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TITLE: Scalability and Safety Studies in Clinical-Grade Pluripotent-Derived Myogenic Progenitors for Therapeutic Application in DMD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

As proposed in the application, during the first year we performed a series of purification studies using CD54 antibody to identify the conditions for the optimal purification of iPAX7 human iPS cell-derived myogenic progenitors. These experiments resulted in the identification of the optimal ratio of antibody and microbeads for the MACS-mediated purification of our cell product with minimal residual of CD54-negative cells (Fig. 1-B). In addition, in order to identify the minimal media components required for the maintenance of their muscle regenerative potential, we tested multiple media conditions for the generation and expansion of CD54+ cells from iPAX7 iPS cells (Fig. 1C). These studies are ongoing as they require intramuscular transplantation in recipient animals followed by analysis 2 months post-injection. We observed that removal of horse serum has no impact on the proliferation and differentiation of CD54+ cells into muscle. To assess safety of human iPAX7 iPS cells, we performed karyotype analysis (Fig. 1D) and all genome sequencing using the 10X Genomics Chromium platform. Data are in the analysis pipeline and will include comparison to the genomic variants reported for the original cell line generated at NIH.

15. SUBJECT TERMS

ACURO- Animal Care and Use Review Office; cGMP- Current Good Manufacturing Practice; CNV- Copy Number Variation; DMD- Duchenne Muscular Dystrophy; DYS- dystrophin; FACS- Fluorescence-Activated Cell Sorting; FDA- Food and Drug Administration; HRPO/ACURO- Human Research Protection; IND- Investigational New Drug; iPSC- Induced Pluripotent Stem

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION

This application builds on our successful experimental studies developing pluripotent stem cell-derived myogenic progenitors to promote long-term muscle regeneration for Duchene Muscular Dystrophy. The purpose of this project is to optimize manufacturing and purification of our myogenic cell product in compliance with current good manufacturing practice (cGMP). Once this is achieved and validated in preclinical studies, we will be in a strong position to begin IND filing, and a phase 1 safety trial for Duchene Muscular Dystrophy

2. KEYWORDS

ACURO - Animal Care and Use Review Office
cGMP - Current Good Manufacturing Practice
CNV- Copy Number Variation
DMD - Duchenne Muscular Dystrophy
DYS - DYSTROPHN
FACS - Fluorescence-Activated Cell Sorting
FDA - Food and Drug Administration
HRPO/ACURO – Human Research Protection
IND - Investigational New Drug
iPSC - induced Pluripotent Stem Cell
LiPSC-ER2.2 - GMP-manufactured human induced pluripotent stem cell line used in this project
MACS - Magnetic Cell Sorting
NIH – National Institutes of Health
PAX7 - Paired box protein 7, transcription factor, important for muscle development and adult muscle regeneration
pCCL - third generation self-inactivating lentiviral vector
SNP - single nucleotide polymorphism
rtTA - reverse tetracycline-controlled transactivator

3. ACCOMPLISHMENTS

3.a What were the major goals of the project?

Major Goal 1: Optimization of purification strategy using MACS	Proposed (Achieved)
Milestone Achieved: HRPO/ACURO Approval	Month 4-6 (100%)
Milestone Achieved: *in vitro* validation purification protocol	Month 9 (100%)
Major Goal 2: Transplantation studies with MACS-purified myogenic progenitors	Months
Milestone Achieved: *in vivo* validation purification protocol	Month 21 (30%)
Major Goal 3: Scalability studies (growth curves)	Months

Milestone Achieved: Growth rates obtained for all conditions evaluated.	Month 18 (50%)
Major Goal 4: Scalability studies (*in vivo)*	Months
Milestone Achieved: *in vivo* validation scalability studies	Month 19 (30%)
Major Goal 5: Safety of iPAX7 myogenic progenitors	Months
Milestone Achieved: Mapping of integration sites as well as identification of potential SNPs and CNVs	Month 15 (75%)
Major Goal 6: Verification of clinical-grade compatible purification and scale up of LiPSC-ER2.2 iPS cell-derived myogenic progenitors	Months
Milestone Achieved: Generation and *in vitro* validation of clinical grade "compatible" myogenic progenitors	Month 33 (0%)
Major Goal 7: *in vivo* validation of clinical grade "compatible" myogenic progenitors	Months
Milestone Achieved: *in vivo* validation of clinical grade "compatible" myogenic progenitors	Month 36 (0%)

3.b What was accomplished under these goals?

1) Major activities:
Our major activities during the last 12 months involved optimization of MACS-purification as well as optimization of scalability testing different culture conditions. We also began safety studies and transplantation experiments.

