *To transfer the protocols to a product development laboratory at a cGMP-compliant facility, perform optimizations and prepare documentation/batch record*. We continue working with the Gates Biomanufacturing Facility (GBF), to adapt our reprogramming and gene editing protocols to cGMP standards. The completion of this task was slightly delayed due to the Covid19 pandemic. As one of the steps, we developed a Standard Operation Procedure (SOP) for the expansion of gene corrected iPSCs from a single colony to 10^8 cells sufficient to generate 5 x10^6 iPSC-derived keratinocytes. **Fig. 3** depicts schematics of the procedure and the timeline.

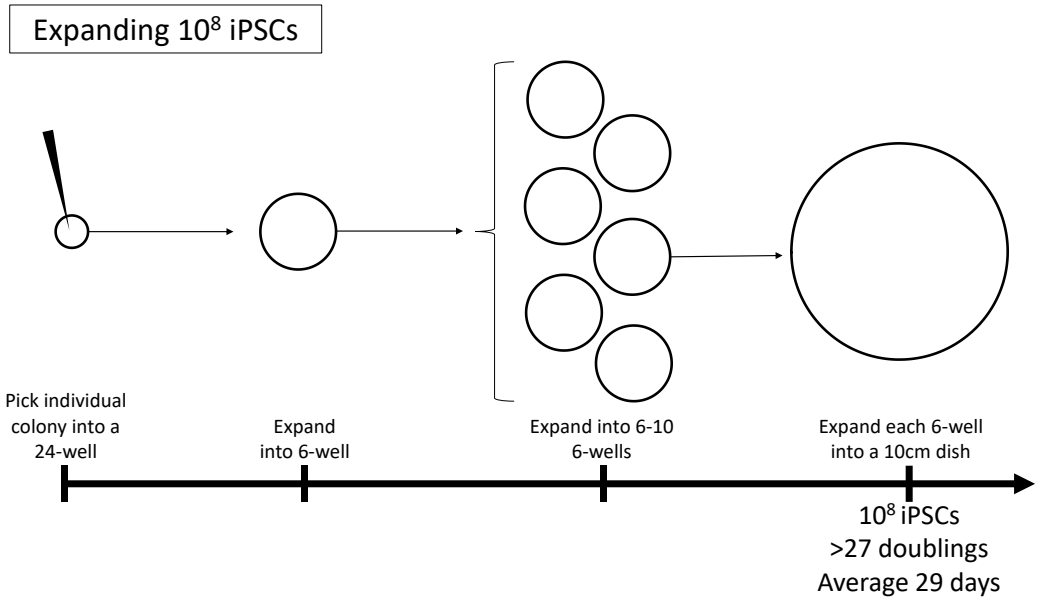

Fig. 3. Schematics of the expansion of gene corrected RDEB iPSCs to generate Process Intermediate #2 for clinical manufacturing of the iPSC-based therapy for EB.

Task 2.1 To confirm reproducibility of keratinocyte differentiation protocol on iPSCs with the corrected COL7A1$^{c.7485+G>A}$ mutation (3 human subjects).

We provided one of our corrected RDEB iPSC lines to Stanford and Columbia to validate the differentiation of these cells into a keratinocyte lineage as proposed in the original application. All three groups have successfully differentiated gene corrected RDEB iPSCs into keratinocytes in multiple differentiation experiments and initiated the validation studies to confirm the restoration of the Col7 expression and the formation of a basement membrane. See previous progress report for the data on Coupling Efficiency.

To assess the maturation process during the differentiation of gene corrected iPSCs into keratinocytes (iKCs), we performed immunofluorescence (IF) staining for keratin 14, keratin 18 and p63 at day 45 during differentiation. At day 45, the iKCs expressed increased levels of keratin 14 and p63 compared with days 7 and 21, and did not express keratin 18, representing features of mature normal human keratinocytes (**Fig. 4**)

