AWARD NUMBER: **W81XWH-18-1-0706**

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

PRINCIPAL INVESTIGATOR: **Dennis R. Roop**

CONTRACTING ORGANIZATION: **University of Colorado Denver**
Aurora, CO 80045-2570

REPORT DATE: **OCTOBER 2019**

TYPE OF REPORT: **Annual report**

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
OCTOBER 2019	Annual report	09/15/2018-09/14/2019

4. TITLE AND SUBTITLE
Translating a Stem Cell-Based Therapy for Epidermolysis Bullosa into the Clinic

5a. CONTRACT NUMBER
W81XWH-18-1-0706

5b. GRANT NUMBER
PR171428

6. AUTHOR(S)
Dennis R. Roop

E-Mail: Dennis.Roop@cuanschutz.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Colorado Denver

12801 E 17th Ave, Aurora, CO 80045

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The proposal develops 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 the first year, we have secured approval for our ACURO protocol to use animals (ACURO Log Number PR171428.e001, approved on 10/01/2019), and are currently waiting for the IRB approvals from our collaborating groups from Stanford and Columbia Universities to address HRPO concerns. While our human subject protocol was awaiting approval by the DOD, we focused on tasks that did not require human subject participation. Specifically, we validated antibodies necessary to accomplish the project; we are also transferring our protocols for the generation of iPSCs and iPSC-derived keratinocytes into the Gates Biomanufacturing Facility (GBF) to implement a pilot cGMP run; and we have finalized the protocol for the differentiation of iPSCs into fibroblasts. We have also assessed several matrices to generate full thickness skin equivalents for the proposed research.

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

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION:

This application proposes to advance 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. In this proposal, we will transfer 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. We 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)	Pending HRPO approval
Task 1.2 To validate the absence of off-target events post-correction	Pending HRPO approval
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)	Not initiated
Task 1.5 To transfer the protocols to a product development laboratory at a cGMP-compliant facility, perform optimizations and prepare documentation/batch record	**Initiated/ In progress**
Task 1.6 To implement a pilot small-scale cGMP-compliant run of the protocol for the generation of a Master Cell Bank 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	**ACURO protocol is approved as of 10/01/2019; HRPO protocol is pending**

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)	Not initiated
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	**Initiated/ In progress**
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	**Completed**
Task 3.2 To examine the functionality of iPSC-derived fibroblasts (12 mice for xenotransplantation)	**Initiated/In progress**
Task 3.3 To develop a cGMP-compatible protocol for the differentiation of *COL7A1*$^{c.7485+G>A}$ corrected iPSCs into fibroblasts	Not initiated
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	**Initiated/In progress**
Task 3.5 To assess wound closure by iPSC-derived composite grafts in immunocompromised mice (180 mice per site)	Not initiated
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.

1. During the first year of the funded project, we secured the approvals of our human subject protocol and our IACUC protocol with our local committees (COMIRB and IACUC respectively). We have also obtained approval from ACURO to use animals in our proposal and have initiated *in vivo* experiments as described in our proposal. We are still waiting for approval of our HRPO protocol, which was caused by the miscommunication between the DOD and our University email systems that resulted in blocking of emails with PDF files attached. The initial HRPO submission was accomplished on 02/11/2019. However, the response from the HRPO committee was not obtained until August 2019. Following the HRPO recommendation, we have modified our COMIRB protocol and obtained an exemption from the human subject research from our local board. Similar determination is currently being requested by our collaborators on this application (Dr. Oro, Stanford University and Dr. Christiano, Columbia University) from their IRB committees.

2. While our human subject protocol has been awaiting approval from the DOD, we have focused on tasks that do not require human subject participation. We have also validated the necessary reagents, including cGMP-compliant reagents, in anticipation of initiation of several tasks. For tasks requiring the optimization of our protocols, we used control induced pluripotent stem cells (iPSCs) generated from completely de-identified pre-existing and publically available specimens obtained from public biorepositories (ATCC and Lonza). These completely de-identified and publically available specimens are exempt from being considered human subject research at the federal level under Title 45, Part 46 of the Department of Health and Human Services Basic Policy for the Protection of Human Subjects. We have also used de-identified keratinocytes

and fibroblasts obtained from these public biorepositories to develop the methodology for the generation of composite skin grafts for the proposal.

3. *Task 1.3 To validate custom-made antibodies to quantify the level of WT Col 7 post-COL7A1$^{c.7485+G>A}$ correction.* For this task, we used 3D skin equivalents generated from completely de-identified and publically available healthy keratinocytes and fibroblasts (Lonza), and validated the antibody that was generated in our laboratory, as well as from several commercial sources. We found that two antibodies available from commercial sources are more specific to type VII collagen (Col7) than the antibodies that we generated **(Fig.1**) We are now waiting for HRPO approval of our human subject protocol to validate these antibodies on patient cell lines.

