

## Plan Overview

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*A Data Management Plan created using DMPTool*

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

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### 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<sup>-/-</sup> (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.

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

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 tissues (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.

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, 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.

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 convert file formats. The program offers multiple output file formats.

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

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

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

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.

No limitations are foreseen.

No restrictions to access will be requested.

There are no human subjects in the proposed study.

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.

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