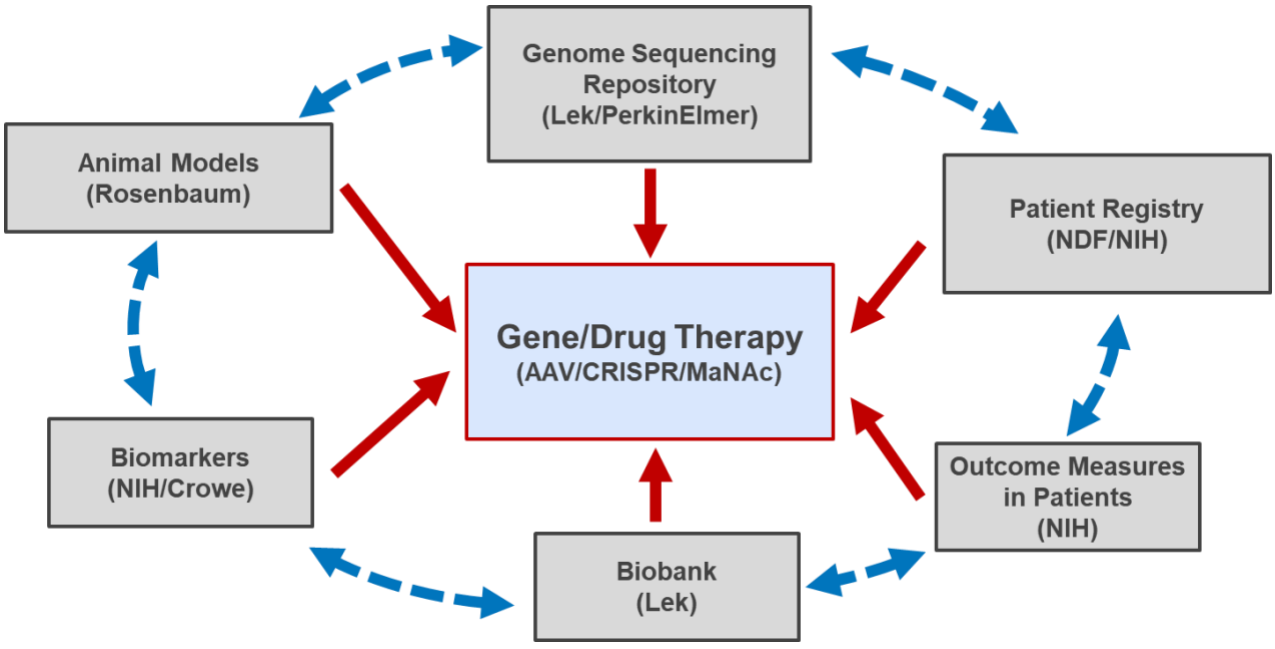
NEUROMUSCULAR DISEASE FOUNDATION
JOIN THE GLOBAL EFFORT TO CURE NEUROMUSCULAR DISEASES

Current Status of Therapy Development

Several factors contribute to the successful development of an effective therapy to HIBM

- Identification and optimization of a therapeutic approach (AAV-mediated gene therapy; gene correction strategies; drug replacement/development therapy)
- Development of outcome measures (critically important in diseases characterized by slow progression like HIBM)
- Identification of biomarkers to be used to validate efficacy as well as identify new drugs that can slow down disease progression.
- Establishing a patient registry to use for clinical trials.
- Generation of animal models to validate therapy efficacy.
- Repositories of data and biological specimens to identify biomarkers/outcome measures.



SUMMARY OF CURRENT PROPOSALS SUPPORTED BY NDF

Project 1. Development of animal models for GNE myopathy (Hadassah/Rosembaum
The goal of the project is to develop a mouse and a zebrafish models lacking expression of GNE. Progress reports submitted to the NDF show that both projects are on their way. Animal models are necessary to assess efficacy of any therapy and establish dose needed to achieve an effect. Furthermore, these models will enable to stimulate the interest and gain the attention of pharmaceutical companies interested in developing and marketing a cure for HIBM. The NDF has been aggressively pursuing the development of multiple models for the disease with the aim of obtaining at least one that shows the phenotype and the characteristics needed to be a useful model. Sofar, the only models available either don't show a consistent phenotype or they die too young to be able to evaluate possible outcomes. The results obtained so far, although still at an early stage, are very encouraging and demonstrate that at least one of the model (zebrafish) has been generated. Currently the NDF is promoting and is helping establishing collaborations among different scientists to ensure that the model becomes a valuable shared resource for the HIBM field.

Project 2. Biobank (M. Lek/ A. Lek)
The goal of this project is to establish a repository of cells from patients, family members and eventually animal models as a resource for the HIBM community of scientists and pharmaceutical companies for research purposes (including identification of biomarkers and to validate therapeutic applications). To date, the Lek lab was able to establish and bank 40 cell lines. All are available as fibroblasts (skin cells) as well as myoblasts (muscle cells). DNA and RNA have been collected and stored for upcoming sequencing and samples have been already shared with other investigators in the HIBM field (Noah Weisleder -Ohio State University, Kelly Crowe -Mount St. Joseph University). Future plans include further collection and storage of samples that contain different mutations from so as to obtain a good representation of all HIBM patients worldwide.