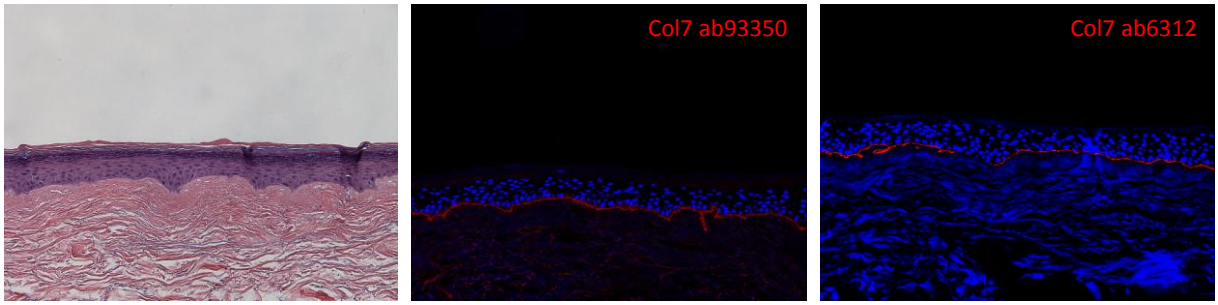


Fig. 1: Identification of antibodies that recognize Col7 in a 3D skin equivalent. The skin equivalent was generated using a collagen plug that was pre-seeded with human healthy fibroblasts obtained from Lonza followed by plating human healthy keratinocytes (Lonza). The plug was then exposed to Airlift medium to promote the formation and stratification of the epidermis. The formed 3D skin equivalent was fixed and sectioned for antibody staining. A variety of antibodies that recognize Col7 were tested using the skin equivalent. Two antibodies obtained from Abcam (ab93350 and ab6312) produced the most specific immunofluorescence staining of the basement membrane, where Col7 is primarily located, with minimal background. H&E (left) and immunofluorescence images (middle and right) were obtained using a 10x objective.

4. *Task 1.5 To transfer the protocols to a product development laboratory at a cGMP-compliant facility, perform optimizations and prepare documentation/batch record*. Given the complexity of cGMP-compliant manufacturing, we have initiated our collaboration with our local cGMP compliant facility, the Gates Biomanufacturing Facility (GBF), to transfer our reprogramming and gene editing protocols. These techniques include: (1) production of RNA reagents for reprogramming and gene editing; (2) quality control tests for these RNA reagents; (3) cell culturing techniques (using de-identified iPSCs while pending the appropriate approvals). Based on the recommendation from the FDA during our Interact meeting organized as part of our CIRM grant, we have also performed an off-target cleavage analysis of our guide RNA (gRNA) that we will use for the correction of the *COL7A1*$^{c.7485+G>A}$ mutation together with modified mRNA (mod-mRNA) encoding Cas9. 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 (**Table 1**). However, most of these potential target sequences are located in either introns or intergenic regions and therefore should not have any impact on the cellular phenotype if unintentionally modified by Cas9. We have further analyzed the potential risk of off target gene editing in cells as a result of our Cas9-mediated *COL7A1* correction strategy. We transfected healthy fibroblasts (Lonza) with Cas9 mod mRNA and our gRNA designed specifically to target *COL7A1*. Genomic DNA was isolated and the top 4 off-targets, scored by cutting frequency determination, were sequenced. No INDELs have been detected in any of the analyzed off-target sites. We are currently developing an *in vitro* strategy to further analyze potential off-target events of our Cas9-mediated gene correction strategy using CIRCLE Sequencing (CIRCLE-Seq). CIRCLE-Seq is a highly sensitive *in vitro*

screening strategy that is currently being used by many researchers to assess the off-target cleavage efficiency of Cas9.

Table 1: Predicted off-target sites of our *COL7A1*-specific gene correction strategy

