RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 03/31/2020

NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Grant Number: 5R01AR044533-25
FAIN: R01AR044533

Principal Investigator(s):
JEFFREY S CHAMBERLAIN, PHD

Project Title: Assembly of the Dystrophin-Glycoprotein Complex

AMY GILES
UNIVERSITY OF WASHINGTON
4333 Brooklyn Ave NE
Box 359472
Seattle, WA 981959472

Award e-mailed to: osp@uw.edu

Period Of Performance:
Budget Period: 04/01/2020 – 03/31/2021
Project Period: 04/19/1997 – 03/31/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $524,344 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF WASHINGTON in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Arthritis And Musculoskeletal And Skin Diseases of the National Institutes of Health under Award Number R01AR044533. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Leslie Littlejohn
Grants Management Officer
NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AR044533-25

Award Calculation (U.S. Dollars)

Salaries and Wages	$170,130
Fringe Benefits	$47,615
Personnel Costs (Subtotal)	$217,745
Materials & Supplies	$60,185
Travel	$2,200
Other	$54,429
Publication Costs	$2,640
Federal Direct Costs	$337,199
Federal F&A Costs	$187,145
Approved Budget	$524,344
Total Amount of Federal Funds Obligated (Federal Share)	$524,344
TOTAL FEDERAL AWARD AMOUNT	$524,344
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$524,344

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
25	$524,344	$524,344
26	$524,344	$524,344

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Arthritis, Musculoskeletal and Skin Diseases Research
CFDA Number:	93.846
EIN:	1916001537A1
Document Number:	RAR044533G
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021
AR	8472466	$524,344	$524,344

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: 2 B / **OC**: 41025 / **Released:** eRA Commons User Name 03/30/2020
Award Processed: 03/31/2020 12:02:01 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AR044533-25

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AR044533-25

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget

period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AR044533. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Sheila Simmons
Email: simmonss@mail.nih.gov **Phone**: 301-594-9812 **Fax**: 301-480-5450

Program Official: Thomas Cheever
Email: Thomas.Cheever@nih.gov **Phone**: (301) 594-5019 **Fax**: (301) 480-1284

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AR044533-25

INSTITUTION: UNIVERSITY OF WASHINGTON

Budget	Year 25	Year 26
Salaries and Wages	$170,130	$170,130
Fringe Benefits	$47,615	$47,615
Personnel Costs (Subtotal)	$217,745	$217,745
Materials & Supplies	$60,185	$60,185
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Publication Costs	$2,640	$2,640
TOTAL FEDERAL DC	$337,199	$337,199
TOTAL FEDERAL F&A	$187,145	$187,145
TOTAL COST	$524,344	$524,344

Facilities and Administrative Costs	Year 25	Year 26
F&A Cost Rate 1	55.5%	55.5%
F&A Cost Base 1	$337,199	$337,199
F&A Costs 1	$187,145	$187,145