Project 3. Whole Genome Sequencing (WGS) (M. Lek/ PerkinElmer)
Of the 100 samples originally contracted with PerkinElmer (PE), a total of 40 samples have been collected and WGS has been carried out accordingly. Samples were obtained from NIH (8 samples, 2 families), India (18 samples), Israel (27 samples), Philadelphia (26 samples). One family (5 samples) overlap with NIH samples. Sequencing of 40 samples completed and raw data was shared by PE. Raw data is currently being processed and analyzed in the Lek lab.

Project 4. Identification of Biomarker (NIH/M. Huizing)
The proposal is focused on identify biomarkers and gene modifiers that could play a role in disease progression. Analyses could identify new players in the disease as well as new potential outcome measures to use in clinical trials to validate the efficacy of the therapy. Currently, there

are 22 GNE-M patients from the Phase 1-ManNAc trial and 12 patients from the Phase 2-ManNAc trial, whose blood has been tested for differences in free Neu5Ac from baseline levels. Quantitative lectin analysis on muscle biopsy slides at baseline and after 30 days of ManNAc therapy in 12 patients in the NIH Phase2-ManNAc trial show significant differences in hyposialylation of GNE-M muscle tissue after 30 days ManNAc therapy compared to baseline suggesting that this assay could be used as possible outcome measure although it requires invasive procedures like collection of muscle biopsies. Current studies are now focused on identify additional biomarkers which require less invasive procedures (Sialylated Blood Glycans, Plasma T-ST Ratios).

Project 5. CRISPR gene editing approaches to GNE (A. Lek)
The project is focused at conducting proof-of-concept studies in cells in vitro and at correcting the M743T mutation in patient fibroblasts. According to the most recent progress report, the cell lines and constructs needed to carry on the project have been successfully generated and results on the feasibility of using CRSPR-mediated gene editing approaches should be obtained by the end of the funded project.

Project 6. Lectin Staining Biomarker for GNE Myopathy (NIH/Crowe)
The goal of this proposal is to test levels of GNEM biomarkers in patient cells in vitro before and following gene therapy. Studies will then be extended to patient muscle biopsies as well as zebrafish models for the disease currently being established in the laboratory of Dr. Stella Mitrani-Rosenbaum and sponsored by the NDF. The results obtained are particularly important because, if successful, will enable to establish outcome measure to be used in clinical trials and are required by the FDA in order to receive approval of the therapeutic approach being tested.

Project 7. Compromised membrane repair as a potential pathologic mechanism in GNEM patients (N. Weisleder)
Exiting preliminary studies provided by Dr. Weisleider suggest that muscle cells isolated from GNE myopathy patients and obtained through the Lek lab) show to be more susceptible to damage than cells isolated from non-affected individual. This finding is particularly important because could explain the slow deterioration of muscles in patients. Furthermore, the studies will help establish important biomarkers and outcome measures to be used in future clinical trials which are critically needed at this stage of clinical development for HIBM. The assays being developed in the Weisleider lab could in fact become integral part of clinical practices in human trials and to identify different stages of the disease. In addition, the assay could be used to prove the efficacy of the gene therapy application being tested thus becoming a valuable tool to ensure FDA approval.

Project 8. Development of FDA-compliant gene therapy assays for GNE myopathy (Children Hospital/Martin)

The project is focused on developing a gene therapy clinical development plan to be used to demonstrate activity of AAV-mediated delivery of GNE into cells and muscles of mice. In particular, the project will establish a potency assay that effectively describes the biological activity of the AAV vector to be used, in this case a AAV.GNE gene therapy vector. This assay must be done annually on clinical lots of AAV to demonstrate that potency has not been lost, and it must also be done to demonstrate that the AAV to be used in patients has the necessary biological activity when it is administered. Dose-response study in wild type (C57Bl/6J) mice with AAV.GNE vector will be used to assess the dose and level of vector genome transduction needed to provide a one-fold elevation in GNE enzyme activity (i.e., functional gene replacement). These studies will provide data needed to determine levels of functional GNE overexpression required for gene replacement in all organs and the number of vector genomes that must be transduced to accomplish such changes.

Project 9. Activation of endogenous mutated GNE product by small compound (Yoshioka/ Nishino in Japan)

Dr. Yoshioka will undertake a drug discovery project aimed at identify compounds capable of activating the GNE/MNK kinase. In silico, computerized models will be used to identify drugs that are already available on the market and that could therefore be repurposed for HIBM patients. The goal is to identify drugs that activate GNE/MNK kinase and therefore elevates supply of sialic acid (SA) to the whole body, including muscles. The use of drugs that are already approved for the use in patients for other diseases may be subjected to less stringed regulatory issues and has the potential to reach the bed side in a shorter period of time compare to new compounds. If proven safe and effective, the drug could be used alone or in combination with gene therapy- mediated approaches to HIBM thus increasing the efficacy of the clinical approach.