MIT Rank	CFD Rank	Mismatches	Mismatch Count	OT Locus Description
MIT-1	CFD-1	GGGATCCACCGTGAGTCCTCGGG ....*............. GGGACCCACCGTGAGTCCTCGGG	1	exon:COL7A1
MIT-2	CFD-4	GGGATCCACCGTGAGTCCTCGGG ......*.**......... GGGATCGAGTGTGAGTCCTCTGG	3	intron:AC019118.2
MIT-3	CFD-8	GGGATCCACCGTGAGTCCTCGGG **..**............ CCGAAACACCGTGAGTCCTCTGG	4	intron:MFHAS1
MIT-4	CFD-26	GGGATCCACCGTGAGTCCTCGGG .*..*...**......... GAGAACCAAGGTGAGTCCTCAGG	4	intergenic:RP11-565A3.2-ADAM20P3
MIT-5	CFD-16	GGGATCCACCGTGAGTCCTCGGG .*.....*.**........ GTGATCCGCACTGAGTCCTCGGG	4	exon:MRC2
MIT-9	CFD-2	GGGATCCACCGTGAGTCCTCGGG *.*...........*.... AGAATCCACCGTGAGACCTCTGG	3	intergenic:NRP2-RN7SKP178
MIT-14	CFD-5	GGGATCCACCGTGAGTCCTCGGG ..*..*...**........ GGAATTCACTTTGAGTCCTCCGG	4	intergenic:RP11-204N11.2-C14orf64
MIT-19	CFD-3	GGGATCCACCGTGAGTCCTCGGG *.......**......*... AGGATCCATAGTGAGTACTCAGG	4	intron:C10orf90

5. *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 have initiated the transfer of our high efficiency differentiation protocol to the GBF. The problem of heterogeneity of cell populations derived during differentiation of iPSCs into epidermal progenitors is a significant safety hurdle toward clinical translation of iPSCs for the treatment of skin diseases. Therefore, we are now focusing on developing a platform for enriching the cultures with iPSC-derived keratinocytes and eliminating undifferentiated iPSCs. Using control de-identified iPSCs, we are developing a protocol for the purification of keratinocytes derived from iPSCs with the CliniMACS Prodigy. The CliniMACS Prodigy® is a closed platform with the capability of providing fully automated and scalable cell manufacturing procedures of various cell types on a single device and within a single process setup. Based on the preliminary data obtained by our collaborator on this application Dr. Oro, we initially focused on using α-CD49f (integrin α6) antibodies for selecting iPSC-derived keratinocytes. However, we have recently performed a more extensive screening of keratinocyte markers suitable for the purification using CliniMACS Prodigy® and found that another integrin –CD104 (integrin β4) may be more selective for keratinocytes (**Fig. 2A**). In addition, we found that undifferentiated iPSCs express integrin α6 at a high level (**Fig. 2B**). We have also performed a spiking experiment mixing human keratinocytes (Lonza) with 5% of undifferentated iPSCs followed by MACS Separation using α-CD49f magentic beads. As expected, we failed to eliminate undifferentiated iPSCs from the mixture when α-CD49f magentic beads were used (**Fig. 3A**). We are currently re-evaluating our purification strategy and using α-CD104 magentic beads for selection of iPSC-derived keratinocytes. In our preliminary experiment, the use of α-CD104 magentic beads selectively enriches for keratinocytes and eliminates iPSCs (**Fig. 3B**).

