

Plan Overview

A Data Management Plan created using DMP Tool

DMP ID: <https://doi.org/10.48321/D1M66W>

Title: Single and Double AAV Somatic Gene Amelioration of Mouse Models of LAMA2 Related Dystrophy

Creator: Peter Yurchenco - **ORCID:** [0000-0003-0387-2317](https://orcid.org/0000-0003-0387-2317)

Affiliation: Rutgers, The State University of New Jersey (rutgers.edu)

Contributor: Laura Palumbo

Funder: National Institutes of Health (nih.gov)

Funding opportunity number: PA-20-184

Template: NIH-Default DMSP

Project abstract:

Laminin-211 (Lm211) is an ~800kDa glycoprotein of neuromuscular basement membranes (BMs) required for BM assembly, structure and functions. Missense and truncating mutations of the LAMA2 gene cause a congenital muscular dystrophy and neuropathy (LAMA2-Related Dystrophy, MDC1A). The clinical spectrum ranges from mild (ambulatory) to severe (non-ambulatory), depending on the mutation, with corresponding mouse models for the disease spectrum. The goal of this application is to develop BM structure-altering therapies in two mouse models. Our strategy employs non-replicating adeno-associated (AAV) delivery of genes coding for small laminin-binding linker proteins that restore missing Lm211 functions of defective or compensating laminins. The approach is built upon our experience studying BM assembly and structure-functions relationships with engineering of novel interactive proteins.

Aim I. The *dy2J/dy2J* mouse, a model for ambulatory dystrophy, bears a mutation within the alpha2LN domain that prevents polymerization. We found the disease phenotype and pathology are substantially ameliorated by AAV9 delivery of a gene coding for a laminin-binding protein (aLNNdDelG2'). **A.** To understand dose-benefit relationships, we will compare mice treated at different doses with evaluation of linker and laminin DNA/protein levels, ambulation, strength, and

histology. **B.** We will determine the degree of amelioration achievable at later AAV delivery ages. **C.** We plan to modify the polyA tail to increase expression.

Aim II. Most LAMA2-RD patients have no Lm-alpha2 expression. They remain non-ambulatory with profound muscle weakness, joint contractures, and life-threatening respiratory impairment, accompanied by neuropathy and seizures. The *Lama2*^{-/-} (*dy3K/dy3K*) mouse serves as a model. Here the Lm-alpha4 subunit is expressed in compensation, mostly as Lm411, and overall trimeric laminin expression is considerably reduced. Preliminary data reveal that muscle and peripheral neuropathy are ameliorated by simultaneous AAV9 expression of two small genes, one coding for aLNNdDelG2' and the other coding for miniagrin (*mag*) to link the Lm411 coiled-coil domain to the alpha-dystroglycan receptor. We plan to **(A)** extend the preliminary analysis to include determination of survival, ambulation, grip-strength, linker/endogenous DNA and protein levels, and histology, **(B)** evaluate the muscle-specific AAVMYO virus as an alternative for *mag*, and **(C)** design and evaluate single laminin-binding linker proteins carrying both needed activities, first in vitro and then in *dy3K* mice.

Start date: 09-05-2024

End date: 08-31-2028

Last modified: 07-08-2024

Copyright information:

The above plan creator(s) have agreed that others may use as much of the text of this plan as they would like in their own plans, and customize it as necessary. You do not need to credit the creator(s) as the source of the language used, but using any of the plan's text does not imply that the creator(s) endorse, or have any relationship to, your project or proposal

Single and Double AAV Somatic Gene Amelioration of Mouse Models of LAMA2 Related Dystrophy

Data Type

Types and amount of scientific data expected to be generated in the project:
Summarize the types and estimated amount of scientific data expected to be generated in the project.

Describe data in general terms that address the type and amount/size of scientific data expected to be collected and used in the project (e.g., 256-channel EEG data and fMRI images from ~50 research participants). Descriptions may indicate the data modality (e.g., imaging, genomic, mobile, survey), level of aggregation (e.g., individual, aggregated, summarized), and/or the degree of data processing that has occurred (i.e., how raw or processed the data will be)

The project is expected to produce collections of individual and summarized mouse measurements of weights, grip-strengths and ambulation (data entered manually into Excel) that are then organized for graphing and statistics in GraphPad Prism-10 (.prism) or SigmaPlot12.5 (.jnb) (similar to Prism). A second type of data are image files generated from scanned paraffin-embedded stained tissue sections (Leica Aperio ImageScopex64 .svs converted to .jpg files), images of immunostained frozen sections of muscle, nerve and other tissues (.ipl files converted to .tif or .jpg), morphometry measurements such as areas, fraction central nuclei, collagen fibrosis (manually entered into Excel files), images of immunoblots and Coomassie blue stained gels (.tif, .psd, .jpg). Image files are often analyzed in ImageJ/Fiji (fraction central nuclei, myofiber and muscle areas, area of fibrosis in muscle; amyelination plaque count and axon/myelination ratios, naked axon fraction in Remak bundles in peripheral nerve). The numerical data are saved in Excel for subsequent graphing and statistical analysis. Electron microscopic images are saved as .tif files or converted to smaller .jpg files. The cell culture data in which laminins and linker proteins are incubated with myotubes and Schwann cells are recorded as multiple immuno-microscopic 10x/20x field images that are converted to tif or jpg files then analyzed in ImageJ/Fiji to assess matrix coverage. These are then outputted into Excel for graphing and statistics.