A. COVER PAGE

Project Title: Assembly of the Dystrophin-Glycoprotein Complex	
Grant Number: 5R01AR044533-25	**Project/Grant Period:** 04/19/1997 - 03/31/2022
Reporting Period: 04/01/2019 - 03/31/2020	**Requested Budget Period:** 04/01/2020 - 03/31/2021
Report Term Frequency: Annual	**Date Submitted:** 02/10/2020
Program Director/Principal Investigator Information: JEFFREY S CHAMBERLAIN , BA PHD **Phone number:** 206-221-5363 **Email:** jsc5@uw.edu	**Recipient Organization:** UNIVERSITY OF WASHINGTON Office of Sponsored Programs 4333 Brooklyn Ave NE SEATTLE, WA 981959472 **DUNS:** 605799469 **EIN:** 1916001537A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: CAROL RHODES University of Washington Office of Sponsored Programs 4333 Brooklyn Ave NE SEATTLE, WA 98105 **Phone number:** 206-543-4043 **Email:** osp@uw.edu	**Signing Official:** AMY GILES 4333 Brooklyn Ave NE Box 359472 Seattle, WA 981959472 **Phone number:** 206-543-4043 **Email:** amygiles@uw.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1. Characterization of methods for AAV-mediated CRISPR/Cas9 delivery to muscles of mdx4cv mice. These studies will compare the use of various Cas9 enzymes to monitor differences in dystrophin and DGC induction & off-target DNA editing. Several strategies for inducing dystrophin & the DGC in mdx4cv mice will be compared for efficiency, including exon deletion, cleavage and non-homologous end-joining (NHEJ) & homology-directed repair (HDR). Methods will also be pursued to optimize gene editing, including comparing different muscle promoters to drive Cas9 expression, varying the ratios of Cas9 to guide RNAs, performing a dose escalation study, monitoring short vs long-term expression and measuring functional benefit of the delivery strategies.
Aim 2. Enhancement of dystrophin expression by CRISPR/Cas9 delivery. The studies in aim 1 should provide important data on major parameters needed to induce full or nearly full-length dystrophin in the murine DMD model. Building upon those results we will examine the effects of varying the relative timing of Cas9 vs guide RNA expression. The use of multiple pairs of guide RNAs will also be tested. Finally, we will examine the effects on gene editing and off target cleavage efficiencies by transiently expressing Cas9 using several different strategies.
Aim 3. AAV mediated transduction of satellite cells. Gene therapies for DMD will require life-long dystrophin/DGC expression. Gene editing has been suggested as a way to achieve permanent dystrophin expression, especially compared with gene replacement therapies where the episomal AAV vector may be slowly lost. However, gene edited myonuclei would also decline in number if there is even low-level muscle turnover, and the half-life of normal myofibers is unknown but almost certainly finite. A permanent correction may therefore require efficient targeting of myonuclei & myogenic progenitors such that edited nuclei are replaced during myofiber aging and/or regeneration. This aim will explore the use of different AAVs for targeting satellite cells. Several strategies will be used to determine the efficiency of progenitor cell targeting and the ability to restore dystrophin during regeneration.
Aim 4: Editing of the dystrophin gene in the canine model for DMD. Studies in mice are useful for optimizing therapeutic strategies but they do not necessarily translate to larger mammals or humans. This aim will address whether methods refined in mice can be adapted for use in a canine model for DMD. The CXMD colony at our Institution carries the intron 6 splice acceptor mutation in the dystrophin gene on an outcrossed golden retriever/beagle strain. Together with Dr. Martin Childers and colleagues we will explore local and systemic delivery of CRISPR/Cas9 and will monitor dystrophin/DGC expression, striated muscle function and potential immune responses vs Cas9. The major strategy will be to genetically delete exons 6-8 of the dystrophin gene, but depending on the results in Aims 1-2, NHEJ & HDR strategies will also be tested.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B5.Training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

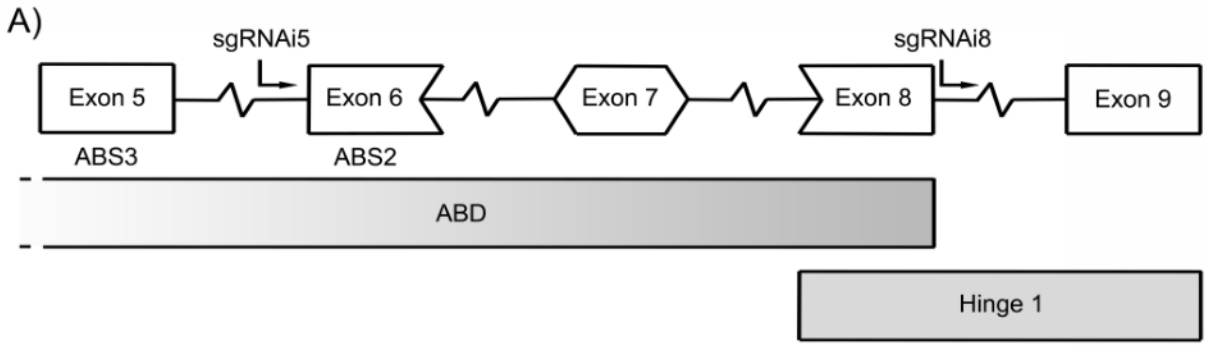
Our plans for the coming year are to submit both papers discussed in B2)both have been delayed as we saw a need for more data). We are also finalizing a solicited review article on muscle gene editing. Experimentally, we have begun exploring the sue of exosomes as a non-viral method to deliver CRISPR/Cas9. However, that approach seems to help with satellite cells, but not with myofibers. We are evaluating whether to continue this approach. We will continue to focus on the satellite cell transduction studies, using reporter genes in satellite cells that can sensitively identify gene editing. Two groups report that AAV9 can target satellite cells, a result we are unable to reproduce. We have discussed this issue with several other gene editing groups, including Eric Olson, and everyone other than the published group agree that they see very little, if any, satellite cell editing with current approaches. We feel the contradictory data from Amy Wagers and Dongsheng Duan is seriously misinterpreted and flawed, and will discuss this in our editing review. We also will continue to explore alternate methods to target satellite cells in vivo in normal and dystrophic mice. We are also beginning to pursue more studies of immune responses vs Cas9. Finally, we are also beginning to test new Cas enzymes, including base modifying enzymes, and prime editing. However, those studies were not originally proposed in our application, and we may need to pursue

additional funding mechanisms to pay a thorough analysis if the preliminary data look promising. Hopefully, our new methods will show enough efficiency that we can adapt them to the canine studies.