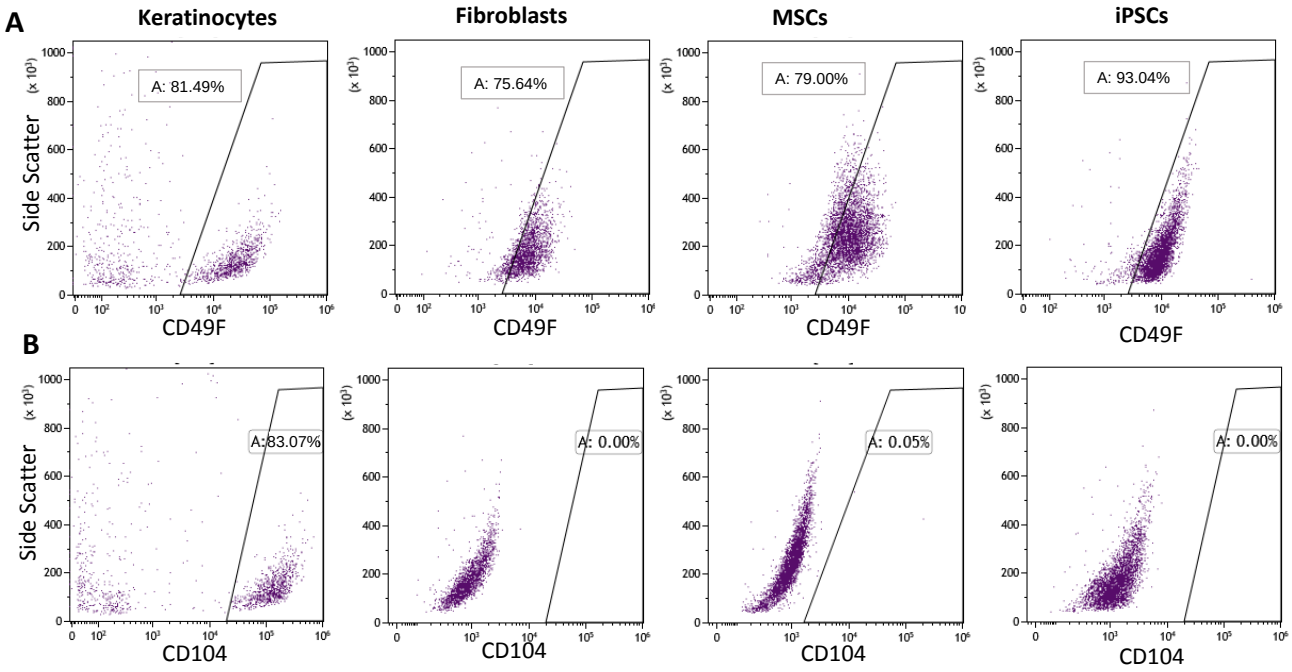


Fig. 2. Identifying a keratinocyte-specific surface marker for the enrichment of iPSC-derived keratinocyte cultures. Healthy human keratinocytes (Lonza), fibroblasts (Lonza), mesenchymal stem cells (MSCs, ATCC) and iPSCs (generated from fibroblasts from ATCC) were stained using anti-CD49f (**A**) and anti-CD104 (**B**) antibodies. CD104 is only expressed in keratinocytes and therefore is a better marker for keratinocyte culture enrichment.

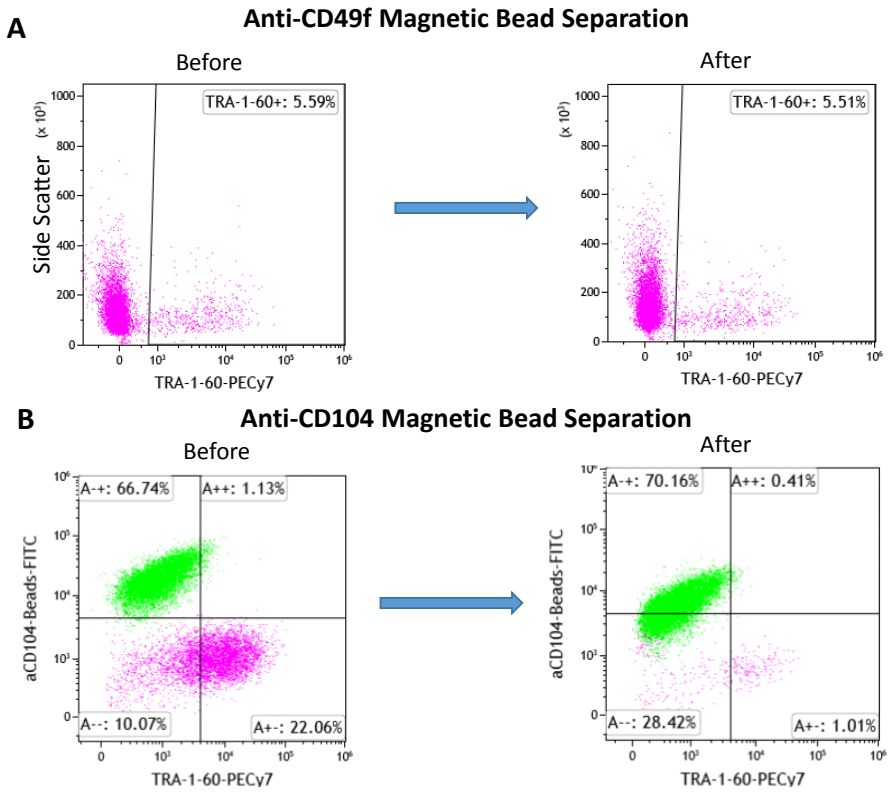
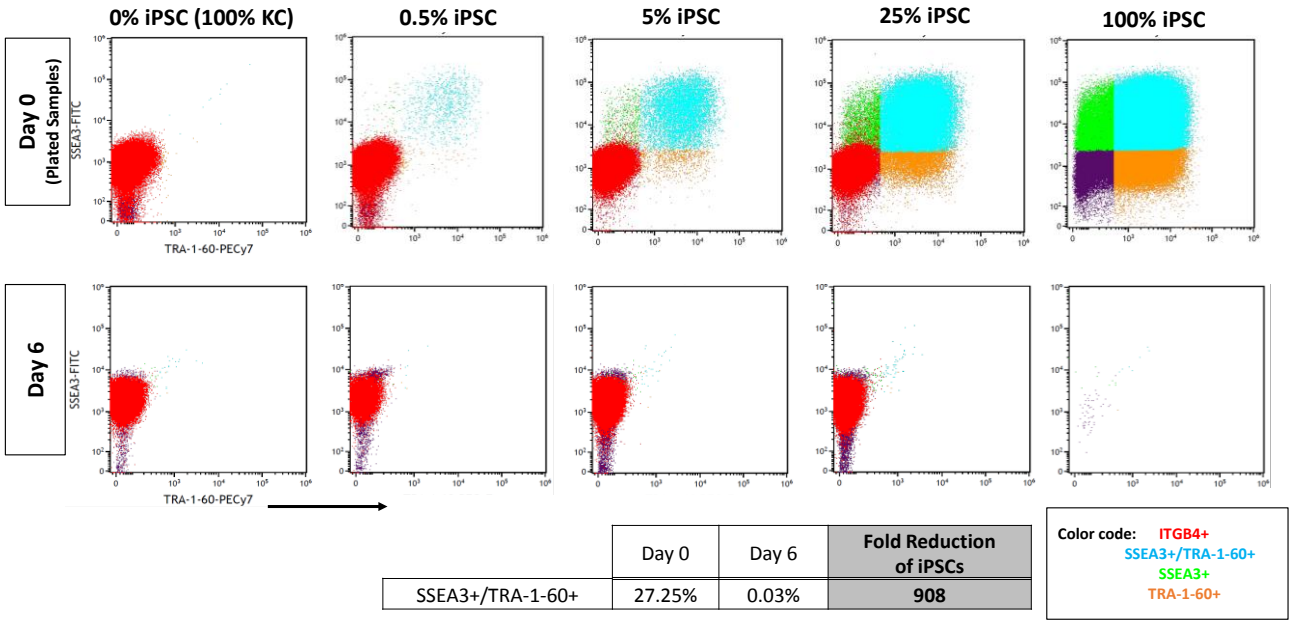