2) Specific objectives:
- To perform *in vitro* and in vivo validation of a MACS-based purification protocol for iPAX7 myogenic progenitors
- To determine optimal culture conditions for the scalability of iPAX7 myogenic progenitors (growth curves)
- To perform *in vivo* validation scalability studies
- To determine the safety of iPAX7 myogenic progenitors

3) Significant findings/developments:
As proposed in the application, during the first year we performed a series of purification studies using CD54 antibody to identify the conditions for the optimal purification of iPAX7 human iPS cell-derived myogenic progenitors. These experiments resulted in the identification of the optimal ratio of antibody and microbeads for the MACS-mediated purification of our cell product with minimal residual of CD54-negative cells (Fig. 1-B). In addition, in order to identify the minimal media components required for the maintenance of their muscle regenerative potential, we tested multiple media conditions for the generation and expansion of CD54+ cells from iPAX7 iPS cells (Fig. 1C). These studies are ongoing as they require intramuscular transplantation in recipient animals followed by analysis 2 months post-injection. We observed that removal of horse serum has no impact on the proliferation and differentiation of CD54+ cells into muscle. To assess safety of human iPAX7 iPS cells, we performed karyotype analysis (Fig. 1D) and all genome

sequencing using the 10X Genomics Chromium platform. Data are in the analysis pipeline and will include comparison to the genomic variants reported for the original cell line generated at NIH.

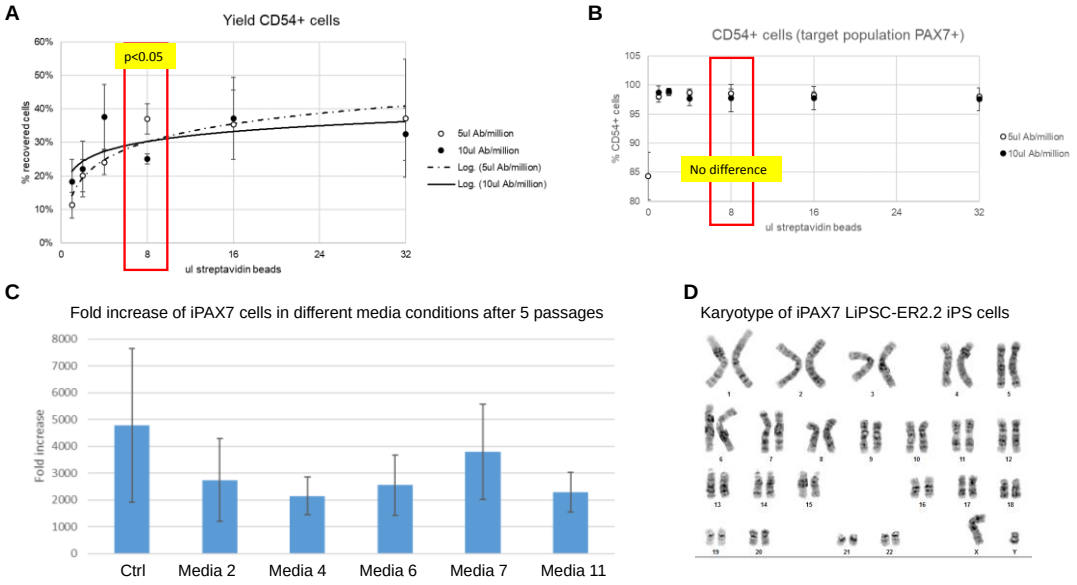


Figure 1. Significant developments. A) Optimization of MACS-mediated CD54+ cell purification. Five and 10ul of biotin-conjugated anti-CD54 antibody/million cells were used in combination with different amounts of streptavidin beads. The optimal recovery was determined using 5ul of antibody and 8ul of streptavidin beads. B) MACS-mediated purification enable enrichment of CD54+ to nearly 100% in all condition tested. C) Fold enrichment of CD54+ cells obtained by differentiating iPAX7-LiPSC-ER2.2 iPS cells using different media compositions. All conditions tested retained the ability of these cells to differentiate *in vitro* and displayed a similar growth potential. D) Karyotype analysis of LiPSC-ER2.2 iPS cells following transduction with pCCL-PAX7 and pCCL-rtTA viruses. Cells displayed a normal karyotype and genomic DNA has been submitted for whole genome sequencing.

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

"Nothing to Report".

3.d How were the results disseminated to communities of interest?

"Nothing to Report".

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

We will complete milestones associated with major goals 2, 3, 4, and 5, as described above. We will complete the growth curve studies as well as the *in vivo* experiments using the optimized media composition and MACS-purification protocol. Assessment of long-term safety following intramuscular transplantation of CD54+ myogenic progenitors will be completed. We will provide a list of viral integration sites in the genome of iPAX7 LiPSC-

ER2.2 iPS cells and eventual genomic variations arising from the viral transduction process.

4. IMPACT

4.a What was the impact on the development of the principal discipline(s) of the project?
"Nothing to Report".

4.b What was the impact on other disciplines?
"Nothing to Report".

4.c What was the impact on technology transfer?
"Nothing to Report".

4.d What was the impact on society beyond science and technology?
"Nothing to Report"

5. CHANGES/ PROBLEMS

5.a Changes in approach and reasons for change
"Nothing to Report".

5.b Actual or anticipated problems or delays and action or plans to resolve them
"Nothing to Report".

5.c Changes that had a significant expenditures
"Nothing to Report".

5.d Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
"Nothing to Report"

6. PRODUCTS

6.a Publications, conference papers, and presentations
"Nothing to Report".

6.b Website(s) or other Internet site(s)
"Nothing to Report".