B2. Accomplishment

We previously demonstrated that muscle-specific CRISPR/Cas9-mediated dystrophin correction can be accomplished in vivo following recombinant adeno-associated viral (rAAV) vector delivery. We have now shown that stable, systemic, long-term gene editing and expression of dystrophin following multi-exon deletion is dependent on preventing skeletal muscle turnover. Skeletal muscles of dystrophic mice display two phases of necrosis and regeneration (turnover): a rapid and severe crisis phase between approximately 3 to 8 weeks of age, followed by a slower and ongoing phase that continues throughout their lifespan. These cycles of degeneration and regeneration result in pronounced detrimental effects on long-term dystrophin expression following gene editing as evidenced by loss of vector genomes and corrected myonuclei in unprotected or partially corrected myofibers. To explore the 'dystrophin level'-dependence of this loss, we observed that co-delivery of a low dose of AAV-CK8e-microdystrophin (μDys) vector with AAV-CRISPR/Cas9 efficiently protected dystrophic skeletal muscles from turnover and significantly increased gene editing efficacy and the persistence of dystrophin expression from corrected nuclei, leading to improved skeletal muscle strength. In contrast, dystrophic cardiomyocytes, which do not undergo cycles of necrosis and regeneration, display persistent dystrophin expression from gene edited myonuclei in the absence of co-delivered micro-dystrophin. Essentially, this means that skeletal muscles are more sensitive to dystrophin levels than cardiac muscles, as has been seen previously with gene replacement methods by us and by others. While some methods of dystrophin editing, such as targeting single sites to effect exon skipping, can be quite efficient (e.g. the approach by Eric Olson's lab), correcting more complex mutations (including the more common ones), such as larger deletions that require editing of multiple exons, *is far less efficient*. Our results emphasize the critical need for highly efficient and rapid editing of dystrophin genes in skeletal muscles.

We have also tested editing in 2 dystrophic dogs. In this study we demonstrated successful correction of dystrophin expression in a large animal model of Duchenne muscular dystrophy following multiple intramuscular injections of the vectors. Here it was necessary to use gene editing to delete 3 or 4 exons of the canine gene (the primary target is a deletion of exons 6-8, but exon 9 often is naturally skipped as well, see figure below). This proof-of-principle study demonstrates that our approach to restore an open reading frame via multi-exon deletion is applicable to large animal models and shows promise for further development of the CRISPR/Cas9 system towards clinical translation into human DMD patients. The efficiency seemed better than in mice, but still generated useful levels of dystrophin expression. We hope to submit both these papers soon, they have been delayed as we keep accumulating additional data.



We have also been exploring some potential drawbacks to gene editing in muscle that suggest needed improvements to the delivery system. In a collaborative study with Bence Gyorgy's lab at Harvard, we showed a high frequency of AAV vector integration into mouse muscle target sites of the CRISPR/Cas9 machinery. In our approach we are targeting two exons of the murine dystrophin gene (exons 52-53) in *mdx*^{4cv} mice in order to delete those exons and restore an open-reading frame in the dystrophin mRNA. This need to effect two double-stranded breaks in the gene (to delete both exons) requires near simultaneous cutting at each guide RNA target. The first issue we encountered is that

a significant percentage of the target sites appear to cut, but then are quickly repaired by non-homologous end joining (NHEJ) BEFORE the second site can be cut. Consequently, the two exon region is not efficiently excised. Furthermore, many of these repaired cut sites were found, upon deep sequencing, to now harbor fragments of the AAV vectors used to deliver Cas9 and the guide RNAs. In other words, the use of AAV to deliver Cas9 is associated with a high degree of insertional mutagenesis whereby the vector integrates into the genome. Normally AAV rarely integrates into genomic DNA, but the rate of integration is enormously amplified by the generation of double-stranded breaks in the target DNA. Whether this presents a safety hazard is a bit unclear. It might in proliferating cells, but in our case, we use muscle-restricted promoter elements that prevent Cas9 expression in non-muscle (and dividing) cells. Thus we only see vector integration in post-mitotic, striated muscle cells, which are unable to from tumors.