Fig. 3. A CD104-based magnetic bead separation efficiently eliminates iPSCs from a culture of keratinocytes. (**A**) Keratinocytes were mixed with 5% of iPSCs and subjected to the magnetic bead separation procedure using anti-CD49f magnetic beads. After elution, the percent of iPSC remained unchanged (right). (**B**) Keratinocytes were mixed with 30% of iPSCs and subjected to the magnetic bead separation procedure using anti-CD104 magnetic beads. After elution, the percent of iPSC decreased to ~1% (right).

As an additional precaution to ensure that no undifferentiated iPSCs (which can result in teratoma formation upon transplantation into the patients) are left in our iPSC-derived keratinocyte culture, we tested if our keratinocyte culture conditions can support the growth/survival of iPSCs. We mixed keratinocytes (Lonza) and iPSCs (generated from de-identified fibroblasts obtained from ATCC) at different ratios (0%, 0.5%, 5%, and 25%). We then cultured these cell mixtures for 6 days using DKSFM medium, which we use to expand our iPSC-derived keratinocyte cultures. At the end of 6 days of culturing, flow cytometry analyses for the expression of the pluripotency markers SSEA3 and TRA-1-60 and the specific keratinocyte marker CD104 (integrin β4) was performed, and the fold reduction of iPSCs in mixed cultures was calculated as compared to the flow cytometry results obtained at the time keratinocytes and iPSCs were plated. We found that our culturing conditions promote ~900 fold reduction in the number of iPSCs (**Fig. 4**), suggesting that even if a few iPSC cells are left after differentiation, they are likely to be eliminated by continous culturing under keratinocyte growth conditions.



	Day 0	Day 6	Fold Reduction of iPSCs
SSEA3+/TRA-1-60+	27.25%	0.03%	**908**

Fig. 4. Keratinocyte-specific culturing conditions eliminate iPSCs from the culture. iPSC were added into cultures of healthy keratinocytes (Lonza) at a different ratio (0.5%, 5%, 25%) and then cultured under keratinocyte specific conditions for 6 days. Keratinocytes alone (100% KC) and iPSCs alone (100%) were also included as controls. Flow cytometry analysis for the expression of pluripotency markers SSEA3 and TRA-1-60, as well as the keratinocyte marker CD104, was performed. The table shows the fold reduction of iPSCs (SSEA3+/Tra-1-60+ cells) under the condition where 25% of iPSCs were mixed with 75% of keratinocytes.

6. <u>*Task 3.1*</u> *To optimize a protocol for the differentiation of iPSCs into a fibroblast lineage*. We used control iPSCs to develop a protocol for the derivation of fibroblasts. We can now successfully generate iPSC-derived fibroblast-like cells that are negative for pluripotency markers and positive for fibroblasts markers (**Fig. 5**).