6.c Technology or techniques
"Nothing to Report".

6.d Inventions, patent applications, and/or licenses
"Nothing to Report".

6.e Other products
"Nothing to Report".

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

7.a What individuals have worked on the project?

Name	Project Role	eRA Commons ID	Person Months	Contribution to Project
Rita Perlingeiro	PI	rperlingeiro	1.80	Overall oversight of the project.
Alessandro Magli	Assistant Professor	amagli	2.4	Dr. Magli was responsible for experimental design. He led the studies involving the optimization of purification and scalability of pluripotent stem cell-derived myogenic progenitors.
James Kiley	Researcher 2	N/A	6.0	Mr. Kiley performed the studies involving the optimization of purification and scalability of pluripotent stem cell-derived myogenic progenitors
Tania Incitti	Postdoctoral Associate	tincitti	1.2	Dr. Incitti performed transplantation experiments.
David McKenna	Co-PI	dmckenna	0.6	Dr. McKenna provided expertise in the design and implementation of studies.

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

"Nothing to Report".

7.c What other organizations were involved as partners?

"Nothing to Report"

8. SPECIAL REPORTING REQUIREMENTS

N/A

Insert Project Title Here

Insert ERMS/Log Number and Task Title Here
W81XWH-17-1-0659

PI: Rita Perlingeiro **Org:** Insert Recipient Organization/Contractor Name Here **Award Amount:** $599,578.94

Study/Product Aim(s)

Aim 1 – To define the optimal purification strategy for the clinical application of pluripotent-derived myogenic progenitors.

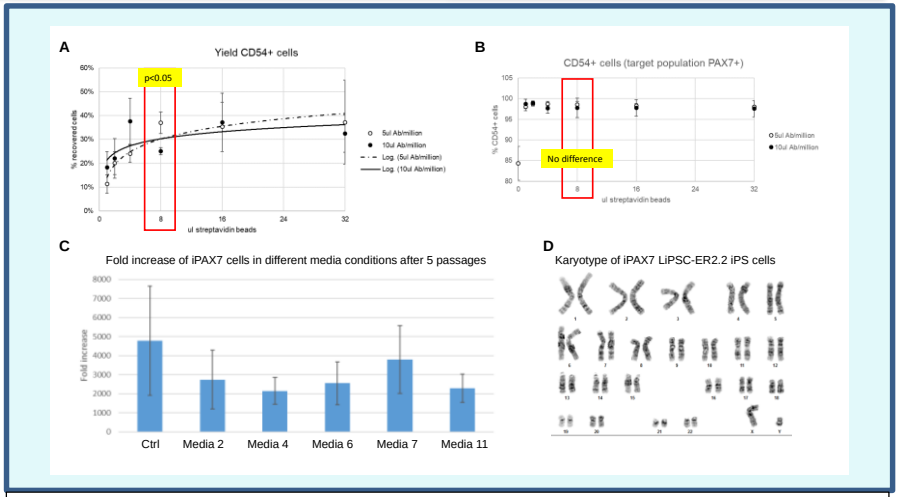
Aim 2 – To develop a GMP-compliant protocol for the expansion of human pluripotent-derived skeletal muscle progenitors to enable clinical application

Aim 3 – To complete a comprehensive risk assessment analysis of Pax7-induced pluripotent-derived myogenic progenitors.

Aim 4 – *To generate and validate clinical grade "compatible" pluripotent-derived myogenic progenitors.*

Approach

Our approach is a skeletal muscle cell therapy from pluripotent stem cells using conditional expression of PAX7.



Accomplishment: We have completed optimization of the MACS-purification approach and made great progress on the scalability studies. Safety studies (genome sequencing) and transplantation are underway. Normal karyotype is shown above .

Timeline and Cost

Activities CY	17	18	19	20
Aim 1.Optimization of purification strategy using MACS				
Aim 2.To develop a GMP-compliant protocol				
Aim 3.Comprehensive risk assessment				
Aim 4. Generate and validate clinical grade "compatible" product				
Estimated Budget ($K)	**$200,000**	**$199,790**	**$199,789**	

Updated: 09.18.18

Goals/Milestones

CY17 Goals – Documentation and purchase LiPSC-ER2.2 iPS cells.

☑ HRPO/ACURO Approvals and purchase of LiPSC-ER2.2 iPS cells.

CY18 Goals – Optimization MACS-purification and scalability studies.

☑ *in vitro* validation of purification protocol.

☑ Tested all growth composition culture conditions.

CY19 Goal – Final optimization of manufacturing.

☐ Growth rates obtained for all growth composition culture conditions evaluated.

☐ *in vivo* validation of purification protocol and selected defined growth condition.

☐ Mapping of integration sites as well as identification of potential SNPs/CNVs.

CY20 Goal – Generation and validation of clinical grade "compatible" approach.

☐ *in vitro* validation of clinical grade "compatible" myogenic progenitors.

☐ *in vivo* validation of clinical grade "compatible" myogenic progenitors.

Comments/Challenges/Issues/Concerns

N/A

Budget Expenditure to Date

Projected Expenditure: $200,000

Actual Expenditure: $212,379