To address the inefficiency problem generated by the need to cut and cleave two different target sites flanking multi-exon targets, we have adapted a method using 4 different guide RNAs and 4 different targets, and delivering these 4 guides on two different vectors injected at two different times. Thus, we deliver one set of guide RNAs to effect cleavage of the relevant region, then follow-up a few weeks later with a second set of guide RNAs located outside the first set. This significantly increased the rate/efficiency of editing. However, this approach requires immune suppression ding the first injection in order to enable a second set of injections. This was accomplished by using conventional immune suppression drugs, but more recently has been successfully performed in mdx/Rag mice (no B or T-cells).

Rigor:

Antibodies. We have generated numerous antibodies vs dystrophin, the dystrophin-glycoprotein complex, and other proteins related to our disease focus. We also reply on an extensive collection of antibodies generated by other labs or available via commercial entities. Antibodies are characterized by western blot and IF studies, this is fairly easily accomplished by testing WT and *mdx*^{4cv} mice for our dystrophin and DGC member antibodies, as they provide positive and negative controls. While formal periodic re-testing is not scheduled, these antibodies are almost always used in experiments with negative and positive controls. Should the antibodies' specificity and/or sensitivity change over time (as of yet unseen), such a change would be readily noticed due to the frequency of their use in well-controlled experiments.

Primary cells: Primary cells used for vector production (293 cells) are characterized by assessing yields and titer of reporter gene vectors that are routinely used in the lab.

Redacted by agreement

Mouse lines: Our mice are regularly verified for genotype/phenotype either by direct sequencing assays or as part of the experimental plan (assayed for dystrophin expression by western blot and immunostaining) for mdx mice, which do not express dystrophin or DGC complexes (the latter at least not at anywhere near normal levels. Control mice are obtained from [redacted] which does routine genotyping. CXMD dogs are always genotyped at birth and again before starting protocols, and all canines are monitored for neutralizing antibody titer vs AAV prior to enrollment in a study.

AAV vectors and plasmids: New plasmids inserts are always sequenced (in house or by synthesis companies when purchased) and tested after each plasmid prep by restriction digestion. AAV vectors are produced in-house in my lab. Vectors are purified on a combination of affinity columns, HPLC, and cesium gradient, depending on the serotype and downstream use. For some experiments such as large animal studies, vector is purified on cesium chloride gradients, which allows for visualization of both full and empty capsids and an empirical assessment of vector quality. Purified vectors are tittered and Q/C'd by HT1080 transduction when not using an MCK promoter, visualized on a protein gel & quantitated by real-time PCR and Southern blot, then tested in muscle by IM injection. This combination of methods allows for quality control that ensures the purity of the protein and the encapsulation of full-length vector genomes. Vector that is more than a year old is re-titered by southern blot and qPCR.

Carrying out the goals of the project (including dealing with success and failure), presenting research updates and writing manuscripts are a major training and professional development aspects of the work the post-docs in my lab do with me. We collaborate with several other labs at the UW in this project. Interacting with these other labs provides additional experience, training and development from their interactions with these labs and observations of how they function and how collaborative science is carried out. My post-docs and I also regularly and extensively discuss career development goals, plans and progress, including publications, learning & teaching opportunities, as well as timelines related to career advancement goals such as job applications, publications and grant submissions, etc. They are required to attend seminars on a regular basis and attend at least one journal club. Everyone in the lab presents their data on a rotating basis at our weekly lab meeting, and we alternate meeting with literature reviews. I like for them to also attend at least one meeting per year, possibly more if they have data for a presentation. There are no graduate students currently on this project.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Ng R, Banks GB, Hall JK, Muir LA, Ramos JN, Wicki J, Odom GL, Konieczny P, Seto J, Chamberlain JR, Chamberlain JS. Animal models of muscular dystrophy. Progress in molecular biology and translational science. 2012;105:83-111. PubMed PMID: 22137430; PubMed Central PMCID: PMC4872622; DOI: 10.1016/B978-0-12-394596-9.00004-4.
Complete	Bengtsson NE, Seto JT, Hall JK, Chamberlain JS, Odom GL. Progress and prospects of gene therapy clinical trials for the muscular dystrophies. Human molecular genetics. 2016 April 15;25(R1):R9-17. PubMed PMID: 26450518; PubMed Central PMCID: PMC4802376; DOI: 10.1093/hmg/ddv420.
Complete	Davey JR, Watt KI, Parker BL, Chaudhuri R, Ryall JG, Cunningham L, Qian H, Sartorelli V, Sandri M, Chamberlain J, James DE, Gregorevic P. Integrated expression analysis of muscle hypertrophy identifies Asb2 as a negative regulator of muscle mass. JCI insight. 2016 April 21;1(5). PubMed PMID: 27182554; PubMed Central PMCID: PMC4863241; DOI: 10.1172/jci.insight.85477.
Complete	Muir LA, Murry CE, Chamberlain JS. Prosurvival Factors Improve Functional Engraftment of Myogenically Converted Dermal Cells into Dystrophic Skeletal Muscle. Stem cells and development. 2016 October;25(20):1559-1569. PubMed PMID: 27503462; PubMed Central PMCID: PMC5035915; DOI: 10.1089/scd.2016.0136.
Complete	Bengtsson NE, Hall JK, Odom GL, Phelps MP, Andrus CR, Hawkins RD, Hauschka SD, Chamberlain JR, Chamberlain JS. Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nature communications. 2017 February 14;8:14454. PubMed PMID: 28195574; PubMed Central PMCID: PMC5316861; DOI: 10.1038/ncomms14454.
Complete	Bengtsson NE, Hall JK, Odom GL, Phelps MP, Andrus CR, Hawkins RD, Hauschka SD, Chamberlain JR, Chamberlain JS. Corrigendum: Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nature communications. 2017 June 23;8:16007. PubMed PMID: 28643790; PubMed Central PMCID: PMC5489999; DOI: 10.1038/ncomms16007.
Complete	Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. Nature communications. 2018 August 29;9(1):3497. PubMed PMID: 30158648; PubMed Central PMCID: PMC6115392; DOI: 10.1038/s41467-018-05843-9.
Complete	Hanlon KS, Kleinstiver BP, Garcia SP, Zaborowski MP, Volak A, Spirig SE, Muller A, Sousa AA, Tsai SQ, Bengtsson NE, Lööv C, Ingelsson M, Chamberlain JS, Corey DP, Aryee MJ, Joung JK, Breakefield XO, Maguire CA, György B. High levels of AAV vector integration into CRISPR-induced DNA breaks. Nature communications. 2019 September 30;10(1):4439. PubMed PMID: 31570731; PubMed Central PMCID: PMC6769011; DOI: 10.1038/s41467-019-12449-2.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?
C.5 OTHER PRODUCTS AND RESOURCE SHARING Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	CHAMBERLAIN, JEFFREY S	BA,PHD	PD/PI	EFFORT	EFFORT	EFFORT			NA
	N	Redacted by agreement	BS,PHD	Co-Investigator						NA
	N		PHD,MS	Co-Investigator						NA
	N			Research Scientist						NA
	N			Research Scientist						NA
	N			Research Scientist						NA
	N			Research Scientist						NA
	N		PHD,MS	Co-Investigator						NA
	N			Research Scientist						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Changes to other support.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Changes to other support
Jeffrey S. Chamberlain
New:

Private Source | Chamberlain JS (PI) | 05/01/2019 – 04/30/2020
Gene Therapy for DMD
This project is to explore directly gene editing as compared with gene replacement for DMD. It is a one-year gift pass-through grant

NIH P30 DK017047 | Kahn S (PI) | 01/01/2019 – 12/31/2019
UW Diabetes Res Cntr—VTMC
This is a subcontract from the UW Diabetes Center for Vector production

PENDING:

Pending Support

Pending Support

EXPIRED

NIH R01 AR40864-31 | Chamberlain JS (PI) | 04/01/1991 – 11/30/2025
Dystrophin replacement in mdx mice
This project is to explore dystrophin expression in satellite cells, to examine reversibility of dystrophy by µDystrophin in old animals, and to develop novel gene delivery vectors for muscle. **Grant expired 2/29/2020;**
Renewal to be resubmitted 3/5/2020

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: UNIVERSITY OF WASHINGTON	605799469	WA-007	850 Republican St HSB K-243b SEATTLE WA 981098055
UNIVERSITY OF WASHINGTON	605799469	WA-007	UNIVERSITY OF WASHINGTON 4333 Brooklyn Ave NE SEATTLE WA 981959472

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No