7. <u>*Task 3.2*</u> *To examine the functionality of iPSC-derived fibroblasts.* We are characterizing our iPSC-derived fibroblasts. We are preparing cells for RNA-sequencing and grafting using our *in vivo* chamber assay to confirm their functionality as described in our proposal. We have just obtained approval of our ACURO protocol and can now initiate *in vivo* experiments. Once we obtain approval for our HRPO protocol to use cells derived from human subjects, we will validate our protocol on genetically corrected RDEB iPSCs.

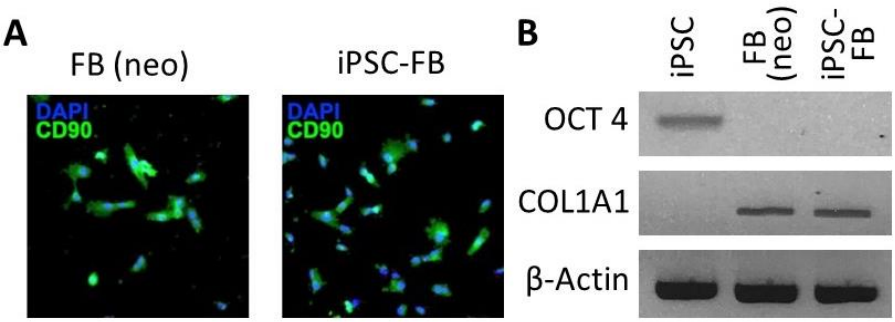


Fig. 5. Fibroblasts derived from iPSCs (iPSC-FB) express appropriate markers as determined by the immunofluorescence staining for CD90 (**A**) and RT-PCR for Col1 (**B**). FB (neo) are control neonatal fibroblasts.

8. *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*. Since our goal is to generate a transplantable full thickness skin equivalent, we tested several available allogeneic accellular dermal matrix products for their compatibility with the process of generating full thickness skin equivalents. Using control de-identified keratinocytes and fibroblasts, we have discovered that ALLODERM™ Regenerative Tissue Matrix supports the generation of human skin equivalents (**Fig. 6**). ALLODERM™ is allograft human dermis, aseptically processed to remove cells and freeze-dried to remove moisture while preserving biologic components and structure of the dermal matrix. While ALLODERM supports the generation of the high-quality epidermis, we still have difficulties in seeding fibroblasts into ALLODERM due to slow and incomplete migration of the fibroblasts through the relatively thick dermis. Therefore, we continue to explore alternative matrices and are currently testing fibrin and collagens to form full-thickness 3D skin equivalents. In our preliminary experiments, we found that using collagen allows us to generate thick dermis with healthy fibroblasts and good-quality epidermis (**Fig. 7**). Since we have now obtained approval for our ACCURO protocol, we are initiating the grafting experiments on a mouse to confirm the formation of functional skin using collagen-based human skin equivalents.

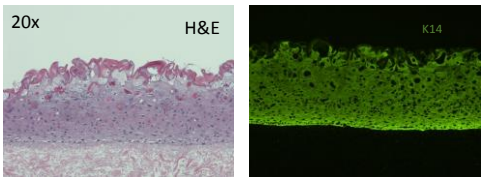


Fig. 6. Generation of a 3D full-thickness skin equivalent using ALLODERM. Healthy fibroblasts (Lonza) were pre-seeded on ALLODERM followed by plating human healthy keratinocytes (Lonza). The tissue construct was then exposed to Airlift medium to induce the maturation of the epidermis. The skin equivalent was fixed and sectioned after 1 month in culture and analyzed for the expression of keratin (K) 14 (right, green) using antibody staining.

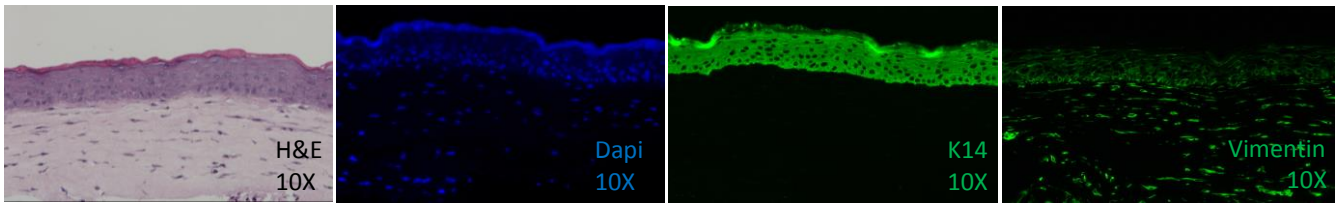


Fig.7 Generation of full thickness skin equivalents using collagen plugs. Skin equivalents were generated as described in Fig. 1. DAPI and vimentin staining indicates fibroblast migration and the formation of the complete dermis. K14 staining marks the generation of a multi-layered epidermis.