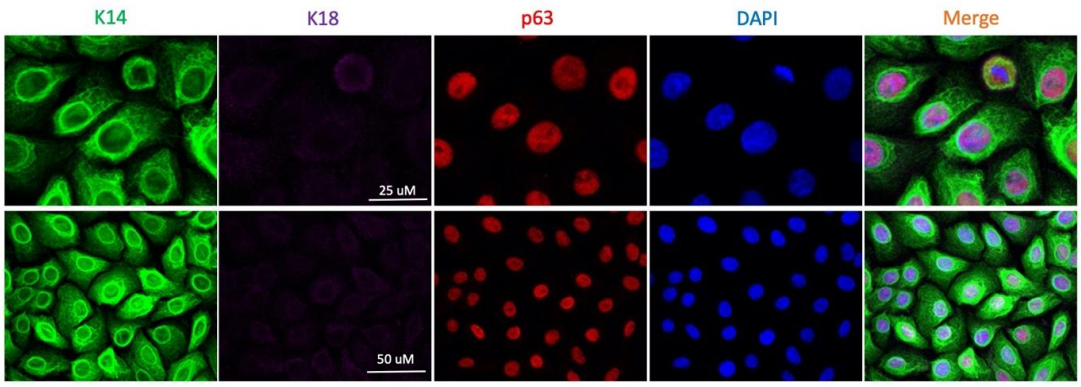

Fig. 4. K14 and p63 expression in iKCs derived from genetically corrected CO2-6 iPSC line. Genetically corrected RDEB IPSCs were differentiated into keratinocytes (iKCs) using 1μM retinoic acid and 5 ng/μL BMP4. At day 45, iKCs were harvested and enriched by selective binding to α-CD104 (integrin β4) magnetic beads. The cells were stained for K14 (green), K18 (purple), p63 (red) and DAPI (blue).

An important demonstration of the function of the iKCs is to show they can stratify and produce Type VII collagen (Col7). We performed multiple organotypic cultures of iKCs as previously published using devitalized dermis (Sebastiano et al. 2013). Indeed, the iKCs formed a stratified epidermis including a K14 positive basal layer, and stratified layers associated with expression of suprabasal differentiation markers K10 and loricrin. Importantly, Col7 was uniformly expressed at the basement membrane zone, consistent with wild type levels and assembly of Col7. This indicates that the COL7A1 gene was corrected and gene corrected RDEB iPSCs could be differentiated into a stratified epidermis in organotypic cultures (**Fig. 5**).

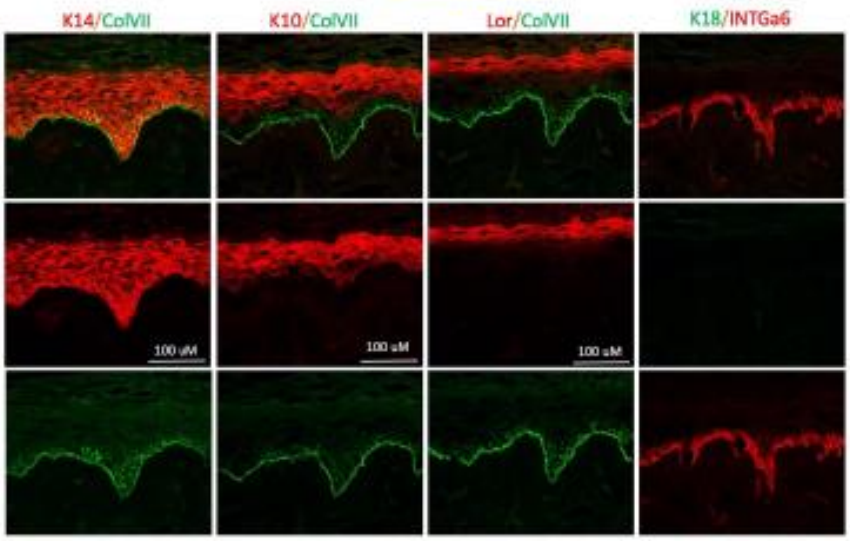

Fig. 5. Organotypic skin cultures of iKCs derived from the CO2-6 iPSC line. Markers Keratin 14 (K14), Keratin10 (K10), Loricrin (Lor), Keratin 18 (K18), and Integrin A6, (INTGA6) define the stratified epidermis in organotypic cultures. Wild type collagen VII was uniformly expressed at the basement membrane zone, consistent with wild type levels and assembly of Col7.

Our collaborators on this project have noticed that occasionally the generation of 3D skin fails. The main reasons for failure include uneven thickness of the devitalized dermis, infection, over-drying of the prep at the air-liquid level. To improve the consistency of the generation of 3D skin equivalents, we have replaced devitalized dermis with an FDA approved acellular dermal substitute, Alloderm. We are currently re-making 3D skin equivalents using Alloderm.