In general, we plan to provide primary numerical data in generally accessible files, i.e. Excel files (xlsx or comma delimited .csv) and will provide primary data image files used for morphometry and other measurements as .jpg, or .tif files. The Excel files are generally less than 5 MB and .jpg image files less than 10 MB. Total files are expected to be less than 5 GB.

Scientific data that will be preserved and shared, and the rationale for doing so: *Describe which scientific data from the project will be preserved and shared and provide the rationale for this decision.*

Data will be shared openly. Data from individual mice will be in replicates, generally 3 or more per condition. Similarly, image analysis will use measurements from 3 or more mice per condition. Final cleaned data used for analyses will be shared. The format of primary data numerical data used to generate graphs and statistics will be provided as Excel files. Image files will be provided as .tif or .jpg files.

Metadata, other relevant data, and associated documentation: Briefly list the metadata, other relevant data, and any associated documentation (e.g., study protocols and data collection instruments) that will be made accessible to facilitate interpretation of the scientific data.

Metadata, i.e. short descriptions of the above data terms and labels, methods, instruments, and results that provide context to enable meaningful interpretation, will be stored along with the primary data as established by the repository. In addition, we will insert very short descriptions into Excel files.

Related Tools, Software and/or Code

State whether specialized tools, software, and/or code are needed to access or manipulate shared scientific data, and if so, provide the name(s) of the needed tool(s) and software and specify how they can be accessed.

Data are analyzed in specific programs with outputs available in mostly standard formats. No codes have been written. A list of programs used in the lab follows:

Excel (.xlsx): Spreadsheet data with Excel program available on most computers.

GraphPad Prism 10: This program is used to tabulate and organize data from Excel. It can convert individual measurements copied from Excel (or directly entered) into a variety of graphs (that we use to superimpose individual data points onto bar graphs of average and deviations). It also offers a considerable menu of statistical packages that are employed to determine significance. The program is offered by Rutgers at reduced rates. The output displays can be jpg and other image files along with text files for statistics.

SigmaPlot 12.5: This program (purchased) can similarly be used analyze data as Prism. Data can be saved as .jnb files or outputted as .tif., .jpg, pdf files.

Adobe Photoshop files with a variety of images can be saved as layered Adobe psd and more general tif files or as flattened image files (e.g. jpg). The pdf files require access to the program.

Our indirect fluorescence microscope (Olympus IX70) image output is in IP-Lab (.IPL) files that we convert into more accessible tif and jpg formats in ImageJ for analysis.

Aperio ImageScope viewer program for Leica scanned slides presents 40x image data as large .svs files. Regions of interest for analysis are converted to tif and jpg files.

ImageJ is also used to analyze images and covert file formats. The program offers multiple output file formats.

Standards

State what common data standards will be applied to the scientific data and associated metadata to enable interoperability of datasets and resources, and provide the name(s) of the data standards that will be applied and describe how these data standards will be

applied to the scientific data generated by the research proposed in this project. If applicable, indicate that no consensus standards exist

Data will be stored in common and open formats such as xlsx, tif, jpg, and commonly used scientific programs such as Prism, SigmaPlot and Aperio formats. Associated metadata will be as required by the intended data repository, Harvard Dataverse, which uses metadata based on DDI Codebook.

Data Preservation, Access, and Associated Timelines

Repository where scientific data and metadata will be archived: Provide the name of the repository(ies) where scientific data and metadata arising from the project will be archived.

Data and associated metadata will be shared and archived in the following open data repository:

Harvard DataVerse (<https://dataverse.harvard.edu>).

How scientific data will be findable and identifiable: Describe how the scientific data will be findable and identifiable, i.e., via a persistent unique identifier or other standard indexing tools.

The Harvard repository assigns datasets a citable, unique, persistent digital object identifier (DOI) to support data discovery, reporting and research assessment.

When and how long the scientific data will be made available: Describe when the scientific data will be made available to other users (i.e., no later than time of an associated publication or end of the performance period, whichever comes first) and for how long data will be available.

The scientific data will be made available to other users no later than the time of an associated publication, or end of the funded period, whichever come first. The data will be made available as long as allowed by the repository.

Access, Distribution, or Reuse Considerations

Factors affecting subsequent access, distribution, or reuse of scientific data: NIH expects that in drafting Plans, researchers maximize the appropriate sharing of scientific data. Describe and justify any applicable factors or data use limitations affecting subsequent access, distribution, or reuse of scientific data related to informed consent, privacy and confidentiality protections, and any other considerations that may limit the extent of data sharing.

No limitations are foreseen.

Whether access to scientific data will be controlled: State whether access to the scientific data will be controlled (i.e., made available by a data repository only after approval).

No restrictions to access will be requested.

Protections for privacy, rights, and confidentiality of human research participants: If generating scientific data derived from humans, describe how the privacy, rights, and confidentiality of human research participants will be protected (e.g., through de-identification, Certificates of Confidentiality, and other protective measures).

There are no human subjects in the proposed study.

Oversight of Data Management and Sharing

Describe how compliance with this Plan will be monitored and managed, frequency of oversight, and by whom at your institution (e.g., titles, roles).

Dr. Peter D. Yurchenco, the Principal Investigator of the proposed project, will manage and oversee compliance. Data backups are done at completion of experiments and analyses. Image files from scanned slides are stored in the cloud-based folder system, BOX, a service provided through Rutgers University. It is equipped with enterprise-grade security. Copies of data files are copied and maintained on a password-protected external hard drive that is updated almost daily, and copied to the P.I.'s password-protected laptop computer kept at the P.I.'s home. Thus, data are maintained on three separate devices.