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."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "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 start presenting our results at national and international meetings, such as meetings organized by DEBRA International. These meetings are usually attended by patients with EB and serve as a great way to enhance patient's awareness of research progress in the field.

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 performed under clinically relevant standards. During the first year, we optimized several important steps of our complex therapy to ensure the successful approval for a clinical trial. The procedure for the purification of functional skin cells is especially important as it can be applied to the treatment of a variety of other skin conditions.

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

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

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. Furthermore, the skin is an ideal target tissue to initially test an iPSC-based therapy: it is readily accessible, easy to monitor, and if an adverse event should occur, the affected cells could be easily excised. Therefore, 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.

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

What was the impact on society beyond science and technology?
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.

During the first year of funding, we faced a significant delay in the approval of our ACURO and HRPO protocols. This delay was caused by the security software on the DOD side that prevented the receipt of our emails (originated from the official University of Colorado webmail service) containing PDF attachments with our protocols. This issue has now been resolved. While the ACURO protocol has been recently approved, the HRPO approval is still pending. To mitigate this delay, during the first year of funding we focused on the tasks that did not require human subject involvement or animal work.

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.

Nothing to Report

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: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **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. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

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".

<u>Example:</u>

Name:	*Mary Smith*
Project Role:	*Graduate Student*

Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Dennis Roop
Project Role: PI
Nearest person month worked: 0.6
Contribution to Project: Dr. Roop oversees the project as a PI.
Funding Support: National Institute of Health, CIRM, EB Charities; institutional support.

Name: Ganna Bilousova
Project Role: Co-Investigator
Nearest person month worked: 2.4
Contribution to Project: Dr. Bilousova prepares regulatory compliance documents and oversees the work related to iPSC differentiation.
Funding Support: National Institute of Health, CIRM, EB Charities, institutional support

Name: Igor Kogut
Project Role: Co-Investigator
Nearest person month worked: 1.7
Contribution to Project: Dr. Kogut oversees the transfer of our technologies into a cGMP compliant facility.
Funding Support: National Institute of Health, CIRM, EB Charities, institutional support

Name: Kiel Carson Butterfield
Project Role: Professional Research Assistant (PRA)
Nearest person month worked: 1.5
Contribution to Project: Ms. Butterfield assists Dr. Kogut in adapting our technologies to cGMP manufacturing.
Funding Support: National Institute of Health, CIRM, EB Charities

Name: Michael Ferreyros
Project Role: Sr. PRA
Nearest person month worked: 3.5
Contribution to Project: Mr. Ferreyros assists Dr. Bilousova in preparing regulatory compliance documents and maintaining cell lines. He is also involved in antibody testing for the project.
Funding Support: National Institute of Health, EB Charities

Name: Patrick S. McGrath
Project Role: Sr. Research Associate
Nearest person month worked: 3.25
Contribution to Project: Dr. McGrath is involved in designing and testing gene editing constructs for the project. He also assists Dr. Bilousova in molecular biology techniques and participates in a cGMP transfer of our technologies.
Funding Support: National Institute of Health, CIRM, EB Charities

Name:	*Shennea McGarvey*
Project Role:	*PRA*
Nearest person month worked:	*2.0*
Contribution to Project:	*Mrs. McGarvey assists Dr. Bilousova in differentiating iPSCs into fibroblasts and keratinocytes.*
Funding Support:	*National Institute of Health, EB Charities*
Name:	*Josiah Fernandes*
Project Role:	*PRA*
Nearest person month worked:	*0.8*
Contribution to Project:	*Mr. Fernandez assists Dr. Bilousova in the generation of 3D skin equivalents.*
Funding Support:	*National Institute of Health, EB Charities*
Name:	*Chann Makara Han*
Project Role:	*PRA*
Nearest person month worked:	*1.4*
Contribution to Project:	*Mr. Han assists Dr. McGrath in analyzing off-target events in our Cas9-mediated gene correction strategy.*
Funding Support:	*National Institute of Health, EB Charities*

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."
If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

1. **R01AR059947-05 and R01AR059947-05S1** that supported Dr. Roop (PI) and Drs. Bilousova and Kogut (Co-Investigators) have been closed.

2. The grant awarded to Drs. Roop and Bilousova from DEBRA International (**25A4282**) titled "A stem-cell based therapy for patients with Epidermolysis Bullosa Simplex" has been also closed.

3. The grant from the Colorado Office of Economic Development & International Trade titled "Validation of the cGMP Manufacture of Cell Therapy Products at the Gates Biomanufacturing Facility", where Dr. Bilousova was a Co-Investigator, has been closed.