Task 2.2 To transfer the protocol for the generation of epidermal progenitors to a product development laboratory at a cGMP-compliant facility, perform optimizations and prepare documentation/batch record. We are working with the Gates Biomanufacturing Facility (GBF) to develop a protocol for the purification of authentic iPSC-derived keratinocytes based on CD104 (integrin β4) expression using the CliniMACS Prodigy. This aim was also slightly delayed due to the pandemic. We initially attempted to sort for integrin $\alpha6^{high}$ cells to enrich for keratinocyte stem cells and eliminate undifferented iPSCs, however, we discovered that iPSCs express integrin α6 as shown by sorting with anti-CD49f (**see Fig. 2 Annual Technical Report submitted 10-12-2019**). In contrast, integrin β4 is only expressed by keratinocytes, and thus it is a better cell surface marker for enrichment of iPSC-derived keratinocyte stem cells and elimination of undifferentiated iPSCs (**see Fig. 3 Annual Technical Report submitted 10-12-2019**). The GBF has initiated the optimization of the CliniMACS protocol. As the first step, a mixture of normal keratinocytes and undifferentiated iPSCs is being used at different ratios to validate the CD104 antibody conjugated to magnetic beads and the procedure. We have provided the GFP with appropriate iPSC lines and normal keratinocytes, and the GBF has already validated the appropriate antibody to be used in subsequent steps.

Task 3.1 To optimize a protocol for the differentiation of iPSCs into a fibroblast lineage. We have optimized the protocol for the differentiation of iPSCs into fibroblasts (**see our previous report**).

Task 3.2 To examine the functionality of iPSC-derived fibroblasts. We have initiated grafting experiments using wt iPSC-derived fibroblasts and are currently starting the differentiation of gene corrected RDEB fibroblasts.

Task 3.3 To develop a cGMP-compatible protocol for the differentiation of COL7A1$^{c.7485+G>A}$ corrected iPSCs into fibroblasts. We collected all certificates of analysis for the reagents used in our differentiation protocol and identified those reagents that are not cGMP compatible. We are currently replacing these reagents with their cGMP-compatible counterparts to make our protocol cGMP-compatible. We are currently validating these reagents during differentiation of wt iPSCs into fibroblasts. In addition, we are introducing changes to the protocol developed in 3.1. The original protocol uses embryoid bodies (EBs) to make iPSC-derived fibroblasts. However, the generation of EBs introduces an extra level of complexity to cGMP-compatible protocols. We are currently modifying our established protocols with the goal to develop a monolayer-based protocol and simplify the cGMP manufacturing of iPSC-derived fibroblasts.

Task 3.4 To generate a composite graft using genetically corrected iPSC-derived keratinocytes and fibroblasts in organotypic cultures and verify type VII collagen deposition. We are currently validating multiple matrices in addition to ALLODERM to generate 3D full thickness skin equivalents for transplantation as described in the previous report. Among matrices we are testing collagen, a combination of collagen I and fibrin, as well as collagen mesh.

Task 3.5 To assess wound closure by iPSC-derived composite grafts in immunocompromised mice (180 mice per site). We have generated several 3D skin equivalents using primary keratinocytes and fibroblasts and different matrices (ALLODERM, collagen and fibrin) and transplanted these equivalents onto immunodeficient mice to identify the best matrix for the generation of human skin *in vivo*. These experiments are currently ongoing. As indicated above, the *in vivo* studies were delayed due to the COVID19 –related closure of the University of Colorado.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue characterizing keratinocytes derived from genetically corrected RDEB iPSCs, including the off-target analysis. We will further optimize our protocol for the generation of iPSC-derived fibroblasts to make it cGMP-compatible. We will also continue working with our cGMP-compliant facility to perform pilot cGMP-compliant manufacturing of genetically corrected iPSCs and iPSC-derived keratinocytes.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We anticipate that our project will develop a stem-cell based therapy for the treatment of epidermolysis bullosa (EB), a group of rare inherited skin blistering diseases. EB derives from genetic mutations in structural proteins of the skin and sentences those afflicted to a life of severe pain and disability due to constant blistering and scarring. The development of a stem-based therapy is a complex process that needs to be reproducible and performed under clinically relevant standards. During this funding period, we have completed the validation of the reproducibility of our simultaneous gene editing and reprogramming protocol that allows us to generate genetically corrected EB skin cells. We have also completed the characterization of these gene corrected iPSCs. We are currently validating the functionality of our genetically corrected EB skin cells. These accomplishments will bring us a step closer toward approval for a clinical trial to treat EB.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Stem cell-based strategies similar to the one proposed in this application, whereby patient cells are genetically corrected and reprogrammed into immature induced pluripotent stem cells (iPSCs) that can be subsequently differentitated into target cell types for transplantation, can be applied to virtially any other currently incurable monogenic disease, including cystic fibrosis, Fanconi anemia, beta thalassemia, etc. However, unlike other monogenic diseases, EB, and especially RDEB, may represent an ideal platform to initially test an iPSC-based therapy due to the orphan nature of EB and its severity. Our success in completing validation and characterization studies of our simultaneous gene editing and reprogramming procedure achieved during this funding period provide feasibility data for the use of this approach to correct genetic disorders affecting other tissues, such as hematopoietic system.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

While many proposed experiments have been successfully accomplished, the completion of many *in vivo* studies and cGMP-compatible runs has been delayed due to the COVID19 pandemic and the closure of research laboratories on the University of Colorado Anschutz Medical Campus (UCAMC). As of July 2020, 100% of research personnel have been approved to return back to laboratory research activity, which allowed us to initiate many experiments that have been postponed. However, the laboratory personnel are required to work on a staggered basis, with no more than 50% occupancy at any given time to maintain social distancing. These restrictions continue to impact our research progress. The potential delay caused by these restrictions will be mitigated by requesting a no cost extension before the completion of Year 3 of the project.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The delay in the initiation of several tasks caused by the COVID19 pandemic reduced our projected expenditure to date. However, we anticipate requesting a no cost extension before the end of the project to mitigate this delay and accomplish all aims of the proposal within the requested budget.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

  Books or other non-periodical, one-time publications.

Nothing to Report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*

- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Dennis Roop
Project Role: PI
Nearest person month worked: 0.66
Contribution to Project: Dr. Roop oversees the project as a PI.
Funding Support: National Institute of Health, EB Charities; institutional support.

Name: Ganna Bilousova
Project Role: Co-Investigator
Nearest person month worked: 2.29
Contribution to Project: Dr. Bilousova prepares regulatory compliance documents and oversees the work related to iPSC generation and differentiation.
Funding Support: National Institute of Health, CIRM, EB Charities, institutional support

Name: Igor Kogut
Project Role: Co-Investigator
Nearest person month worked: 2.22
Contribution to Project: Dr. Kogut oversees a transfer of our technologies into a cGMP compliant facility.
Funding Support: National Institute of Health, EB Charities, institutional support

Name: Kiel Carson Butterfield
Project Role: Professional Research Assistant (PRA)
Nearest person month worked: 5.17
Contribution to Project: Ms. Butterfield assists in adapting our technologies to cGMP manufacturing and generation of gene corrected RDEB iPSCs.
Funding Support: National Institute of Health, EB Charities, institutional support

Name: Christopher Taylor
Project Role: PRA
Nearest person month worked: 3.9
Contribution to Project: Mr. Taylor assists Dr. Bilousova in mouse grafting experiments and maintenance of a mouse colony.
Funding Support: National Institute of Health, EB Charities, institutional support

Name: *Andrii Rozhok*
Project Role: *Instructor*
Nearest person month worked: *3.18*
Contribution to Project: *Mr. Rozhok develops a platform to analyze whole-genome sequencing data.*
Funding Support: *Institutional support*

Name: *Josiah Fernandes*
Project Role: *PRA*
Nearest person month worked: *2.77*
Contribution to Project: *Mr. Fernandez assists Dr. Bilousova in the generation of 3D skin equivalents and differentiation of iPSCs.*
Funding Support: *National Institute of Health, EB Charities, institutional support*

Name: *Chann Makara Han*
Project Role: *PRA*
Nearest person month worked: *1*
Contribution to Project: *Mr. Han assists Drs. Kogut and Bilousova in analyzing iPSCs and detecting off-target events in the Cas9-mediated gene correction strategy.*
Funding Support: *National Institute of Health, EB Charities, institutional support*

Name: *Maryna Pavlova*
Project Role: *RA*
Nearest person month worked: *2.23*
Contribution to Project: *Dr. Pavlova works on the differentiation of iPSCs into keratinocytes and xenografting. She also assists Dr. Bilousova in the generation of 3D skin equivalents.*
Funding Support: *National Institute of Health, EB Charities*

Name: *Michael Ferreyros*
Project Role: *Sr. PRA*
Nearest person month worked: *1.6*
Contribution to Project: *Mr. Ferreyros assists Dr. Pavlova in in vivo experiments.*
Funding Support: *National Institute of Health, EB Charities, institutional support*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No changes

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

No changes.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*
Included with the report.

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

None