4. The grant from EB Charities that funded the EB Consortium, including Drs. Roop, Kogut and Bilousova, was closed and replaced with two other awards (see below) without affecting the percent effort of the PI and Co-Investigators on the current DOD-funded project.

5 A new grant from the EB Charities was awarded titled " Developing an iPS Cell-based Therapy for Epidermolysis Bullosa Simplex". Goals: To develop a stem-cell based therapy for Epidermolysis Bullosa Simplex (EBS) patients using K14 corrected induced pluripotent stem cells and adapt the procedure to cGMP-compliant standards. No overlap.
Aims: (1) To optimize the purification of keratinocytes differentiated from gene-edited EBS iPSC using the CliniMACS Prodigy; (2) To determine if the "Spray on Skin" system can deliver keratinocytes to blisters induced on our conditional mouse model of EBS.
Start and end date: 05/01/2019 - 4/30/2020
Contact: Alex Silver; alexsilver@gmail.com

6. Two new grants from the EB Charities titled Developing a Therapeutic Approach for Delivering Stem Cells Systemically to Treat Fragile Internal Epithelia in RDEB and Testing a "Spray on Skin" Approach as an Alternative Method for Delivering Keratinocytes and Fibroblasts for RDEB" (**25A3910/AWD-163717**). The goals of these proposals is to optimize the spray-on-skin delivery system using mouse primary keratinocytes and fibroblasts on a conditional Recessive Dystrophic Epidermolysis Bullosa (RDEB) mouse model. An additional goal is to adapt the systemic use of mouse MSCs to treat a conditional RDEB mouse model as a pre-requisite for developing an MSC-based systemic therapy for RDEB patients. No overlap.
   Aims: (1) To optimize the delivery of mouse keratinocytes and fibroblasts to blistered skin using a "Spray on Skin" delivery system; (2) To determine if the "Spray on Skin" system can deliver keratinocytes and fibroblasts to blisters induced on a novel conditional mouse model of RDEB; (3) To optimize the systemic delivery of mouse $GFP^+/HCELL^+$ MSCs.
   Start and end date: 01/01/2019 - 12/31/2019
   Contact: Alex Silver; alexsilver@gmail.com

7. Dr. Roop (PI) and Drs. Bilousova and Kogut (Co-Investigators) have been recently awarded a U01 grant from the NIH (U01 AR075932) titled "Exploring Alternative iPS Cell Therapies for Recessive Dystrophic Epidermolysis Bullosa". This award also includes matching funds from EB Charities since applications funded under RFA-HL-18-030: Regenerative Medicine Innovation Projects (RMIP) Investigator-Initiated Studies (Collaborative U01) as part of the 21st Century Cures Act were mandated to have 1:1 matching funds for the total amount of the award. The goal of the proposal is to explore two novel induced pluripotent stem cell (iPSC) therapies for the severe recessive dystrophic form of epidermolysis bullosa (RDEB). We propose to extend the studies that are already underway in the EB Consortium and assess an alternative approach for delivering genetically corrected RDEB iPSC-derived skin cells using the RECELL "spray-on-skin" device developed by Avita Medical. We will also determine if systemic delivery of COL7A1-corrected iPSC-derived MSCs facilitates healing of damaged oral mucosa, promotes Col7 deposition in the skin and enhances engraftment of iPSC-derived skin cells. There is no overlap with the current proposal and the percent effort was adjusted from other projects without affecting the current DOD-funded project.
   Aims: (1) Generation of *COL7A1*-corrected iPSC-derived fibroblasts and MSCs under cGMP-compliant conditions; (2) Exploring the feasibility of using a "spray on skin" device to deliver *COL7A1*-corrected iPSC-derived keratinocytes and fibroblasts. (3) Assessing the ability of systemically delivered MSCs to improve wound healing in the skin and internal epithelia. (4) Generating preliminary safety and efficacy data for the FDA.
   Start and end date: 07/15/2019 - 07/14/2021
   Contact: Alexey Belkin; Alexey.belkin@nih.gov

Although several new grants were awarded, several older grants were closed for the PI and Co-Investigators on the current proposal. As a result, there were no significant changes in the percent effort of the key personnel and the PI. There is also no scientific overlap between the current proposal and these new grants as described above.

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. The current proposal is a collaboration between members of the EB iPS Cell Consortium. Other institutions include Stanford University and Columbia University. Dr. Oro (Stanford) and Dr. Christiano (Columbia) are sub-award PIs on the current application as indicated in the original application and approved statement of work. No other new collaborations have been established.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:
Included with the report.

9. APPENDICES: