An Open-Label Phase 2 Study of ManNAc in Subjects with GNE Myopathy

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Collaborating Institutions	None

PROTOCOL SUMMARY

Full Title:	An Open-Label Phase 2 Study of ManNAc in Subjects with GNE Myopathy
Principal Investigator:	Nuria Carrillo, M.D.
Sample Size:	N= 12
Accrual Ceiling:	30, with estimated 60% ineligible during screening
Study Population:	Subjects with GNE myopathy, either gender, age 18-80 years.
Accrual Period:	January 2015- June 2015
Study Design:	Phase 2, open-label, single-center study of ManNAc in subjects with GNE myopathy. The study will characterize the safety, tolerability and PK of multiple doses of ManNAc and long-term safety and efficacy of ManNAc.
Study Duration:	Start Date: January 2015. Projected End Date: July 2018
Primary Objective:	To assess the long-term safety, tolerability, pharmacokinetics, pharmacodynamics and biochemical effect of oral ManNAc to subjects with GNE myopathy.
Secondary Objectives:	To evaluate the effect of ManNAc on clinical measures and biomarkers of GNE myopathy and to identify clinical endpoints suitable for subsequent clinical trials.
Endpoints:	Safety evaluations, PK, PD, muscle strength, muscle MRI, functional measures, patient-reported outcomes, and biomarkers.

List of Abbreviations

Abbreviation	Explanation
AMAT	adult myopathy assessment tool
ARM	Advancement of Research for Myopathies
ADL	activities of daily living
AE	adverse event
ALT	alanine aminotransferase
α-DG	alpha-dystroglycan
AST	aspartate aminotransferase
AUC	area under the curve
AUC_{EXT}	percentage of the AUC that is extrapolated beyond the last measurable concentration
AUC_{INF}	area under the plasma concentration time curve from time 0 (predose) to infinity
AUC_{LAST}	AUC from time 0 to time of last measurable plasma concentration
BID	twice daily
BUN	blood urea nitrogen
CBC	complete blood count
CC	Clinical Center
CD	Clinical Director
CFR	Code of Federal Regulations
CK	creatine kinase
Cl	chloride
CL/F	apparent systemic clearance
CLIA	Clinical Laboratory Improvement Amendments
C_{MAX}	maximum observed plasma concentration
CMP	cytidine 5'-monophosphate
CMP-Neu5Ac	CMP-sialic acid
CO_2	carbon dioxide
CPK	creatine phosphokinase
CRF	clinical report forms
CRIS	Clinical Research Information System
CRU	clinical research unit
CTCAE	Common Terminology Criteria for Adverse Events

CTDB	Clinical Trial Database
CV	coefficient of variation
DEX-M74	Drug product of ManNAc
DMRV	Distal Myopathy with Rimmed Vacuoles
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGTP	gamma-glutamyl transpeptidase
GlcNAc	N-acetylglucosamine
GLP	Good Laboratory Practice
GMBB	Genetics and Molecular Biology Branch
GNE	human gene encoding the GNE protein, when mutated, causes GNE myopathy
Gne	murine gene encoding the mouse Gne protein
GNE	UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase
Gne	murine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase enzyme
HAP	Human Activity Profile
hCG	human chorionic gonadotropin
HED	human equivalent dose
HIBM	Hereditary Inclusion Body Myopathy
HIPAA	Health Insurance Portability and Accountability Act
hr	hour
IB	Investigator Brochure
IBM2	Inclusion Body Myopathy 2
IBMFRS	Inclusion Body Myositis Functional Rating Scale
ICF	informed consent form
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous
IVIG	intravenous immunoglobulin
K	potassium

Kg	kilograms
LC-MS/MS	liquid chromatography and tandem mass spectrometry
LD	lactate dehydrogenase
MAD	multiple ascending dose
ManNAc	N-acetyl-D-mannosamine
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
MGB	Medical Genetics Branch
MMT	Manual Muscle Testing
MTD	maximum tolerated dose
MVIC	maximal voluntary isometric contraction
n	number
Na	sodium
NCATS	National Center for Advancing Translational Sciences
NDF	Neuromuscular Disease Foundation
Neu5Ac	N-acetylneuraminic acid or sialic acid
NHGRI	National Human Genome Research Institute
ng	nanogram
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NOAEL	no observable adverse effect level
PD	Pharmacodynamics(s)
PDS	Pharmaceutical Development Service at NIH
PEP	phosphoenolpyruvate
PI	Principal Investigator
PIB	powder in bottle
PII	personally identifiable information
PK	pharmacokinetic(s)
PROs	patient-reported outcomes
PSA-NCAM	polysialylated neuronal cell adhesion molecule
PT	prothrombin time
PTT	partial thromboplastin time

QMA	Quantitative Muscle Assessment
RBC	red blood cell
RNA	ribonucleic acid
SAD	single ascending dose
SAE	serious adverse event
SD	standard deviation
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SRC	Safety Review Committee
STIR	short tau inversion recovery
$t_{½}$	terminal-phase half-life
T_{MAX}	time to C_{MAX}
TRND	Therapeutics for Rare and Neglected Diseases
UDP-GlcNAc	uridine diphospho-N-acetylglucosamine
Vz/F	apparent volume of distribution, terminal phase
WBC	white blood cell
λ_z	elimination rate constant

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1. PRECIS

GNE myopathy, previously known as hereditary inclusion body myopathy (HIBM), is a rare, autosomal recessive myopathy with onset in early adulthood that is characterized by progressive muscle weakness and atrophy, which leads to wheelchair use and dependent care. The causative gene, *GNE*, encodes the rate-limiting enzyme in the biosynthesis of sialic acid. While the exact pathophysiology of GNE myopathy remains unknown, decreased sialic acid production and subsequent hyposialylation of muscle glycoproteins are thought to be key factors leading to muscle deterioration in GNE myopathy. This hypothesis is supported by prevention of disease after administration of N-acetyl-D-mannosamine (ManNAc) in mouse models of GNE myopathy. A first-in-human, Phase 1 single ascending dose study evaluated the safety, pharmacokinetics, and pharmacodynamics of a single dose of 3,000, 6,000, or 10,000 mg drug product-grade ManNAc in subjects with GNE myopathy (ClinicalTrials.gov NCT01634750; IND No.78,091). ManNAc was safe and well-tolerated in all subjects who participated in this study. In this Phase 2, open-label, single-center study we propose to administer ManNAc orally to 12 subjects for days (30 months). The objectives of the study are to assess the safety, tolerability, pharmacokinetics, pharmacodynamics and biochemical efficacy of orally administered ManNAc in GNE myopathy subjects and to evaluate disease-related biomarkers and relevant clinical endpoints. In the first phase of pharmacokinetic assessment, two cohorts of 6 subjects will receive ManNAc at doses of 3,000 mg twice a day (6,000 mg per day) or 6,000 mg twice a day (12,000 mg per day) for 7 days while admitted to the NIH Clinical Center to assess PK and safety. Safety and tolerability will be assessed on an individual basis. In the second phase of the study, all subjects will receive treatment with ManNAc at a dose of 6,000 mg twice daily for the remainder of the study. Follow-up safety and efficacy evaluations will occur at 42 days, and at 91 (3 months), 182 (6 months), 365 (12 months), 548 (18 months), 730 (24 months) and 912 (30 months) days. Safety lab evaluation will be performed also at 456 (15 months), 638 (21 months) and 820 (27 months) days either at the NIH clinical center or subjects' home laboratory or physician's office. Final dosing will occur at the 30-month visit. After the final dose, all Grade $\geq$2 AEs at least possibly related to study drug will be followed to resolution. Safety will be evaluated by adverse events (AEs), clinical laboratory tests, vital signs, and physical examinations. PK will be assessed for plasma ManNAc and Neu5Ac. Biochemical efficacy will be measured by change in the sialylation of proteins and clinical efficacy will be assessed using a battery of clinical assessments deemed to be relevant based on disease natural history.

2. OBJECTIVES AND SPECIFIC AIMS

2.1. Primary Objectives

The primary objectives of this study are:

- To assess the safety and tolerability of multiple doses of orally administered ManNAc to subjects with GNE myopathy;
- To examine plasma pharmacokinetics (PK) and pharmacodynamics (PD) of multiple doses of orally administered ManNAc to subjects with GNE myopathy;
- To determine the biochemical effect of ManNAc, as assessed by sialylation of proteins.

2.2. Secondary Objectives

The secondary objectives of this study are:

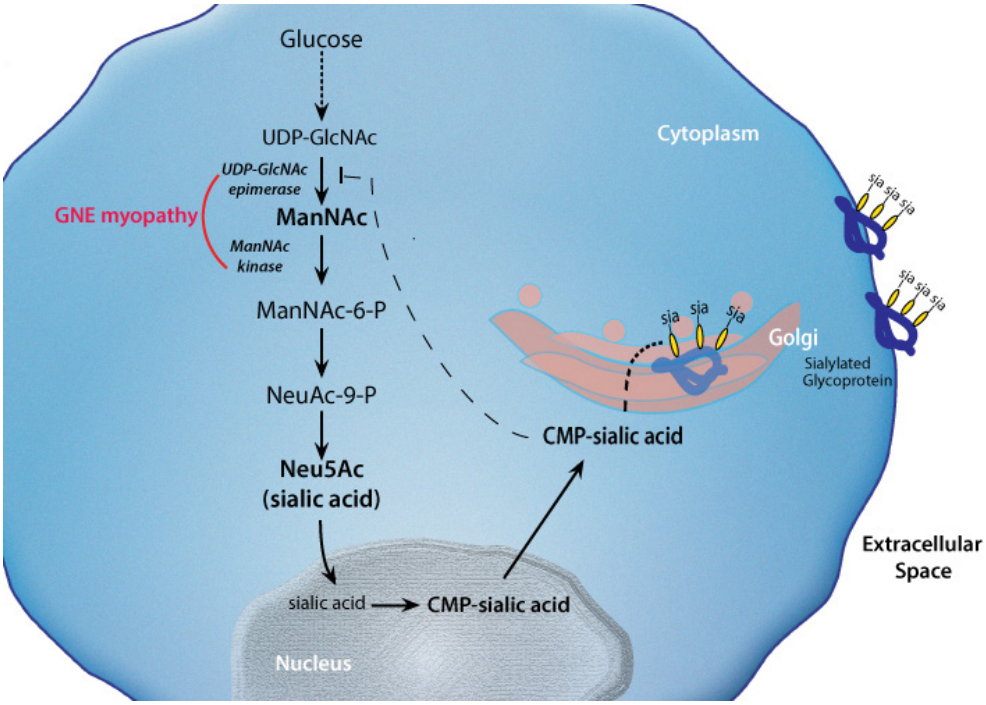
- To evaluate the effect of ManNAc on disease-relevant biomarkers;
- To measure the effect of ManNAc on clinical aspects of GNE myopathy and identify clinical endpoints suitable for subsequent clinical trials.

3. BRIEF RATIONALE AND BACKGROUND

3.1. GNE Myopathy

GNE myopathy is a rare autosomal recessive muscular disease[1-3] with an estimated prevalence of ~6:1,000,000[4]. Previous names of the disease include Hereditary Inclusion Body Myopathy (HIBM), Inclusion Body Myopathy 2 (IBM2), Distal Myopathy with Rimmed Vacuoles (DMRV), Nonaka Myopathy or quadriceps-sparing myopathy (Q-IBM). To avoid confusion, an international consortium has recently unified the nomenclature to GNE myopathy[5]. Patients present in early adulthood with distal weakness (foot drop), secondary to anterior tibialis muscle involvement. The disease slowly progresses to cause atrophy and weakness of the distal muscles of the lower extremities, followed by thigh muscles, with relative sparing of the quadriceps[1, 6]. The upper extremities are affected typically 5-10 years after onset of symptoms[1]. Findings on muscle biopsy typically include 'rimmed" vacuoles, lack of inflammation and hyposialylation. This relentlessly progressive disease results in marked disability, including wheelchair use within 1 or 2 decades after the onset of symptoms, and ultimately dependent care in some patients[1, 7]. There is no treatment available for GNE myopathy, but there is encouraging preclinical evidence that sialylation-increasing therapies, including sialic acid or N-acetyl-D-mannosamine (ManNAc) could provide benefit for affected individuals (Section 3.3).

Figure 1. Sialic Acid Biosynthetic Pathway



GNE myopathy is caused by biallelic mutations in *GNE*, the gene that encodes the rate-limiting bifunctional enzyme in sialic acid biosynthesis, UDP-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase[8] (Figure 1). Over 150 *GNE* gene mutations have been reported in GNE myopathy patients worldwide, in either the epimerase or the kinase encoding domains of the enzyme[4]. N-acetylneuraminic acid (Neu5Ac, generally referred to as 'sialic acid') is the most abundant mammalian sialic acid and the precursor of most other sialic acids. Sialic acids are negatively charged terminal sugar moieties on glycoproteins and glycolipids[9, 10], where they mediate several biological processes and play essential roles in disease processes[9-11]. Sialylation appears to be critical for mouse development, since the *Gne* knock-out results in embryonic lethality in the mouse model[12]. *GNE* mutations decrease sialic acid production and, consequently, sialylation is decreased, i.e., the incorporation of sialic acid into glycoproteins and glycolipids[13, 14]. Hyposialylation appears to be a major cause of the myopathy of GNE deficiency, since administration of sialic acid or its precursor, ManNAc, prevents or arrests the development of disease in mouse models of

GNE myopathy[15, 16]. Although overall sialylation in GNE myopathy cells and tissues may appear normal[17, 18], specific glycoproteins and glycolipids are hyposialylated in GNE myopathy muscle, including alpha-dystroglycan (α-DG)[19], polysialic acid on neural crest adhesion molecule (PSA-NCAM)[20], neprilysin[21], the GM3 ganglioside[22], or O-linked glycans in general[23] (See Figure 8).

3.2. ManNAc Description and Pathway

N-acetyl-D-mannosamine monohydrate (ManNAc) has a molecular weight of 239.2 g/mol. ManNAc will be used in this study as *drug product* of ManNAc. ManNAc is a naturally occurring uncharged monosaccharide, the first committed precursor for the biosynthesis of Neu5Ac (sialic acid), and a substrate of the GNE enzyme (Figure 1). Neu5Ac is a negatively charged molecule, which consists of ManNAc in an ether linkage with D-pyruvic acid. In contrast to Neu5Ac, which is a negatively charged molecule, ManNAc is the only neutral molecule in the sialic acid biosynthesis pathway. As such, it is expected to readily reach the intracellular space, as confirmed by *in vitro* studies[2, 24].

3.3. Natural History Study

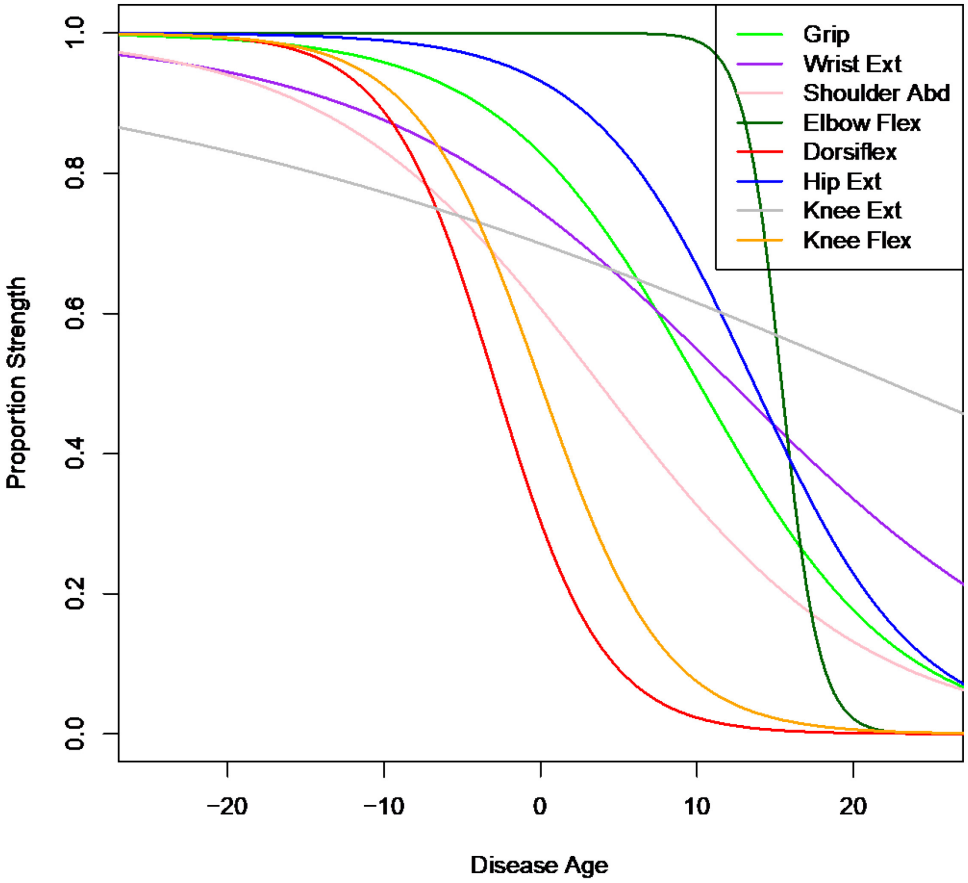
Patients with GNE myopathy have been followed on a prospective, single-center Natural History Study, NIH Protocol 11-HG-0218 "A Natural History Study of GNE Myopathy" (ClinicalTrails.gov Identifier: NCT01417533), hereafter referred to as the "Natural History Study" at the NIH Clinical Center (NIH CC). This study started in September 2011 to define the progression, potential endpoints and biomarkers of GNE myopathy.

3.3.1. Progression Model GNE Myopathy

A disease progression model was developed utilizing the natural history data of 32 GNE myopathy subjects, to determine the average rate of decay of selected muscle groups. The primary endpoint used was Quantitative Muscle Assessment (QMA)[25]. For each available QMA measurement a corresponding predicted muscle score is calculated based on the subject's age, gender and BMI[26] and the proportion of QMA muscle score relative to the predicted score, referred to as proportion of strength, was used. The primary endpoint of the disease progression model is the proportion of strength across the primary muscle groups: 1) Ankle dorsiflexion (anterior tibialis muscle), 2) Knee flexion (hamstrings), 3) Grip, 4) Hip extension, and 5) Knee extension (quadriceps), and 6) Shoulder abduction (deltoid).

The stage of disease varies among subjects, so the model accommodates subjects at varying stages of the disease by estimating a disease age for each subject based on their decline in the proportion of muscle strength (QMA muscle score relative to predicted muscle score) within 5 primary muscle groups. Disease age zero is defined when knee flexion is .50 of the maximum muscle strength. Given this estimate of disease age we are able to align the subjects and estimate an expected decay of each of the 5 primary muscle groups relative to one another and as a function of disease age (Figure 2). This allows us to understand the rate of progression of muscles groups relative to one another and to make subject-specific predictions of their decline.

Figure 2. Expected Progression of Muscle Groups



The expected proportion of muscle strength for each of the 5 primary muscle groups is plotted on the y-axis and the disease age is plotted on the x-axis. Note the slow progression of Knee Extension (quadriceps) when compared to other muscle groups.
Abbreviations: Dorsiflex: Ankle Dorsiflexion; Flex: Flexion; Ext: Extension

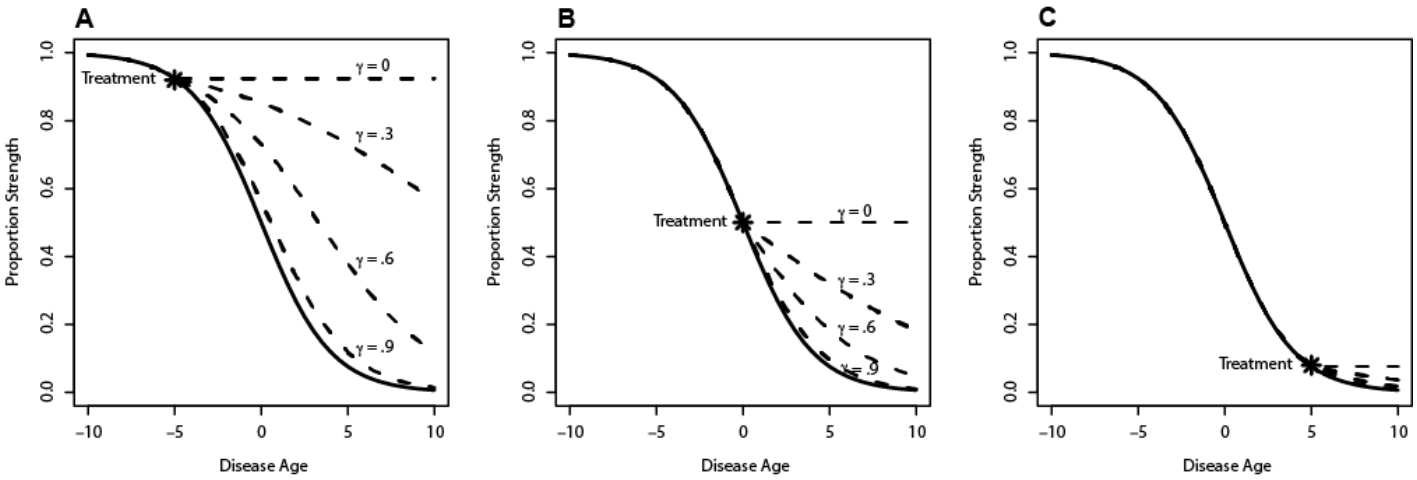
3.3.1.1. Determining Treatment Effect using the Progression Model GNE Myopathy

The disease progression model can be used to determine whether an intervention changes the rate of disease progression compared to the natural progression under no intervention. The rate of decay for each muscle (at any given disease age) is estimated in the model. To determine whether a therapy affects disease progression, the model incorporates a treatment effect parameter (referred to as gamma, γ) that determines whether the intervention slows the rate of decline compared to no intervention. If γ is equal to 1 there is no treatment effect (as the rate of progression is the same with intervention than with no intervention). If γ is less than 1, an intervention slows the rate of progression as compared to no intervention. If γ = 0.5, the treatment slows the rate of muscle progression by 50%. If γ equals zero, the treatment has stopped the rate of progression (Figure 3).

We have determined that using muscle strength as measured by QMA as a primary endpoint and the progression model as primary analysis, significantly increases power and reduces the number of subjects required to determine treatment effect in clinical trials when compared to other tested endpoints. To inform the design of the clinical trial to determine efficacy of a potential therapy for GNE myopathy, utilizing the primary efficacy endpoint and analysis described above, simulations of the design under multiple scenarios were performed. Scenarios included different treatment effects (γ), number of patients, duration of treatment, randomization and frequency of visits. For each scenario 1,000 trials were simulated. It was determined that a trial enrolling 50 subjects, a 3:1 randomization (treatment:placebo),

duration of 2 years and follow-up visits every 6 months would have the following conditional probabilities of trial success: if the treatment slows the rate of decline by ≥50% (γ≥0.5), 25% (γ =0.25) and 0% (γ =0), the probabilities of trial success would be ~98.8%, 75.5% and <1%, respectively. Therefore, the probabilities of trial success are conditional of an assumed effect size. By extending the current study, we will gain understanding of the magnitude of effect size of ManNAc, giving us important information to refine the design of our upcoming pivotal clinical trial.

Figure 3. Interpretation of treatment effect (γ) in the Disease Progression Model



The solid line corresponds to progression of muscle decline without an intervention. The dashed-lines represent different treatment effects, γ. If γ is equal to 1 there is no treatment effect. If γ is less than 1, the treatment slows the rate of progression. If γ = 0.5, the treatment slows the rate of muscle progression by 50%. If γ equals zero, the treatment has stopped the rate of progression.

3.4. Nonclinical Studies of ManNAc

3.4.1. Efficacy of ManNAc in Mouse Models

There is *in vitro* and *in vivo* evidence that ManNAc restores sialic acid production and increases the sialylation of hyposialylated glycoproteins and glycolipids (glycans) in disease models of GNE myopathy. Selected studies of ManNAc are summarized below. Additional details are located in the Investigator Brochure (IB).

- A gene-targeted knock-in *Gne* mouse homozygous for the Persian-Jewish GNE myopathy founder mutation, p.M743T (hGNE2 protein nomenclature, GenBank accession NP_001121699; in previous GNE nomenclature called p.M712T)[5], died within 72 hours after birth. Oral administration of 1,000 mg/kg/day of ManNAc (Human Equivalent Dose (HED) 5,600 mg/day) to pregnant and nursing mice rescued survival of mutant pups beyond 72 hours and was associated with increased enzymatic activity of UDP-GlcNAc 2-epimerase[27].
- A transgenic mouse model for a founder mutation in Japanese patients on a *Gne* null background (*Gne*$^{-/-}$h*GNE*D207V-Tg) (originally called *Gne*$^{-/-}$h*GNE*D176V-Tg), recapitulated the muscle phenotype of GNE myopathy, developing progressive muscle weakness starting at 10 weeks and by 40 weeks of age showing significant changes in muscle pathology and biochemistry similar to that of GNE myopathy patients. Prophylactic continuous oral treatment of these mice with ManNAc at 20 mg/kg/day (HED 112 mg/day), 200 mg/kg/day (HED 1120 mg/day), and 2,000 mg/kg/day (HED

11,200 mg/day), starting at week 5 resulted in prevention of muscle weakness and atrophy (reduced or absent rimmed vacuoles and intracellular amyloid inclusions), improved survival, and improved sialylation of membrane-bound glycans in muscle and other tissues. All treatment groups had an improved muscle phenotype and increased sialylation after ManNAc administration[16].

3.5. Clinical Use of ManNAc

3.5.1. 1966 Study in Humans

There is a single 1966 report of 6 subjects receiving single oral doses of ManNAc. Two subjects received single 5,000 mg oral doses and 4 subjects received single 10,000 mg oral doses. ManNAc was reported to have a "pleasant bland sweetish" taste and no side effects were reported[28].

3.5.2. Anecdotal Use of ManNAc

There is some anecdotal evidence of GNE myopathy patients using ManNAc (in doses up to approximately 12,000 mg per day) from a non-pharmaceutical source and without medical supervision. In general, ManNAc was well tolerated. Available information on these patients is provided in the IB.

3.5.3. Clinical Study 12-HG-0207

This single-ascending dose (SAD) study (ClinicalTrials.gov Identifier: NCT01634750) that evaluated the safety, tolerability, PK, and PD of ManNAc in subjects with GNE myopathy started in September 2012 and completed dosing in May 2013. ManNAc was used in this study at doses of 3,000 mg, 6,000 mg and 10,000 mg. Cohort 1 included 6 subjects randomized in a 2:1 ratio to receive either ManNAc (n=4) or mannitol as placebo (n=2). Cohorts 2 and 3 included 8 subjects each randomized in a 3:1 ratio to receive either ManNAc (n=6) or placebo (n=2). PK analysis from the SAD study showed that ManNAc was well-absorbed and free Neu5Ac concentrations in plasma increased after ManNAc administration and were maintained for 24-48 hours (Table 1).

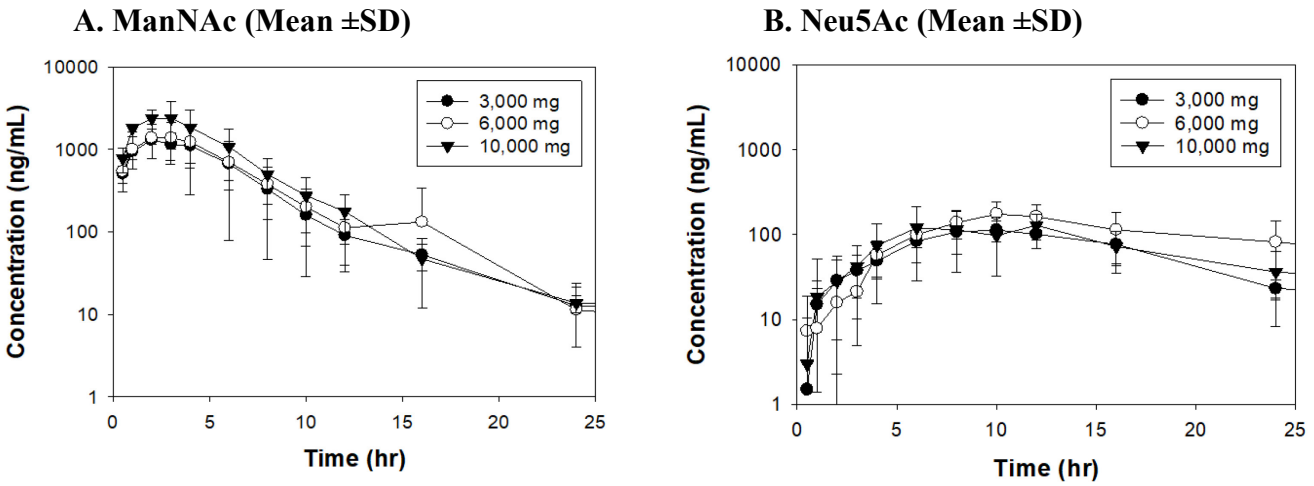
3.6. Pharmacokinetics and Pharmacodynamics

Quantification of ManNAc and Neu5Ac in human plasma was performed using a validated liquid chromatography and tandem mass spectrometry (LC-MS/MS) method with an assay range of 10 to 5,000 ng/mL. As part of the SAD study, mean plasma ManNAc levels observed in plasma controls (n=6) was 43.5 ng/mL (SD 4.47; CV 10.3%). The mean pre-dose plasma ManNAc concentration was 53 ng/mL in GNE myopathy subjects (n=24). The mean plasma Neu5Ac concentration in plasma controls (n=6) and pre-dose in GNE myopathy subjects (n=24) was 130 ng/mL, and 150 ng/mL, respectively. Plasma ManNAc and Neu5Ac concentrations did not increase in GNE myopathy subjects that received mannitol as placebo (n=6), indicating that mannitol had no effect on plasma ManNAc or Neu5Ac concentration.

Oral ManNAc was absorbed rapidly and exhibited a short half-life (~2.4 h). Following administration of a single dose of ManNAc, there was a significant and sustained increase in plasma unconjugated free sialic acid (Neu5Ac) (T_{max} of 8-11 h). Neu5Ac levels remained above baseline 48 h post-dose in subjects who received a dose of 6,000 or 10,000 mg (Figure 4). Since the excretion of Neu5Ac is very rapid, with 70% of the sialic acid is found in the urine within 30–60 min after intragastric administration[16], our findings strongly suggest that orally-administered ManNAc is effectively used as a substrate for intracellular Neu5Ac biosynthesis in subjects with GNE myopathy, including subjects homozygous for kinase domain mutations in the *GNE* gene. These findings provide further evidence that kinases other than ManNAc

kinase, which is deficient in GNE myopathy, can phosphorylate ManNAc[29]. Following a single dose of ManNAc, *in vivo* exposures (C_{max} and AUC) of ManNAc increased less than dose-proportionally (Table 1), suggesting a rate-limiting effect in either the membrane transport of ManNAc or on the enzymatic activity of the Neu5Ac biosynthesis pathway. The PK parameters of a single dose of ManNAc administered orally (fasted) to subjects with GNE myopathy are shown in Table 1.

Figure 4. ManNAc and Neu5Ac Plasma Concentration after Single Oral Dose of ManNAc Administered to Fasting GNE Myopathy Subjects



Mean (±SD) corrected concentration-time profiles. Correction was made by subtracting ManNAc or Neu5Ac concentration (C0) at t=0 for each subject.

Table 1. PK and PD Parameters for Single Dose of Oral ManNAc to Fasting GNE Myopathy Subjects

Dose	T_{max}[1]	C_{max}[2]	C_{max} Ratio		Mean $AUC_{0-\infty}$	AUC Ratio	
(mg)	(hr)	(ng/mL)	Dose Compared	Ratio	(ng*hr/mL)	Dose Compared	Ratio
Plasma ManNAc							
3,000	2	1,451	6,000 mg/3,000 mg	0.9	8,244	6,000/ 3,000 mg	1.2
6,000	2.5	1,336	10,000 mg/6,000 mg	1.9	9,552	10,000/6,000 mg	1.5
10,000	2	2,585	10,000 mg/3,000 mg	1.8	14,161	10,000/3,000 mg	1.7
Plasma Free Neu5Ac							
3,000	10	115	6,000 mg/3,000 mg	1.6	2147	6,000/ 3,000 mg	1.9
6,000	11	181	10,000 mg/6,000 mg	0.8	4171	10,000/6,000 mg	0.7
10,000	8	143	10,000 mg/3,000 mg	1.2	3007	10,000/3,000 mg	1.4

[1]Median; [2] Geometric Mean. PK calculations were conducted with baseline adjustment by subtracting ManNAc or Neu5Ac concentrations at t=0 for each subject.

3.7. Biomarkers

3.7.1. Intracellular Biomarkers

Plasma concentrations of free Neu5Ac in subjects with GNE myopathy did not differ significantly from controls (see Section 3.5.3.2) likely because the intracellular biosynthesis only accounts for a proportion of the plasma sialic acid. Therefore, the measurement of intracellular biomarkers may be a more sensitive measurement in subjects with GNE myopathy. CMP-sialic acid is the end-product of the sialic acid biosynthesis pathway. This nucleotide-activated form of sialic acid is the donor of sialic acid to nascent glycoconjugates in the Golgi apparatus (Figure 1) and therefore may serve as a marker of successful restoration of the intracellular synthesis of biologically active sialic acid. The cellular CMP-sialic acid level is approximately 4-fold greater than free sialic acid. An LC-MS/MS method is being developed and validated to measure intracellular CMP-sialic acid and Neu5Ac levels in human white blood cells (WBC) pellets.

3.7.2. Lectin Staining of Muscle Biopsies

Hyposialylation of muscle proteins is thought to play a major role in GNE myopathy, re-sialylation of muscle proteins would prove biological efficacy. Furthermore, hyposialylation has been correlated with decreased muscle performance in heterozygous GNE knockout mice[30]. Lectin staining has been useful to demonstrate hyposialylation particularly of O-linked glycans in the muscle of the mouse model and subjects with GNE myopathy[1, 23, 31]. Sialylation of muscle glycans recovered to normal levels in our GNE myopathy mouse model after ManNAc therapy[31]. This method is the only reported reproducible, quantifiable assessment of muscle glycan hyposialylation in GNE myopathy to date. We feel that these convincing lectin-staining data justify acquiring muscle biopsies from GNE myopathy subjects in this study at baseline and after 3 months of therapy (see Section 5.2.4.)

3.7.3. Plasma Sialylated Proteins

Proteins that become sialylated in the Golgi and secreted in the plasma may serve as markers of the protein sialylation, in particular those with mucin-like O-glycan structures. We have data that show the ratio of the plasma O-glycan structures Thomson-Friedenreich (T)-antigen versus Sialyl T-antigen (T/ST ratio) is abnormal in subjects with GNE myopathy[32]. We have anecdotal evidence that the plasma T/ST ratios normalized in GNE myopathy subjects that received sialylation-increasing therapies (including IVIG, off-label ManNAc, sialic acid). Exploratory glycan analysis can also be performed in subjects' blood as a way of identifying hyposialylated proteins that may serve as biomarkers.

3.8. Safety of ManNAc

3.8.1. Nonclinical Safety Evaluation

3.8.1.1. 90-Day Rat and Dog Oral Toxicology Studies

The safety of ManNAc has been demonstrated by nonclinical 90-day toxicology studies conducted to support the use of ManNAc in rats (IND 78,091_vol08_11.2.4.3A_2012 06 27.pdf) and dogs (IND 78,091_vol11_11.2.4.6A_2012 06 27.pdf). Adverse effects noted were mainly gastrointestinal and included loose feces, mild mucosal hyperplasia of the colon, mild increase in alanine transaminase (ALT) and minimal to mild hepatocellular hypertrophy. The doses and length of the trial proposed in this protocol are supported by these 90-day toxicology studies (see Appendix L).

Table 2. Maximum Recommended Starting Dose Based on 90-Day Toxicology Studies

Species	NOAEL (mg/kg)	Conversion factor	HED (mg/kg)	Safety factor	MRSD (mg/kg)	MRSD *70 kg (mg/day)
Rats	12,000	*0.16	1,920	10	192	13,440
Dogs	6,000	*0.54	3,240	10	324	22,680
To convert Animal Dose in mg/kg to Human Equivalent Dose (HED) in mg/kg, multiply animal dose by conversion factor based on Body Surface Area. Maximum Recommended Safe Dose (MRSD) in mg/kg = HED/safety factor						

Given the findings in the toxicology studies, effects that may be seen in humans include loose stools, anemia, decreased albumin, increased BUN, increased transaminases and abnormal urinalysis, all of which are monitored as part of this protocol (See 5.2.2). Given our prior experience on the Single Ascending Dose trial (See 3.7.2.1) loose stools may be expected.

3.8.1.2. 26-Week Rat and 39-Week Dog Oral Toxicology Studies

The safety of ManNAc has been demonstrated by nonclinical toxicology studies of 26-weeks in rats and 39-weeks in dogs (Summarized in Appendix L; Tables 6 and 7):

- HLS study number BEQ0001 "Toxicity Study by Oral Gavage Administration to Beagle Dogs for 39 Weeks Followed by a 4 Week Recovery Period", Huntingdon Life Sciences (final report issued October 17, 2014): The administration of ManNAc (ManNAc) was well tolerated and did not result in any evidence of treatment-related adverse toxicity in Beagle dogs after 39 weeks of oral gavage administration with N-Acetyl-D-mannosamine monohydrate (ManNAc) at doses of up to 4,500 mg/kg/day (1,500 mg/kg/dose three times daily), or after the 4 week recovery period.
- HLS study number BEQ0005 "Toxicity Study by Oral Gavage Administration to Sprague Dawley Rats for 26 Weeks Followed by a 4 Week Recovery Period", Huntingdon Life Sciences (final report issued December 9, 2014)

Table 3. Maximum Recommended Starting Dose Based on Long-Term Toxicology Studies

Species	NOAEL (mg/kg/d)	Conversion factor	HED (mg/kg/d)	Safety factor	MRSD (mg/kg)	MRSD *70 kg (mg/day)	Target Organs
Rats	12,000	*0.16	1,920	10	192	13,400	GI, liver, adrenal, kidney
Dogs	4,500	*0.54	2,430	10	243	17,000	GI, liver, kidney
* Equivalent surface area conversion factor Maximum Recommended Safe Dose (MRSD) in mg/kg = HED/safety factor							

Given the findings in the toxicology studies, effects that may be seen in humans include loose stools, anemia, decreased albumin, increased BUN, increased transaminases and abnormal urinalysis, all of which are monitored as part of this protocol (See 5.2.2). Given our prior experience on the Single Ascending Dose trial (See 3.7.2.1) loose stools may be expected.

3.8.2. Clinical Safety

3.8.2.1. 12-HG-0207 (ClinicalTrials.gov Identifier: NCT01634750)

On the single-ascending dose (SAD) study**,** ManNAc was safe and well tolerated. All adverse events (AEs) observed in the study were Common Terminology Criteria for Adverse Events (CTCAE) Grade 1 or 2. There were no serious adverse events (SAEs). The frequency of AEs in Cohorts 1 and 2 (75% and 33%, respectively) was not higher than the frequency of AEs in subjects who received placebo (83%). Half of the subjects at the received 10,000 mg dose, had a single, self-resolving episode of loose stools (Grade 1) shortly after ingestion of ManNAc, which was not associated with abdominal pain or other gastrointestinal symptoms. Diarrhea was not reported at lower doses (Cohorts 1 and 2) or in the placebo group and was likely caused by unabsorbed ManNAc in the gastrointestinal tract, suggesting that absorption of ManNAc is saturated when giving 10,000 mg as a single dose. Therefore a single dose of 10 grams was considered the Maximum Tolerated Dose. See Appendix F for a list of study AEs.

3.8.2.2. 15-HG-0068 Open-Label Phase 2 Study (ClinicalTrials.gov Identifier: NCT02346461)

There have only been no Serious Adverse Events observed as part of this Open-Label Phase 2 Study. The SRC review included safety through 24 months on all subjects. The reviewed safety information included adverse events (AEs), ECGs, vital signs, physical assessments and clinical laboratory data. Eight subjects remain on 6 grams twice daily. Four subjects have withdrawn consent, two of which discontinued due to gastrointestinal tolerability, another planning on getting pregnant and another was withdrawn due to noncompliance. AEs for the cohort from the latest SRC review are in Appendix G. Due to 8 out of 12 subjects participating in this trial, demonstrated gastrointestinal tolerability issues or AEs related to ManNAc and 16% had tolerability issues significant enough to discontinue their participation, we have attempted to better understand the etiology of the decreasd tolerability. The clinical manifestations together with PK evaluations, strongly suggest that unabsorberd ManNAc in the gastrointestinal tract is the likely cause. As discussed below in Section 3.9.1, we believe absorption of ManNAc can be increased by 20% by giving the same daily dose of ManNAc (12 grams) divided three times (4 grams TID) instead of twice daily (6 grams BID).

One subject in the study had Grade 3 hypertryglyceridemia which was determined to be probably related to ManNAc. As higher triglycerides were also seen in toxicology studies (see Table 10), hypertriglyceridemia is a risk associated with ManNAc.

3.8.3. Preliminary Findings 15-HG-0068 Open-Label Phase 2 Study

3.8.4. Pharmacokinetic Model for ManNAc and Sialic Acid in GNE Myopathy Subjects

Serial-sampled plasma ManNAc and Neu5Ac concentrations obtained from the SAD study (see Section 3.5.3) and from this Open-Label Phase 2 study were used to develop a semi-mechanistic population model to simultaneously characterize ManNAc and Neu5Ac plasma concentrations following single or repeated oral doses of ManNAc to GNE myopathy subjects. The model showed that oral bioavailability of ManNAc decreased with increase in oral dose, likely due to decreased absorption. Simulations using this PK model also demonstrated that administering the same daily dose divided in three doses (4,000 mg every 8 hours) rather than twice daily can improve tolerability, increase bioavailability by ~20%, and boost ManNAc and Neu5Ac exposures (See Table 4). Based on this, we will evaluate the PK of 4 grams TID dosing during the 30-month visit.

Table 4. Simulations of ManNAc Dose and Frequency using the Semi-Mechanistic Population PK Model for GNE Myopathy.

ManNAc dose and frequency	ManNAc CSS ave Mean (ng/mL)		Neu5Ac CSS ave Mean (ng/mL)	
	on Day 1	On Day 30	on Day 1	on Day 30
4,000 mg TID	923	1150	189	965
6,000 mg BID	878	952	219	826
12,000 mg QD	693	698	254	645

*Based on simulation run N=90 in a structural PK/PD model.
Abbreviations: CSS, steady state concentration; TID, three times daily; BID, twice daily; QD, once daily.

3.8.5. Muscle Strength

One of the secondary objectives of the Phase 2 study is to evaluate suitable endpoints for future trials. Because there is muscle atrophy and replacement of muscle by fibro-fatty tissue as part of the disease process, reversal of disease progression is unlikely. Rather, a slowing in the rate of progression is the expected treatment effect.

As part of this, muscle strength measured by QMA has been collected at every visit. All 12 patients participating in the open-label study were prior participants of the Natural History Study, and therefore, their 'natural history' rate of progression (prior to ManNAc administration) has been determined.

To evaluate the performance of the disease progression model when incorporating treatment effect, data from the Phase 2 was tested in the model, to compare the rate of progression after initiation of ManNAc to the rate of progression before intervention. At 182 days (6 months), a preliminary analysis shows a posterior mean of γ=0.49 (95% CI: .02-1.35). At 182 days (12 months), another preliminary analysis shows the current mean estimate for γ is approximately 0.61 (95% CI (0.09, 1.27), and the posterior probability that γ is less than 1 is 0.89. The most recent analysis at 548 days (18 months), shows a γ=0.55 with a posterior probability that γ is less than 1 is 0.966. This means, the model estimates a 45% slowing in the rate of decline on subjects receiving ManNAc with a probability that there has been slowing in progression of 96.6%.

4. STUDY DESIGN

4.1. Study Overview

This is a Phase 2, open-label, single-center study of ManNAc in subjects with GNE myopathy. The study will characterize the safety, tolerability and PK of multiple doses of ManNAc (Pharmacokinetic Phase), intermediate (90-day), and long-term (912 days; 30 months) safety and efficacy of ManNAc (Safety and Efficacy Phase). Twelve subjects will be enrolled into the trial.

Before dosing, subjects will undergo baseline evaluations that include clinical laboratory tests, muscle biopsy, Quantitative Muscle Assessment (QMA), muscle MRI, muscle biopsy and patient-reported outcomes (PROs).

In the pharmacokinetic (PK) phase, subjects will receive one of two doses: 3,000 mg (Cohort A) or 6,000 mg (Cohort B) ManNAc twice daily (BID) for total daily doses of 6,000 and 12,000 mg, respectively for 7 days while admitted to the NIH Clinical Center to assess for PK and safety. Safety and tolerability will be assessed on an individual basis.

In the Safety and Efficacy Phase, if ManNAc was safe and well-tolerated in the PK phase, subjects on Cohort A will be escalated to 6,000 mg BID on Day 7, and safety of the new dose-level will be assessed until discharge on Day 10. Subjects on Cohort B will be maintained at 6,000 mg BID, and if well tolerated they may be discharged on Day 7. After discharge, subjects will self-administer ManNAc at a dose of 6,000 BID for a total dosing duration of 912 days (30 months).

Follow-up visits to the NIH Clinical Center will occur on Day 45 (±7), Day 85 (±7), Day 180 (±14), Day 365 (±14), Day 548 (-30), Day 730 (-30 days) and Day 912 (-30 days). PK, PD and safety will be evaluated at all visits. Muscle biopsy will be performed only at the baseline and Day 85 visits.

Follow-up phone calls for safety will occur at approximately Days 28, 63, 120, 240, 300, 400, 638, and 820 (±30 days). After Day 912 all Grade $\geq$2 AEs at least possibly related to study drug will be followed to resolution.

4.2. Sample Size

A sample size of 12 GNE myopathy subjects was selected for this study to provide information on the safety, PK and biochemical efficacy of ManNAc.

4.3. Visit Schedule

Table 5. Brief Visit Schedule

Window	Description	Relative Day	
		Min	Max
Day -30 to -1	Screening Phase	-30	-1
Day -7 to 0	Baseline Phase	-7	0
Day 1-90	Treatment Phase		
Day 1-7	PK Phase (inpatient)	1	7
Day 7-90	Safety/Efficacy Phase	7	90
Day 45 (± 7 days)	Follow-up Evaluation	38	52

Day 85 (± 5 days)	3 month Follow-up evaluation	80	90
Day 180 (± 14 days)	6 month Follow-up evaluation	166	194
Day 365 (± 14 days)	12 month Follow-up evaluation	351	365
Day 450 (± 30 days)	Optional for blood draw		
Day 548 (- 30 days)	18 month Follow-up evaluation	534	562
Day 638 (± 30 days)	Safety labs and phone or email follow-up	608	668
Day 730 (- 30 days)	24 month Follow-up evaluation at NIH CC	700	730
Day 820 (± 30 days)	Safety labs and phone or email follow-up	790	850
Day 912 (- 30 days)	30 month Follow-up evaluation at NIH CC	882	912

See Appendix C for the Schedule of Events and Appendix D for blood and urine sampling schedule.

4.4. Dose and Frequency

4.4.1. Dose Levels

The ManNAc doses to be investigated (3,000 and 6,000 mg BID for a total daily dose of 6,000 and 12,000 mg, respectively) are based on the safety and PK data collected in NIH Study 12-HG-0207 (See Section 3.5.3). In that study, a single dose of 10,000 mg ManNAc was associated with diarrhea and there was no difference in the C_{max} or AUC of plasma Neu5Ac between the doses of 6,000 or 10,000 mg, suggesting saturation in the enzymatic rate of the Neu5Ac biosynthesis pathway or the transport of ManNAc into the cell. Therefore, the maximum single dose of ManNAc to be used in this study will be 6,000 mg per dose, for a total of 12,000 mg per day.

The administration of maximum dose of 12,000 mg ManNAc per day for 90 days is covered by the 90-day toxicology studies performed in rats and dogs. As mentioned in Section 3.8 and shown in Appendix L, effects on the gastrointestinal tract were the most prominent toxicology findings, and included loose and discolored feces, mild liver enlargement, and mucosal hypertrophy of the colon. Given these findings, the NOAEL was determined to be 12,000 mg/kg/day in rats and 6,000 mg/kg/day in dogs. It was determined that the human equivalent dose (HED) is 1,714 mg/kg/day, or approximately 12,000 mg/day based on the NOAEL observed in rats, the most sensitive species, with a 10-fold safety factor. Administration of a maximum dose of 12,000 mg ManNAc for up to one year is covered by the long-term toxicology studies performed in rats (26 weeks) and dogs (39 weeks), as shown in Appendix M.

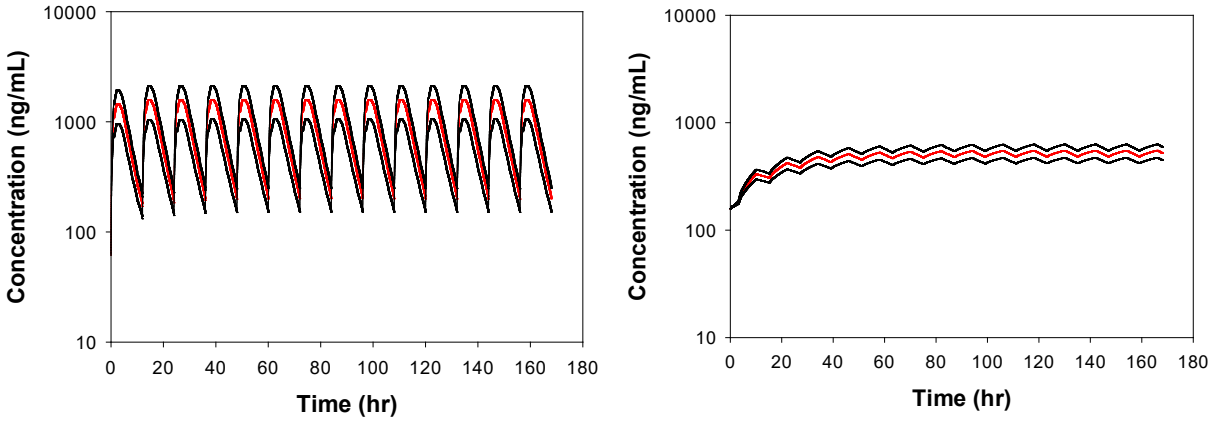
If any safety issues occur during the pharmacokinetic phase (Day 1-10) either detected clinically or based on laboratory values, those subjects will be continued on a lower dose for the safety and efficacy phase of the trial.

4.4.2. Dose Frequency

A ManNAc dose frequency of every 3,000 or 6,000 mg every 12 hours (BID) was selected based on the sustained elevation of plasma free Neu5Ac with a T_{max} of 8-11 hours (Table 1), and levels returning to baseline by 24 hours after a single dose of 3,000 mg of ManNAc but remaining above baseline values by 48 hours after single dose of 6,000 or 10,000 mg of ManNAc (Figure 4) and supported by simulated plasma concentration-time profiles of ManNAc and free Neu5Ac after 6,000 mg of ManNAc BID (12,000 mg per day) were generated to estimate the time to reach steady-state after multiple doses (Figure 5). No

significant accumulation of ManNAc is predicted following BID dosing of 6,000 mg of ManNAc due to the short half-life of ManNAc (2-3 hours), and supported by the rat and dog toxicokinetic reports; however, an accumulation for free Neu5Ac is desired and expected due to its longer half-life (Figure 5), although it does not increase above a certain threshold, as evidenced in the toxicokinetic reports in rats (IND 78,091_vol08_11.2.4.3A_2012 06 27.pdf) and dogs (IND 78,091_vol11_11.2.4.6A_2012 06 27.pdf), likely due to the feedback inhibition in the sialic acid biosynthetic pathway (Figure 1).

Figure 5. Simulated Concentration-Time Profiles for Plasma (A) ManNAc and (B) Free Neu5Ac with ManNAc 6,000 mg BID in Subjects with GNE Myopathy



4.5. Dose Administration

4.5.1. Inpatient Drug Administration

Inpatient drug administration will be performed with the purpose of determining PK parameters. The study drug will be prepared in individual doses of powder in bottle (PIB) by the NIH Pharmacy and sent to the inpatient unit. The study drug will be administered orally by the CC nursing staff BID on Days 1-7, or TID starting on Day 912-914 (+/- 30 days). Subjects will take study drug on an empty stomach, at least 2 hours before a meal and 1 hour after a meal. Water may be taken at all times.

For the morning dose on Days 1 and 7, and Days 912-914, the dose will be given after an overnight fast. Breakfast on Days 1 and 7 may be eaten 1 hour after the morning dose is given. The sample schedule below will be followed, whenever possible; when there is a modification in dosing or other unforeseen delay, the schedule will be adjusted accordingly:

Day 1		**Day 2-6 (days with PK trough only)**	
Midnight-0900:	Water only	0500-0600	Breakfast. No food after 0700
0800:	Morning DOSE	0600-0900	Water only
0800-0900:	Water only	0800:	Morning DOSE
0900-1800:	Regular diet (no restrictions)	0800-0900:	Water only
1800–2000:		0900-1800:	Regular diet (no restrictions)
2000:	Water only	1800–2100:	Water only
2000–2100:	Evening DOSE	2000:	Evening DOSE
2100:	Water only	2000–2100:	Water only

	Bedtime snack (if desired)	2100:	Bedtime snack (if desired)
Day 7		**5-7 days before Day 912**	
Midnight-0900: 0800: 0800-0900: 0900:	Water only Last DOSE Water only May resume regular diet		No ManNAc intake
Days 912-914			
Midnight-0800: 0800: 0800-0900: 0900-1400: 1400-1600: 1600: 1600-1700:	Water only Morning DOSE Water only Regular diet Water only Afternoon DOSE Water only	1700-2200: 2200-2400: 2400: 2400-:	Regular diet (no restrictions) Water only Evening DOSE Water only

4.5.2. Drug Administration after Discharge

After completion of PK studies on Day 1-7, subjects will be discharged and study drug will be self-administered while not admitted to the NIH CC. The study drug will be provided as a canister of bulk powder that will be measured with a Spatula Balance™. During the inpatient stay, subjects will be taught proper dosing and administration techniques. Subjects will take the study drug BID within 15 minutes of breakfast and dinner.

Subjects returning to their 30-month visit will be asked to stop the intake of ManNAc for 5-7 days before admission to perform PK testing of TID dosing.

4.5.3. Drug Administration During Terminal Half-Life PK Determination

Terminal half-life will be determined during the Day 85 visit. While the subject is inpatient, the study drug will be withheld for 72 hours with the purpose of determining terminal half-life PK parameters.

Day 85			
Midnight-0 0: 0800: 0800-0900: 0900:	Water only Terminal half-life DOSE Water only May resume regular diet		

4.6. Concomitant Medications

Subjects must be willing to stop treatment with ManNAc, sialic acid, IVIG, and/or other supplements containing sialic acid (e.g., St John's wort, sialyllactose) 90 days before initial dosing and remain off these medications for the duration of the trial. Prescription medications and supplements will be

maintained at a constant level throughout the duration of the study, unless dose adjustment is clinically necessary. Such adjustments will be recorded in the patient's CRF.

4.7. Safety Evaluations

Safety will be assessed by adverse events (AEs), vital signs, physical examinations, and clinical laboratory tests (See 5.2.2) and classified based on the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03.

4.8. Dose Escalation

Subjects in Cohort A will receive 3,000 mg of ManNAc BID for 7 days before they are escalated on Day 7 to receive 6,000 mg BID for the rest of the trial. The evaluation of a dose escalation/de-escalation for an individual will initially be reviewed by the Principal Investigator, according to the criteria in Table 5 and reviewed by the Safety Review Committee (SRC) when the Cohort is completed or safety issues arise. The safety information to be reviewed will include AEs, physical exams, vital signs, and clinical laboratory data.

4.8.1. Escalation and Continuation Rules for Cohort A

Subjects in Cohort A will receive 3,000 mg of ManNAc BID for 7 days before they are escalated to receive 6,000 mg BID for the rest of the trial:

a. Subjects in Cohort A will be escalated to 6,000 mg BID on Day 7 if no serious AEs and no more than 2 Grade 2 drug-related AEs occur.

b. If subjects experience ≥2 Grade 2 drug-related AEs or ≥ 1 drug-related SAE at 3,000 mg BID, the SRC will be convened to make escalation/de-escalation decisions based on Table 5.

c. The subjects will continue on 6,000 mg BID until they experience ≥2 Grade 2 drug-related AEs or ≥ 1 drug-related SAE. In this case, the SRC will be convened to make decisions based on Table 5.

4.8.2. Initiation and Continuation Rules for Cohort B

The SRC will review all available safety data for Cohort A of subjects prior to making a decision to initiate Cohort B. The SRC will convene when at least 14 days of safety have been collected on the last subject dosed in Cohort A.

a. Cohort B will be initiated at 6,000 mg BID provided that there are <2 subjects in Cohort A that experienced ≥2 Grade 2 drug-related AEs or ≥ 1 drug-related SAE.

b. If subjects experience ≥2 Grade 2 drug-related AEs or ≥ 1 drug-related SAE, the SRC will be convened to make decisions based on Table 6.

Figure 6. Dosing Escalation/De-escalation

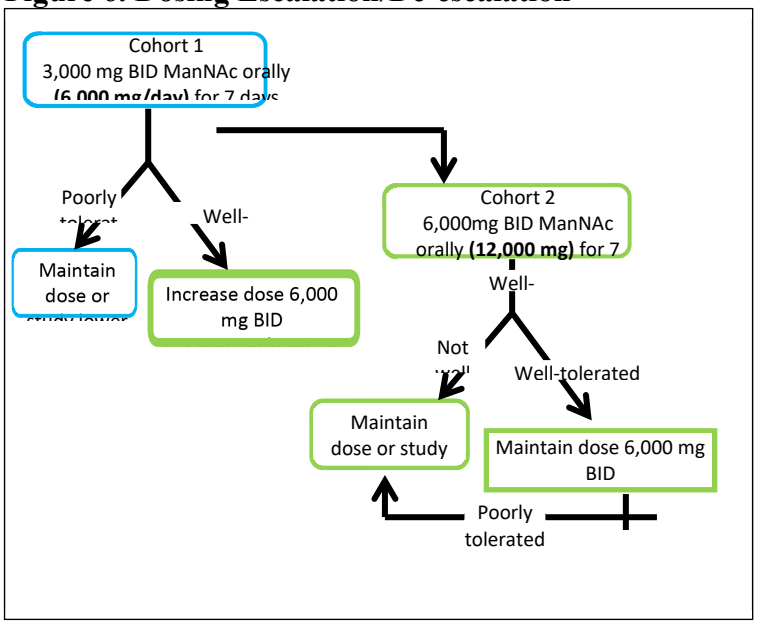


Table 6. Dose Escalation/De-escalation Rules	
Safety Profile*	**Safety Review Committee Decisions***
No SAEs and Any number of CTCAE Grade 1 drug-related AEs or ≤ 1 CTCAE Grade 2 drug-related AE	Continue per protocol
2 CTCAE Grade 2 drug-related AEs	Continue per protocol, or study a lower dose§,
≥ 3 CTCAE Grade 3 drug-related AEs	Study a lower dose§, or Withdraw the subject, and Convene SRC to evaluate the risk/benefit profile for dose escalation of other subjects
Any CTCAE Grade 4 AE; or ≥ 1 drug-related SAE Determination that the limit of safety and/or tolerability has been reached	Withdraw the subject, and Evaluate the risk/benefit profile in other subjects for dose escalations to or above the dose resulting in Grade 3 toxicity, and Discontinue study if 2 subjects develop the same Grade 3 toxicity or if any subject develops a Grade 4 toxicity
The safety decisions in this table apply only to safety and tolerability in an individual subject. If a decision is made that the dose escalation stopping criteria have been met, this does not preclude the use of the remaining subjects to evaluate the safety, PK, and PD effects of lower doses of ManNAc with the assessments described in this protocol. *Note: Subjects from both Cohort A and Cohort B on 6,000 mg BID count toward the number of subjects experiencing toxicity. § Lower doses to study may include 4,500 mg BID, 3,000 mg BID or 2,000 mg BID depending on dose level where toxicity occurred.	

4.9. Dose Modification

If a subject experiences >2 Grade 2 drug-related AE's or any Grade 1 or Grade 2 drug-related AE's that limit tolerability, the SRC will be convened and dose modification considered with a goal of improving tolerability.

4.10. Subject Compliance

Subjects will have directly observed therapy of drug doses administered while inpatient. CC nursing staff will document doses in CRIS.

A diary will be provided to subjects so that AEs, compliance, concomitant medications and other issues can be recorded during the treatment phase while they are at home (Appendix K). The diary will be in paper format, and will be reviewed by a member of the study team during every study visit.

Adverse events, compliance with study procedures, concomitant medications and other issues will also be recorded by a member of the study team during the treatment phase on CRFs.

4.11. Randomization and Blinding

There will be no blinding or randomization.

5. DESCRIPTION OF PROCEDURES

A Schedule of Events is provided in Appendix C, and the blood and urine sampling schedule in Appendix D.

5.1. Medical Information

A medical history, weight, height, vital signs, and physical exam will be conducted after admission to the NIH CC and enrollment into the study. For subjects who are co-enrolled on the Natural History protocol 11-HG-0218, data collected for the Natural History study may be used in the screening/baseline phase if collected within 30 days prior to dosing Day 1.

Physical examinations will be standardized and performed by a credentialed nurse practitioner or physician who is an investigator in this protocol. This will document baseline status and ensure that the subject's medical health is adequate to undergo the proposed research procedures.

Subjects will undergo clinical evaluations while admitted by CC nursing staff or a member of the study team. Vital signs will be performed according to the unit schedule while inpatient and at least daily while outpatient. Medical evaluations and vital signs will be performed at other times if clinically indicated, or if the ongoing review of the data suggests a more detailed assessment of the vital signs is warranted.

All medical information, including physical examination, AEs, compliance with study procedures, concomitant medications, and other issues will be recorded during the treatment phase on CRFs by the study team.

5.2. Diagnostic Studies

During the interventional phase, blood and urine will be collected for safety, PK, and PD evaluations. Safety will also be assessed by AEs, physical examinations, vital signs, and clinical laboratory tests. No radiation or sedation will be used as part of this study unless clinically indicated. Other diagnostic studies may be performed, if clinically indicated.

5.2.1. Procedures

5.2.1.1. Muscle Biopsy

Muscle biopsies will be performed for research purposes. Muscle biopsies will only be performed at the baseline and 91 day (3-month) visits; therefore a maximum of 4 incisions will be done per patient. Muscles to be biopsied include 1) the biceps brachii in the upper extremity and 2) a lower extremity muscle that has been determined as suitable for biopsy by MRI. Two biopsy sites are necessary to have a homogenous sampling from all subjects (through the biceps brachii, a muscle that is easily accessible and only affected in the advanced stage of the disease), and to further test if ManNAc supplementation could reverse the STIR-bright areas that are consistently seen in GNE myopathy subjects. Both upper (biceps brachii) and lower extremity muscle biopsies will be performed at the same time to decrease the subject's discomfort. Repeat MRI at 91 days (3 months) will guide the selection of the muscle to be biopsied for the 91 day (3-month) visit (see Section 5.2.1.1.1). A total of four muscle biopsies will be performed for each subject: two biopsies at baseline and two biopsies at 91 days (3 months).

5.2.1.1.1. Selection of Muscle Biopsy Site

The selection of muscles to be biopsied will be performed by identifying the following characteristics on muscle MRI: 1) Minimal or no fatty replacement and 2) Presence of hyperintense short tau inversion recovery (STIR). Fatty tissue is not informative and STIR hyperintensity is a signal of disease activity. Whenever possible, we will avoid performing biopsies of the quadriceps muscle, as disease characteristics are not likely to be significant in this muscle group. An MRI will be repeated before the 91 day (3-month) muscle biopsy to identify the area for biopsy; if feasible, the 91 day (3-month) biopsy will be the contralateral muscle biopsied at baseline.

5.2.1.1.2. Method for obtaining muscle biopsy

Muscle will be obtained by open muscle biopsy. This procedure is used because it allows safe access to muscles while carefully avoiding local blood vessels and nerves.

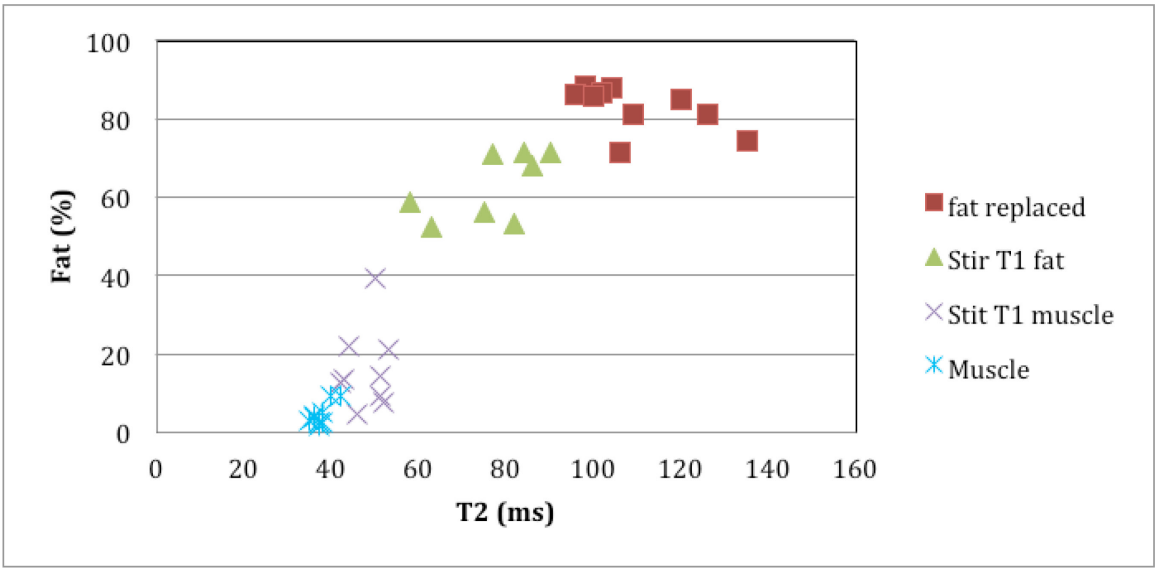
An open-muscle biopsy is a minor surgical procedure performed by a qualified neurologist or neurosurgeon with expertise in this technique. It is performed in the operating room under local anesthesia. After the skin is cleaned and draped, the skin will be infiltrated with local anesthetic which may cause mild discomfort. After a small (approximately 1.5 inches long) skin incision, the desired tissue (muscle) is isolated and biopsied. Two clamped muscles samples, each no greater than 0.5 inches (longitudinal axis) X 0.25 inches (cross-sectional diameter), will be obtained from each biopsy site. The incision site will be closed by a method according to the surgeon's preference.

The biopsy samples will be placed on sterile, saline-dampened gauze and will be processed immediately after collection.

5.2.1.2. Muscle Magnetic Resonance Imaging and Spectroscopy (MRI/MRS)

Muscle MRI/MRS will be performed at baseline, 91 days (3 months) and 365 days (12 months) in lower extremity muscles to monitor areas of disease activity. Findings are characterized by T2-weighted STIR hyperintensity of muscles actively affected, followed by fatty-fibrous replacement on T1-weighted imaging[33]. MRS will noninvasively monitor muscle metabolism and degree of involvement (Figure 7).

Figure 7. MRS of GNE Myopathy Muscle



5.2.1.3. Quantitative Muscle Strength Assessment (QMA)

Muscle strength will be evaluated at baseline and at every follow-up visit utilizing the Quantitative Muscle Strength Assessment (QMA) which measures maximal voluntary isometric contraction (MVIC) in kilograms (kg) using a fixed frame dynamometer, a strain gauge tensiometer, and a computer-aided acquisition system (Aeverl Medical, Gainesville GA, USA) to measure maximal strength[25] in 13 muscles groups. The muscle strength in kg is compared to predicted strength[26, 34-36] for age, gender, height and weight to generate a proportion of predicted strength value.

5.2.1.4. Other Rehabilitation Medicine Assessments

Other assessments may be performed at baseline and follow-up visits and will include muscle strength using the 10-point manual muscle testing (MMT-28) scale, Adult Myopathy Assessment Tool (AMAT), 6-min walk test (may be replaced by 10-meter walk test), timed up-and-go test, forward/functional reach test. The Jebsen Standarized Hand Function test will be done at baseline, 365 days (12 months) and 730 days (24 months).

5.2.1.5. Activity Monitor

Changes in daily activity will be measured using a Fitbit One™ activity monitor (Fitbit Inc., San Francisco, CA) in ambulatory subjects who also possess a compatible home computer. Recent studies have demonstrated that consumer-grade activity monitors reliably measure activity (steps taken, stairs climbed, and distance walked) in persons with disabilities when compared to the Step Activity Monitor, which has been validated in various disabled populations for more than a decade. The Fitbit will allow measurement of activity at a substantially reduced cost. Subjects will wear the device on a waistband, in a pocket, or with a necklace or wristband. They will use the monitor throughout the study. The subjects will register the device's software on their home computer in an anonymized fashion. They will use a study identifier for their name and will not use their own home address. Anonymized e-mail addresses will be created for subjects by the research team. Subjects' activity (walking, steps taken, distance walked) will be automatically uploaded to the anonymous profile. Fitbit data from each subject will be migrated to the study's database (Fitbase) for future analysis.

5.2.1.6. Patient-Reported Outcomes

Patient-Reported Outcomes will be collected at the time of study enrollment (baseline) and at each return visit unless otherwise indicated in the Table below. These will include the Human Activity Profile (HAP), the Inclusion Body Myositis Functional Rating Scale (IBMFRS), the Activity Balance Confidence (ABC) Scale, the Patient-Reported Outcomes Measurement Information System (PROMIS) questionnaires, Global Rate of Change questionnaire, AMAT Anchor, and Functional Questionnaire.

Questionnaire	Concept	Burden
Human Activity Profile (HAP)	Energy expenditure/ physical fitness	94 items/ 5-7 minutes
Inclusion Body Myositis Functional Rating Scale	Function specific	10 item/ <5 minutes
Activity Balance Confidence	Risk for falls	10 items <5 minutes

Global Rate of Change	Assess patient-reported changes between start of trial and follow-up visits	4 items/ <5 minutes Only at follow-up visits
PROMIS questionnaire	Emotional and psychosocial well-being, and fatigue and sleep	32 items/ ~10 minutes
AMAT Anchor	Patient-reported outcome for muscle strength measure validation	6 items/ <5 minutes Only visit Day 85
Functional Questionnaire	Assess device use, employment status, disability status	4 items/ <5 minutes Only at baseline

5.2.2. Clinical Laboratory Studies

Clinical laboratory samples will be collected at pre-dose (baseline) and for safety evaluations on Days 2, 5, 8, and 10 (±1) and at 456 (15 months), 638 (21 months) and 820 (27 months) days (at the NIH clinical center or subjects' home lab or physician) and at every follow-up visit (see Appendix D). For subjects who are co-enrolled on the Natural History protocol 11-HG-0218 and combine a natural history visit with this study, clinical laboratory tests collected for the Natural History study may be used as the pre-dose evaluation for this trial if within 30-days prior to initial dosing. Clinical laboratory tests will be evaluated and noted in a CRIS progress note. Laboratory results outside of CC normal parameters but not of clinical significance will be noted in the CRIS progress note.

5.2.2.1. Hematology/Chemistry

Blood samples for the clinical laboratory tests will be collected by CC phlebotomy or nursing staff using either an indwelling peripheral catheter or by venipuncture. Testing will include the following:

- Hematology: complete blood count (CBC) with differential, platelet count, reticulocyte count and erythrocyte sedimentation rate (ESR), C-reactive protein, prothrombin time (PT), and partial thromboplastin time (PTT).
- Serum chemistry: acute care panel (sodium, potassium, chloride, carbon dioxide, glucose, creatinine, blood urea nitrogen (BUN)), mineral panel (albumin, calcium, magnesium, phosphorus), hepatic panel (alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin), lipid panel, creatine kinase, c-reactive protein, and cystatin-C.

5.2.2.2. Pregnancy Testing

A urine human chorionic gonadotropin (hCG) test will be required at screening/baseline in female subjects with childbearing potential and be repeated within 7 days prior to starting administration of study drug. Subjects will not be eligible for the study if the pregnancy test is positive. Urine hCG will be repeated at every follow-up visit.

5.2.2.3. Urinalysis

Urinalysis will assess urine protein, glucose, ketones, hemoglobin, urobilinogen, leukocyte esterase, nitrite, red blood cells (RBC's) and WBC's.

5.2.3. Pharmacokinetics

Plasma concentrations of ManNAc and free Neu5Ac will be quantified using validated LC-MS/MS methods (Y. Shi *et al*., unpublished data). The method has been validated thoroughly to support clinical trials and has a calibration range of 10 to 5000 ng/mL.

We will administer multiple doses of ManNAc to achieve steady state. Plasma concentrations of ManNAc and free Neu5Ac will be measured at 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours after the first study drug administration (Day 1). On Day 2-7, and 45 (±7), morning C_{trough} (i.e., immediately before administering the morning dose of the study drug) measurements will be performed. C_{trough} will be collected pre-dose on Days 180, 365 and prior to the final dose of study drug. (See Appendix D for time points).

At the 30 month visit, plasma samples for ManNAc and Neu5Ac PK under TID dosing will be collected at 0 (pre-dose), 2, 4, 6, 8, 24 and 48 hours after the first study drug administration on TID dosing. Plasma will be collected right before ManNAc dose (trough) at the 8, 24 and 48 hour timepoints. Patient will avoid ingestion of ManNAc for 5-7 days prior to pre-dose PK draw on TID dosing.

The primary PK parameters that will be assessed for both plasma ManNAc and free Neu5Ac include:

- Maximum observed plasma concentration (C_{max}),
- Time to C_{max} (T_{max}),
- Area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$),
- Area under the plasma concentration-time curve from time 0 to time of last measurable plasma concentration (AUC_{last}),
- Area under the plasma concentration-time curve within the dosing interval (AUC_t),
- Plasma elimination half-life ($t_{½}$).

A pre-dose sample may be evaluated to investigate allelic variants of drug metabolism enzymes, if variable PK data are encountered in subjects.

5.2.4. Lectin and Antibody Labeling of Muscle Biopsies

Frozen slides acquired from subjects' muscle biopsies will be stained with fluorescent-labeled lectins and imaged by (quantitative) confocal microscopy. As mentioned, we and others have demonstrated by lectin staining that in particular O-linked glycans in GNE myopathy subjects and in a GNE myopathy mouse model are hyposialylated[1, 23, 31]. Sialylation of muscle glycans recovered to normal levels in our GNE myopathy mouse model after ManNAc therapy[31]. This method is the only reported reproducible, quantifiable assessment of muscle glycan hyposialylation in GNE myopathy to date (Figure 8).

5.2.5. Potential Biomarkers

5.2.5.1. Intracellular Biomarkers

We will use white blood cell pellets to explore the intracellular concentrations of ManNAc, Neu5Ac and CMP-Neu5Ac after administration of ManNAc. CMP-Neu5Ac, the end product of the sialic acid biosynthesis pathway and the donor of sialic acid to nascent glycoproteins and glycolipids in the Golgi apparatus, may be a useful marker of improved intracellular sialic acid biosynthesis (Figure 1). An LC-MS/MS method is being developed to quantify ManNAc, Neu5Ac and CMP-Neu5Ac in human WBCs. Samples from this study will be stored and tested when the method is validated.

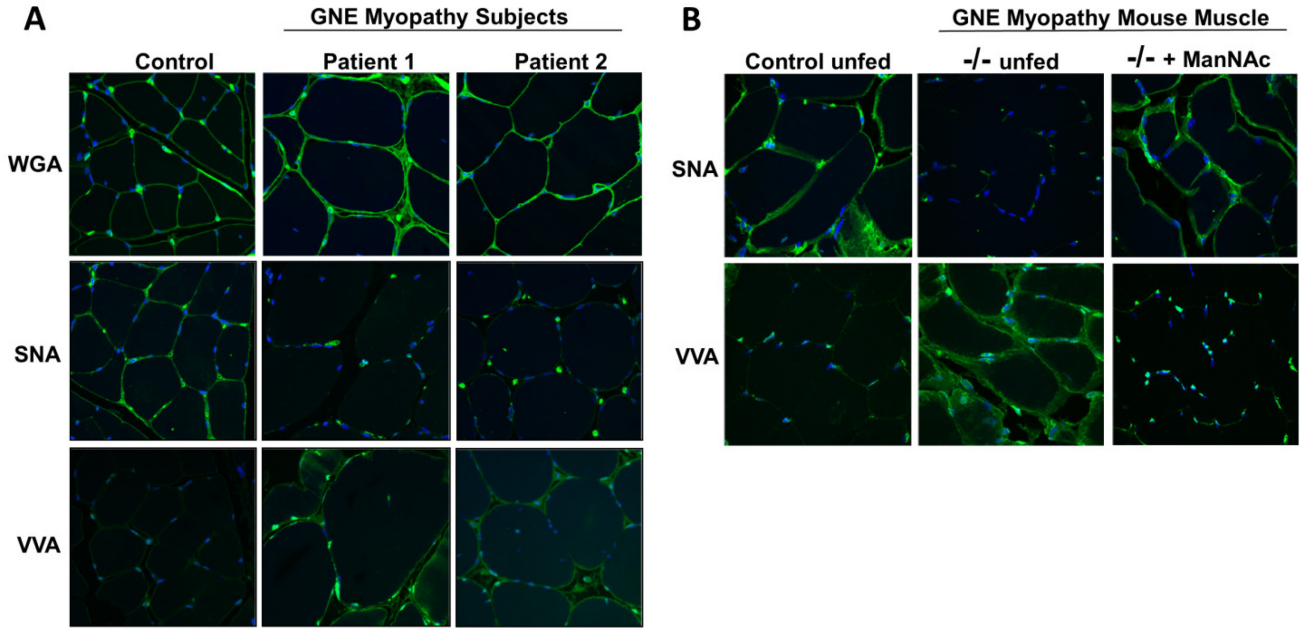
5.2.5.2. Blood Biomarkers

Plasma sialylated proteins are being explored as potential biomarkers, in particular those with mucin-like O-glycan structures. We have data that show the ratio of the plasma O-glycan structures Thomson-Friedenreich (T)-antigen versus Sialyl T-antigen (T/ST ratio) is abnormal in subjects with GNE myopathy[32]. We have anecdotal evidence that the plasma T/ST ratios normalized in GNE myopathy subjects that received sialylation-increasing therapies (including IVIG, off-label ManNAc, sialic acid). Exploratory glycan analysis will be performed on subjects' blood to identify biomarkers and assess their response to ManNAc therapy.

5.2.5.3. Urine Biomarkers

Exploratory glycan analysis will be performed on subjects' urine to identify (sialylated) biomarkers and assess their response to ManNAc therapy.

Figure 8. Muscle Lectin Histochemistry



A. Human muscle sections from biceps (Control and Patient 1) and gastrocnemius (Patient 2) were stained with three lectins (green) informative for sialylation status and co-stained with the nuclear dye DAPI (blue). GNE myopathy muscle specimens show selective hyposialylation compared to control muscle, demonstrated by apparent normal staining of WGA (*Wheat Germ Agglutinin*, binding to most sialic acid groups, independent of linkage), but decreased staining of SNA (*Sambucus Nigra Agglutinin*, predominantly binding sialic acids in a α(2,6)-linkage to their underlying monosaccharide; this linkage is abundant on O-linked glycans). In addition, staining of VVA (*Vicia Villosa Agglutinin,* predominantly binding terminal GalNAc, without sialic acid attached, O-linked to serine or threonine residues of glycoproteins) was increased in GNE myopathy muscle specimen compared to control, indicating hyposialylation of O-linked glycans (see also refs[1, 23, 31, 32].

B. Mouse muscle sections (gastrocnemius) from ~ 6 months old GNE myopathy mice (-/-, unfed) showed similar hyposialylation of O-linked glycans as human GNE myopathy subjects (see A) when compared to an age-matched control (Control unfed). Oral supplementation of ManNAc (1 g/kg/day) or for 12 weeks restored the muscle sialylation status back to the normal range (-/- + ManNAc), demonstrated by increased SNA and decreased VVA signal intensities similar to control specimens (see ref [32]).

All images are 1D projections of confocal Z-stacks. All imaging was performed at the same microscope intensity settings per lectin per species (with a 63 × objective).

5.3. Biological Specimens

5.3.1. Samples for Safety, Pharmacokinetic, and Pharmacodynamic Evaluations

Blood and urine will be collected for safety, PK and PD evaluations (see Appendix D for timepoints). The blood draw for safety labs at Day 450, 638 and 912 (±30) will be performed at the NIH clinical center, or the subject's home physician office or local laboratory. Samples will be stored without personally identifiable information (PII) per the lab manual. Collection, handling, and shipping procedures for plasma samples for PK are provided in the Laboratory Manual.

5.3.2. Research Samples

At the discretion of the investigator, blood and urine (first morning void) may be collected for future research. The amount of blood that may be drawn for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. This is in compliance with the NIH Clinical Center guidelines.

Blood, urine, and derivatives thereof (e.g., RNA/DNA, platelets, red blood cells, white blood cells, urine exosomes) collected under this protocol will be stored for future research not specifically outlined in this protocol. Such research could involve, but is not limited to, molecular, proteomic, biochemical, glycomic, and metabolomic analyses of these samples. Future research using these samples will relate to GNE myopathy.

For subjects also enrolled in the ongoing Natural History study (11-HG-0218), sample volume safety limits will take into account both protocols.

Table 7. Blood Volume

Sample (Day)	Hematology[1]	ESR	PT/PTT	Chemistry[2]	PK	PD	Future Research	Total daily volume
Volume	3	3	4.5	4	3	20	10-50	--
D0	3	3	4.5	4		20	50	84.5
D1					30	80		110
D2					3			3
D3	3	3	4.5	4	3	20	20	57.5
D5					3			3
D7	3	3	4.5	4	30	20	30	94.5
D9	3	3	4.5	4		20	20	54.5
D45	3	3	4.5	4	3	20	50	87.5
D85	3	3	4.5	4	3	20	40	77.5
D90					30	20		50
D91					3			3
D92	3	3	4.5	4	3	20	40	77.5
D93					3			3
D180	3	3	4.5	4	3	20	40	77.5
D365	3	3	4.5	4	3	20	40	77.5

D450	3	3	4.5	4				14.5
548	3	3	4.5	4	3	20	40	77.5
638	3	3	4.5	4				14.5
730	3	3	4.5	4	3	20	40	77.5
820	3	3	4.5	4				14.5
912	3	3	4.5	4	3	-	-	17.5

5.3.3. Collaborating Laboratories

After establishing appropriate material transfer agreements and/or contracts, biospecimens collected under this protocol may be sent to the following investigators:

Table 8. Collaborations

Sample	Laboratory	Location
PK and intracellular PD	Alliance Pharma	Malvern, PA 19355
Hematology, ESR, PT/PTT and Chemistry	Quest Diagnostics	Multiple locations

Additional laboratories can be added by amendment of this protocol, if they require a material transfer agreement.

5.4. Approved Drugs

No approved drugs will be used as part of this study.

5.5. Unapproved Drugs

ManNAc drug substance grade ManNAc and other forms of ManNAc are not approved for any indication. The Investigational New Drug (IND) application number for this product is IND #78091.

5.5.1. Identity of Investigational Product

ManNAc (N-acetyl-D-mannosamine monohydrate; trade name ManNAc) will be supplied as a powder by New Zealand Pharmaceuticals. The study drug will be prepared by the NIH Clinical Center Pharmacy as powder-in-bottle. The pharmacy will add 200 mL of water, shake to dissolve the drug, and re-label the bottles appropriately for delivery to the subjects. See Appendix E.

5.5.2. Packaging, Labeling, and Storage of Clinical Supplies

ManNAc will be supplied as a powder, packaged in 8 oz. amber plastic prescription bottles (Berry Plastics Part # PB-8) with a removable label and labeled as an investigational product.

Storage conditions for the study drug will be 2 to 8 °C. For additional details, refer to Appendix E.

5.5.3. Drug Accountability

The study drug will be registered and tracked with the NIH Pharmaceutical Development Service (PDS). Drug supply will be managed by the NIH CC Pharmacy Department. The NIH pharmacy is responsible for the accountability of the investigational product. This will include documentation of receipt, storage, and dispensing of the investigational agent. The NIH pharmacy will return or destroy (per instruction of the IND sponsor) unused investigational drug at the conclusion of the study.

An order for study drug administration will be entered into CRIS by the study staff. The NIH Pharmacy will prepare and send the study drug to the unit. After administration of the study drug, the CC nursing staff will log the administration into CRIS. The empty container will be discarded.

5.6. Results Given to Participants

Subjects enrolled in this study will be told of their medically relevant laboratory results and the results of consultations. They will eventually be informed of the overall results of the study.

5.7. Questionnaires

A diary will be provided to subjects so that symptoms can be recorded after the administration of study drug (Appendix K). Patient reported outcomes mentioned in Section 5.2.1.6 will be administered as questionnaires.

5.8. Genetic Counseling

In cases where subjects have not received genetic counseling in the past, the Investigator or one of the genetic counselors in the Office of the Clinical Director will perform this service.

5.9. Criteria for Withdrawal

Subjects will be informed that they have the right to withdraw from the study at any time for any reason without prejudice to their medical care.

Subjects may be withdrawn from the study for any of the following reasons:

- Subject request
- Subject is unwilling or unable to comply with the protocol
- Medical reason, at the discretion of the Investigator

In addition, the Sponsor may terminate this study at any time for safety or administrative reasons. The Sponsor will terminate the study if the occurrence of AEs or other findings suggests an unacceptable risk to the health of the subjects.

Subjects who withdraw from the study early should undergo all safety evaluations prior to discharge from the unit. Subjects who withdraw will be replaced. The reason for subject withdrawal must be recorded in the subject's CRF. The Investigator must notify the Sponsor immediately when a subject has been discontinued/withdrawn due to an AE. Subjects who withdraw from the study may request that samples be destroyed.

6. DESCRIPTION OF STUDY POPULATION

6.1. Estimated Number of Participants

This study will enroll 12 subjects with GNE myopathy divided into two dosing cohorts (cohort size = 6) assigned to receive ManNAc at either 3,000 mg BID or 6,000 mg BID for a 7-day pharmacokinetics study, for a total of 6,000 mg or 12,000 mg per day, respectively. All patients receive 6,000 mg BID for the remainder of the study beyond 7 days. Accrual ceiling will be set at 30 subjects to allow for subject replacement if needed. We anticipate enrollment of the 12 subjects within 1 year.

6.2. Eligibility Criteria

6.2.1. Inclusion Criteria

1. Subject is age 18-60 years, inclusive, and of either gender.
2. Subject has a diagnosis of GNE myopathy based upon a consistent clinical course and identification of two *GNE* gene mutations.
3. Subject must be willing to stop any treatment with ManNAc, sialic acid, intravenous immunoglobulin (IVIG), and/or other supplements containing sialic acid (e.g. St. John's wort, sialyllactose) 90 days prior to dosing and remain off such treatment for the duration of the trial.
4. Subjects must have a body mass index (BMI) between 18 and 30 $kg/m^{2,}$ with a bodyweight of >50 kg
5. Subjects must meet one of the criteria below on at least one of the following muscle groups: 1) ankle dorsiflexion, 2) knee flexion, 3) hip extension, 4) grip, 5) elbow flexion, 6) shoulder abduction:
    a. 20-75% of predicted strength measured by QMA at baseline, or
    b. If predicted muscle strength above 75%, a documented change of at least 10% per year.
6. Subject has the ability to travel to the NIH Clinical Center for admissions.
7. Subject has an INR ≤1.5 and must have stopped warfarin and other anticoagulants 2 weeks prior and after muscle biopsy procedures. Aspirin and clopidogrel should be stopped 3 days and 5 days before the procedure, respectively.
8. Subject must be able to comply with requirements of the protocol, including blood collection, drug administration, muscle MRI/MRS, muscle biopsy and muscle strength assessments.
9. If a woman of reproductive age, subject must be willing to use an effective method of contraception for the duration of the trial.
10. Subject must be able to provide informed consent.

6.2.2. Exclusion Criteria

1. Subject had a clinical significant infection or medical illness 30 days prior to the first protocol visit.
2. Subject has a psychiatric illness or neurological disease that would interfere with the ability to comply with the requirements of this protocol. This includes, but is not limited to,

uncontrolled/untreated psychotic depression, bipolar disorder, schizophrenia, substance abuse or dependence, antisocial personality disorder, panic disorder, or behavioral problems, which interfere with effective communication.

3. Subject has hepatic laboratory parameters (AST, ALT, GGTP) or renal laboratory parameters (creatinine, BUN) greater than 3 times the upper limit of normal.
4. Subject has known adverse reactions to anesthetic or sedatives utilized for muscle biopsy.
5. Subject is anemic (defined as Hematocrit <30%) or has platelets <100,000 or white blood cell count less than 3,000.
6. Subject shows evidence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematological, metabolic, or gastrointestinal disease, or has a condition that requires immediate surgical intervention.
7. Subject is pregnant or breastfeeding at any time during the study.
8. Subject has received treatment with another investigational drug, investigational device, or approved therapy for investigational use less than 90 days prior to the first protocol visit.
9. Subject has hypersensitivity to ManNAc or in the judgment of the investigator, has a condition that places the subject at increased risk for adverse effects.
10. The presence of persistent diarrhea or malabsorption that could interfere with the subject's ability to absorb drugs or to tolerate ManNAc therapy.

6.2.3. Justification of Inclusion/Exclusion Criteria

The rationale for the study population is that individuals with GNE myopathy will likely process sialic acid differently from healthy normal volunteers due to the hyposialylation characteristic in GNE myopathy, and therefore the PK of ManNAc in healthy normal volunteers may not be predictive of PK in GNE myopathy subjects.

Subjects must be willing to stop treatment with ManNAc, sialic acid, IVIG, and/or other supplements containing sialic acid (e.g. St. John's wort, sialyllactose) 90 days before dosing and remain off these medications for the duration of the trial.

Children are excluded because GNE myopathy generally does not typically manifest in childhood and juvenile toxicology studies have not been performed. Subjects with certain psychiatric illnesses will be excluded because their symptoms could interfere with compliance or communication with health care personnel and therefore the integrity of the trial.

Subjects with coagulation defects or taking anticoagulants are at higher risk of bleeding with muscle biopsy. Only those subjects that can safely stop taking such medications, as determined by their treating physician, will be eligible for this trial.

6.3. Location of Study

NIH Clinical Center.

6.4. Recruitment Strategies

Subjects will be recruited from patient organizations such as the Neuromuscular Disease Foundation (NDF) and Advancement of Research for Myopathies (ARM); from groups of subjects known to the Investigators, from individuals previously enrolled in the following studies:

- Protocol 11-HG-0218: "A Natural History Study of Patients with GNE Myopathy"
- Protocol 12-HG-0207: "A Phase 1 Randomized, Placebo-Controlled, Double-Blind, Escalating Single-Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of ManNAc in Subjects with GNE Myopathy or Hereditary Inclusion Body Myopathy (HIBM)"

The study will include females in this early stage of drug development as encouraged by regulatory agencies. Pregnancy tests will be performed before dosing (see 5.2.2.2 and Appendix B).

We will attempt to recruit all minority subjects we are aware of, including individuals from the Iranian Jewish community. A recruitment letter will be mailed to several large minority medical centers to aid with the ascertainment of under-represented subjects. These are listed with a sample of the recruitment letter in Appendix J. These will include:

1. Meharry Medical College
   1005 Dr. D.B. Todd, Jr., Blvd., Nashville, TN 37208
   Phone: (615) 327-6111

2. Morehouse School of Medicine
   720 Westview Drive S.W. Atlanta, GA 30310-1495
   Phone: (404)-752-1500

3. Howard University
   2400 Sixth Street, NW, Washington, DC 20059
   Phone: 202-806-6100

4. Charles R. Drew University of Medicine and Science
   1731 East 120th Street Los Angeles, CA 90059
   Phone: (323)-563-4800

6.5. Existing Sample/Data Sets

Not applicable.

6.6. Financial Compensation

None.

7. STATISTICAL CONSIDERATIONS

7.1. Statistical Plan

An independent statistical analysis will be performed after the study is completed, queries are resolved and the database has been locked.

7.1.1. General Reporting Conventions

Standard descriptive statistics will include the number (n), mean and standard deviation (SD), median, minimum, and maximum values for continuous variables. For categorical variables, the frequency of subjects will be provided along with the percentage based on the number of subjects with available data. Unless otherwise indicated, summaries will be based on observed data (i.e., no missing data will be imputed).

Any statistical testing and/or provided confidence intervals (CI) are provided as an aid in interpreting study results and are not intended as claims. Results from this study may be used in designing future studies.

Summaries will be provided by treatment group for the individual ManNAc treatment groups (6,000 mg and 12,000 mg), a combined ManNAc group and the combined control subjects from all two cohorts.

Analyses will be generated using SAS® version 9.2 or higher. Analysis on QMA will be performed using R®.

7.2. Statistical Methods and Evaluations

7.2.1. Demographic and Baseline Characteristics

Baseline and demographic characteristics including age, gender, race, ethnicity, height, weight, BMI, and genotype information will be summarized descriptively by treatment group. The medical history summary will include the number and percent of subjects with one or more findings by body system and treatment group.

7.2.2. Safety Evaluations

All safety data will be listed. AEs, SAEs, unanticipated problems, AEs related to the drug (possible, probable, or definite) and CTCAE Grade 3 and 4 AEs will be summarized overall. These summaries will include the number of events and the number and percentage of subjects with a given event. AEs will also be summarized by system organ class (SOC) and treatment group to identify AEs that occur at a higher frequency in subjects treated with ManNAc at different dose levels.

7.2.3. Pharmacokinetic Analysis

Plasma concentrations of ManNAc and free Neu5Ac will be summarized descriptively by collection time points, and figures will be provided to plot the mean concentrations over time by dose along with individual subject results. Reporting will be carried out according to ICH guidelines (ICH-E3).

The following PK parameters may be estimated using noncompartmental analyses to assess the concentration-time data: C_{max}, T_{max}, AUC_{last}, AUC_t, $AUC_{0-\infty}$, $t_{1/2}$ and C_{trough} plasma concentrations.

In addition, the C_{max} geometric mean and corresponding 95% confidence interval may also be provided.

Day 1 and Day 8 data for C_{max} and AUC_t may be compared to determine the accumulation ratio of the compound.

7.2.4. Pharmacodynamics

Different dose levels will be evaluated by a statistical dose-response model of the relationship between dose and the rate at which plasma free Neu5Ac returns to baseline levels.

The following PD parameters may be summarized and analyzed by treatment group: Intracellular ManNAc, Neu5Ac, CMP-Neu5Ac and plasma sialylation (may include T/ST ratios). A similar statistical dose-response model may be used to evaluate these parameters, if appropriate.

7.2.5. Lectin Staining on Muscle Biopsies

The fluorescence will be quantified in 5 different images per slide with ≥ muscle cells per field and analyzed as a change from baseline to 90 days using a paired t-test.

7.2.6. Quantitative Muscle Strength

In our disease progression model specified in 3.3.1, the rate of progression for each muscle was determined. Given baseline data for each subject, we can then predict the percentage of strength for each primary muscle group for each subject at their follow-up visits. To investigate if ManNAc affects disease progression we will provide descriptive statistics of the predicted percentage of strength for each primary muscle group versus the measured percentage of strength at every visit. We will also more formally explore the possibility of the ManNAc causing a change in the rate of decay of the muscles over a 24-month period by introducing a proportional treatment effect on the rate of decline (γ) into the disease progression at the time of treatment. If γ is equal to 1 there is no effect of the treatment on the disease progression model and the treatment effect model is the same as the normal disease progression model. If γ is less than one the treatment slows the rate of decay of the muscle group and if γ is equal to zero the treatment has stopped the decay of the muscle group altogether. If γ is greater than one the treatment increases the rate of decay of the muscle group. Under this treatment effect model we will report the posterior mean, and 95% credible interval for γ as well as the posterior probability that the treatment slows the rate of decay of the muscle groups (γ is less than one).

7.2.7. Other Rehabilitation Medicine Evaluations

Changes in clinical measurements will be analyzed as a change from baseline to last visit using a paired t-test or Wilcoxon rank sum test based on parametric quality of the data.

7.3. Determination of Sample Size

Eligible subjects will be enrolled into one of 2 cohorts of 6 subjects each, to receive ManNAc at doses of 3,000 mg BID (n=6) or 6,000 mg BID (n=6). Given the rarity of disease and the intent of this study—determine pharmacokinetics, safety and efficacy using blood based and tissue biomarkers, 12 subjects should be suitable.

7.4. Interim Analysis

Interim analyses are planned after each cohort has completed the pharmacokinetic phase, and after each cohort has undergone evaluation at days 182, 365, 548, 730 and 912 (6, 12 18, 24, and 30 months).

7.5. Deviation from Original Analysis Plan

Any deviation from the original statistical plan will be described in the final clinical study report.

8. POTENTIAL BENEFITS

8.1. Direct benefits to participants

The benefits of ManNAc are unknown. There may be no direct benefits to subjects participating in this study.

8.2. Collateral Benefit to Participants

The results of clinical testing performed at NIH will provide data for each subject's medical record. The study team may serve as a resource for consultation with each subject's physician.

8.3. Benefits to Society

This study will improve understanding of the safety and PK of ManNAc and its results will inform future trials to evaluate the efficacy of ManNAc in individuals with GNE myopathy.

9. LIKELIHOOD AND SERIOUSNESS OF HARMS AND MEANS TO MAXIMIZE SAFETY

Efforts will be made to reduce physical and psychological stress by carefully explaining the study's procedures to the individual subjects.

Individual procedures and associated personal risks are briefly described below.

9.1. Therapeutic Interventions

9.1.1. Risks of ManNAc/ManNAc

A single dose of ManNAc was safe and well tolerated as part of NIH study 12-HG-0207. The only drug-related adverse event in this study, loose stools shortly after receiving the 10,000 mg as a single dose of ManNAc, was likely caused by unabsorbed drug in the gastrointestinal tract. As part of this study, the maximum single dose to be given will be 6,000 mg, which was determined to be safe and well tolerated on NIH study 12-HG-0207 (See Appendix F).

One subject participating in study 15-HG-0068 had Grade 3 hypertryglyceridemia which was determined to be probably related to ManNAc. As higher triglycerides were also seen in toxicology studies (see Table 10), the intake ManNAc includes the risk of hypertriglyceridemia.

As mentioned in Section 3.6 and shown in Appendix L, effects on the gastrointestinal tract were the most prominent in the 90-day toxicology studies performed in rats and dogs, and included loose and discolored feces, mild liver enlargement and mucosal hypertrophy of the colon. There have been no serious adverse events through 24 months of follow-up visit for subjects taking ManNAc. Two subjects have withdrawn consent due to gastrointestinal tolerability issues. One subject had a dose reduction due to tolerability concerns associated with grades 1 and 2 flatulence and diarrhea, and the subject has had their symptoms reach a tolerable level at the reduced dose.

9.2. Diagnostic Interventions

9.2.1. Medical Evaluations

There are no significant risks associated with the physical evaluations, or collection of vital signs. Multiple independent evaluations may be stressful.

9.2.2. Serum Pregnancy Testing

There are no risks involved with taking a serum pregnancy test. Subjects will be informed of a positive test. The subject will not be eligible to participate in the study if she is pregnant.

9.2.3. ECG

There are no significant risks associated with performing an ECG.

9.2.4. Placement of a Peripheral Intravenous Line

A peripheral intravenous line may be placed to allow for blood collection. Risks associated with a peripheral intravenous line include a small risk for infection, phlebitis, extravasation, infiltration, air embolism, hemorrhage, and formation of a bruise.

Because of the risk of insertion-site infection, the catheter needs to be removed within 72 hours.

9.2.5. Phlebotomy

Multiple blood draws will be necessary during this study. Phlebotomy is a risk/discomfort of this study. An anesthetic cream such as EMLA may be used. Infection and bruising are possible at the site of the blood draw. The amount of blood to be drawn will not exceed the NIH CC blood draw limits of 550 mL over any 8-week period. Nursing staff will monitor blood-drawing volumes. If blood limits are an issue, testing will be prioritized by the investigators. Safety laboratory tests will take priority over investigational testing. If a subject is concurrently enrolled in another protocol, the total blood volume will not exceed the above limit.

9.2.6. Muscle Biopsy

Muscle biopsies will be obtained when technically feasible. A separate NIH Clinical Center surgical consent will be used for this procedure. The open muscle biopsy will leave small scars on the skin; therefore subjects may have up to four scars, one per biopsy. The rare complications of local infection or bleeding will be guarded against by sterile procedures, and careful hemostasis. The risk of these events is minimized by having the procedure performed by a neurosurgeon or neurologist trained to perform muscle biopsies and under sterile conditions with adequate anesthesia.

To further decrease the risk of bleeding associated with muscle biopsy, anticoagulants, aspirin, clopidogrel and other medications that increase the risk for bleeding are stopped. Only subjects that can safely discontinue such medications, as determined by their treating physician, will be eligible for this study. Pre-surgery and post-surgery instructions, including care of the incision site, will be provided to the subject.

9.2.7. Muscle MRI

Potential risks from MRI include injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in their eyes. Subjects will be screened for these conditions prior to the test, and if they have any of these conditions, they will not have an MRI scan. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room. Women who are pregnant are excluded from the MRI. Therefore, all women of childbearing potential will have a pregnancy test performed, which must be negative, before proceeding. Subjects will be asked to complete an MRI screening form, and to sign a separate MRI consent for each MRI. There are no known long-term risks or consequences of MRI scans.

Holding still during the MRI scan and being placed in a relatively small space can be uncomfortable or create anxiety in some subjects, if desired a mild anxiolytic may be prescribed during the test. MRI evaluation does not involve any radioactivity.

9.2.8. Activity monitor

The only reported adverse event associated with these devices is skin irritation when it is worn against the skin. If this occurs, the device can be moved to an alternate site that is not in direct contact with skin.

9.3. Radiation

Only medically indicated radiation exposure will be incurred.

9.4. Sedation

None.

9.5. Psychological Harms

The main psychological issue will be accepting the possibility that ManNAc administration is not beneficial.

9.6. Risk to Family Relationships

Subjects will have a known genetic condition. No further genetic testing will be performed for this study, therefore there is no risk to family relationships.

9.7. Discrimination

Subjects enrolled in this protocol already have the diagnosis of GNE myopathy, with its attendant risks of insurance and employment discrimination. To the fullest extent possible, the investigators will not disclose to third parties any information about the participants without their expressed consent.

10. PRIVACY AND CONFIDENTIALITY OF MEDICAL INFORMATION AND BIOLOGICAL SPECIMENS

10.1. Participant Identifiers Attached to Data

Clinical data will be collected using subjects' names in a "shadow" file, maintained in a locked cabinet. The Principal Investigator and designated Associate Investigators will hold keys to the locked cabinet. However, to maintain subject privacy, all CRFs, study drug accountability records, study reports, communications, and research blood samples will be identified by the assigned subject number and/or a barcode. The linking code, as well as all other data, will be maintained in records in the password-protected database CTDB.

10.2. Clinical/Demographic Information

This will be part of the clinical data kept in the medical record and in password-protected database CTDB.

10.3. How might information make specific individuals identifiable?

Subjects enrolled in this study are from all over the world. It is doubtful that any demographic information could identify an individual. Subjects will be notified that registration information, results, and other information about this study will be submitted to ClinicalTrials.gov, a publicly available trial registry database; however, protected health information of individual subjects will not be used.

10.4. Access to the key for subject identification

The code to subject identities, as well as other subject data, will be kept in the password-protected database CTDB. Access to the code will be restricted to the Principal Investigator, Dr. Nuria Carrillo and designated Associate Investigators.

10.5. Will pedigrees be published?

Pedigrees are not essential for this drug trial.

10.6. Will results be provided to participants?

All medically relevant data will be given directly to the subjects as they become available. In addition, the clinical research data of the individual subject, obtained in our CLIA-certified lab, will be provided to interested subjects.

10.7. Will Identifiable Information be Released to Third Parties?

The Principal Investigator will grant the NIH monitor(s), auditor(s), and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

The investigators will not disclose to third parties any information about the participants without their expressed consent.

10.8. Sharing of Data/Samples with Other Researchers

If sent to outside laboratories, samples will be coded and subject identifiers will be removed. The code number can be linked to a name only by the Principal Investigator and a designated Associate Investigator. If a biospecimen is sent to a clinical laboratory for testing, identifiers will not be removed and the result will become part of the NIH medical record.

10.9. Additional Features to Protect Confidentiality

The protocol and associated data will be stored on the CTDB. The CTDB is a web-based application that supports flexible data capture and reporting. The system is hosted at the NIH in Bethesda, MD. It is accessible via the Internet through the NIH firewall, on both Mac and Windows based computers. An Oracle relational database is utilized to capture and secure data entered through the web interface. The following features allow for the safe and secure collection of research variables: an application firewall, data encryption and SSL certificates, HIPAA requirements, logical access controls, audit trails. Furthermore, only preauthorized users can access the CTDB. The Principal Investigator can determine the Associate Investigator's data access privileges.

11. ASSESSMENT OF RISK/BENEFIT RATIO

The physical risks of phlebotomy, blood collection, muscle biopsy and ManNAc administration appear reasonable compared with the burden of the disease GNE myopathy. The benefits of this study relate to the future availability of a therapy for GNE myopathy. The benefit of such a treatment could be enormous for the community.

12. COLLECTION, MONITORING, ANALYSIS AND REPORTING OF ADVERSE EVENTS AND PROTOCOL DEVIATIONS

12.1. Adverse Events

AE definitions and classifications can be found below. Our safety monitoring plan is based on experience from the Phase 1 SAD trial of ManNAc (Section 3.5.3 and Appendix F), the 90-day toxicology studies in rat and dogs (See Appendix L) and the long-term toxicology studies in rats and dogs (Appendix M). We will monitor clinical laboratory tests including hemoglobin, liver function tests (See 5.2.2) and other clinical testing as medically indicated.

12.1.1. Monitoring and Reporting of Adverse Events

Subjects will be evaluated and interviewed to identify AEs during the course of the study. AEs may also be identified through clinical examinations and laboratory tests. Events occurring after administration of the first dose of study medication will be recorded on the AE CRF. All AEs occurring from the time the informed consent is signed through the final dose of study drug must be documented, recorded and reported. After the final dose, all Grade ≧2 AEs at least possibly related to study drug will be followed to resolution.

A laboratory abnormality should be reported as an AE if it requires an intervention. Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments, or concomitant treatment. In addition, any medically important laboratory abnormality may be reported as an AE at the discretion of the investigator. This could include a laboratory result for which there is no intervention, but the abnormal value suggests disease or organ toxicity. The Investigator will evaluate all AEs with respect to Seriousness, Severity (intensity or grade), and Causality (relationship to study agent and relationship to research) according to the following guidelines.

The Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary will be used for coding AEs.

The Investigator will grade the severity of each AE according to the "Common Terminology Criteria for Adverse Events (CTCAE)" in effect at the time of protocol activation which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related:

- clear temporal association
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related:

- temporal association
- follows a suspected response pattern (based on similar agents)

- a more likely alternative etiology unlikely

Possibly Related:

- temporal association
- evidence exists for alternative etiology

Unlikely Related

- no clear temporal association

OR

- good evidence for a more likely alternative etiology

Not Related

- no temporal association

OR

- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

Pregnancy: Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. In the event of pregnancy the following steps will be taken:

- Discontinuation of the study agent
- Withdraw from the study but continue in follow up for safety
- Report to safety oversight committee - SRC and IRB
- Advise research subject to notify the obstetrician of study agent exposure
- Safety data will be collected until completion of the pregnancy

12.1.2. Review of Safety and Tolerability

The evaluation of a dose for an individual will initially be reviewed by the PI, according to the criteria and on an ongoing basis by the SRC.

12.2. Monitoring and Reporting Plan

AEs, protocol deviations, unanticipated problems (UP), and serious adverse events, are defined in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, AEs and Protocol Deviations", Appendix G). All AEs occurring from the time the informed consent is signed through the end of the follow-up period will be documented, recorded and reported.

An **adverse event (AE)** is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device whether or not considered related to the drug product or device.

Serious unanticipated problems that are at least possibly related to the research and suggest a greater risk or harm than previously known, **serious protocol deviations** will be reported to the IRB and CD (Clinical Director) as soon as possible but not more than 7 days if ***serious*** or 14 days if ***not serious***, after the PI first learns of the event.

Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event.

Protocol deviations that represent non-compliance (see SOP 16A) will be reported to the IRB and CD within 7 days if serious and 14 days if not serious.

Not serious protocol deviations that do not represent non-compliance will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the eventpart of the Continuing Review.

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected AEs will not be reported to the IRB unless they occur at a rate greater than that known to occur in GNE myopathy. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

At each contact with the subject, information regarding unanticipated problems and adverse events will be elicited by appropriate questioning and examinations. All UPs and AEs will be: immediately documented in the subject's medical record/source document; appropriately recorded on the NIH Problem Report Form and on the Adverse Event Case Report Form (AE CRF) or in the electronic database; and reported to appropriate entities (e.g., IND Sponsor, IRB, FDA, etc.), within the specified time limits.

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected AEs will not be reported to the IRB unless they occur at a rate greater than that known to occur in GNE myopathy. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems.

The following items will be reported to the NHGRI IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events, except expected AEs and deaths granted a waiver of reporting.
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events that in the opinion of the investigator should be reported
- Any protocol-specific reporting requirements (as applicable).

AEs that occur following enrollment of the subject (by signing the informed consent) are followed until the final outcome is known or until the end of the study follow-up period. SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a

final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF (if the CRF is still open). SAEs that occur after the study follow-up period and that are reported to and are assessed by the Investigator to be possibly, probably, or definitely related must be reported to the safety oversight committee as described above.

12.2.1. Notification about Serious Adverse Reaction and Serious and Unexpected Suspected Adverse Reactions

Investigators must report any SAE to the Sponsor immediately, whether or not considered drug related, including those listed in the protocol or Investigator Brochure.

All SAEs, regardless of relationship to the study drug will be reported to the FDA within 7 days in an expedited report. Investigators must also notify FDA of any unexpected fatal or serious adverse events as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

The report must include an assessment of whether there is a reasonable possibility that the drug caused the event.

12.2.2. Reporting to Regulatory Agencies and IRB

We will follow the reporting requirements as described in NIH HRPP SOP 16 “Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations”.

The Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary will be used for the coding of AEs.

12.3. Safety Monitoring Committee/ Data Safety and Monitoring Board (DSMB)

12.3.1. Safety Review Committee (SRC)

An SRC will be convened for the purposes of monitoring individual subject and overall cohort safety. It will be composed of the Sponsor, the Principal Investigator, an independent physician, a member of the NHGRI IRB, and other members as needed. The SRC will meet on a regular basis to review safety data for active subjects in the trial. The SRC will make decisions regarding continuation, termination, and modification of the protocol. The SRC will review all AE data of subjects in each cohort to determine dose escalation/de-escalation for each cohort.

12.3.2. Data and Safety Monitoring Board (DSMB)

The NHGRI DSMB will review all AEs and SAEs on a yearly basis. If 1 or more drug related SAE(s) are observed, the DSMB will perform an earlier review.

13. ALTERNATIVES TO PARTICIPATION

There are no approved therapies for GNE myopathy. Ultragenyx Pharmaceuticals is conducting clinical trials to evaluate the use of extended release sialic acid (SA-ER) in subjects with GNE myopathy.

14. CONSENT PROCESS

Written informed consent is required from each subject prior to any testing under this protocol, including screening tests and evaluations. The informed consent form (ICF), as specified by the NIH IRB, must follow the Protection of Human Subjects regulations listed in the Code of Federal Regulations, Title 21, Part 50.

The background of the proposed study and the benefits and risks of the procedures and study must be explained to the subjects. The PI or an Associate Investigator will obtain consent and provide the subject with a copy of the signed and dated ICF. Confirmation of a subject's informed consent will also be documented in the subject's medical record prior to any testing under this protocol, including screening tests and evaluations.

For the convenience of enrolled subjects, if an amended consent is not available at the time of a subject's visit to the NIH CC, we will complete the re-consenting process over the telephone. An informed consent form will be mailed to the subject, and the subject and a witness will sign, then return via pre-paid mail where an investigator will sign and date (SOP 12.15 Obtaining Consent by Telephone). No amended procedures will be performed and no drug provided until a signed ICF is received.

14.1. Who Will Obtain Consent?

The PI, or an associate investigator on the study, will obtain consent from the subjects enrolled in this protocol.

14.2. Setting of Consent

Consent will be obtained at the NIH Clinical Center when feasible.

14.3. Consent Information

The ICF will be provided to the subjects as a separate document.

14.4. Protections for Vulnerable Participants

Adult subjects who are unable to provide informed consent will not be enrolled in the study.

14.5. Special Circumstances

Short Form Consents will be used for the enrollment of a non-English speaking subject. When the PI uses the English version of the informed consent document as the written summary, the version of the short form consent must match the required informed consent elements in the protocol consent document as required by 45 CFR 46 and the Food and Drug Administrative Amendments Act (FDAAA). Version 2 will be used, which reflects the required elements of informed consent required by 45 CFR 46, in addition to the Food and Drug Administrative Amendments Act (FDAAA) when the protocol is known to meet the definition of an "applicable clinical trial". The following sentence is generally located on the English consent at the end, but before the last page containing "Other Pertinent Information."

A description of this clinical trial will be available on http://www.Clinicaltrials.govhttp://www.Clinicaltrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

15. FINANCIAL COMPENSATION

None

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APPENDIX A. PROTOCOL SYNOPSIS

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Clinical Director, National Human Genome Research Institute
10 Center Drive, Building 10, Room 10C-103, Bethesda, Maryland 20892-1851
Office: (301) 402-2739
Fax: (301) 402-2740

Name of investigational product:
ManNAc

Name of active ingredient:
N-acetyl-D-mannosamine (ManNAc)

Title of study:
An Open-Label Phase 2 Study to Evaluate the Pharmacokinetics, Safety and Efficacy of ManNAc in Subjects with GNE Myopathy

Study center:
NIH Clinical Center
10 Center Drive
Bethesda, MD 20892

Principal Investigator:
Nuria Carrillo, MD
Therapeutics for Rare and Neglected Diseases (TRND)
National Center for Advancing Translational Sciences (NCATS)
10 Center Drive Drive, Building 10, Room 9N256, Bethesda, MD 20892
Office: (301) 402-2324
Fax: (301)-402-0006

Phase of development: 2

Objectives:

Primary:

- To assess the safety and tolerability of multiple doses of orally administered ManNAc to subjects with GNE myopathy;
- To examine plasma pharmacokinetics (PK) and pharmacodynamics (PD) of multiple doses of orally administered ManNAc to subjects with GNE myopathy;
- To determine the biochemical effect of ManNAc, as assessed by sialylation of proteins.

Secondary Objectives

The secondary objectives of this study are:

- To evaluate the effect of ManNAc on disease-relevant biomarkers;
- To measure the effect of ManNAc on clinical aspects of GNE myopathy and identify clinical endpoints suitable for subsequent clinical trials.

Study Design:

This is an open-label Phase 2 study to evaluate the safety, tolerability, PK, and PD of ManNAc in subjects with GNE myopathy.

A total of 12 subjects will be recruited to receive ManNAc (n=6 per cohort) twice daily. In the first phase of pharmacokinetic assessment, subjects will receive ManNAc at doses of 3,000 mg twice a day (6,000 mg per day- Cohort A) or 6,000 mg twice a day (12,000 mg per day – Cohort B) for 7 days while admitted to the NIH Clinical Center to assess PK and safety. Safety and tolerability will be assessed on an individual basis. In the second phase of the study, all subjects will receive treatment with ManNAc at a dose of 6,000 mg twice daily for the remainder of the study. Follow-up safety and efficacy evaluations will occur at 6 and 12 weeks, and at 182, 365, 548, 730, and 912 days (6, 12, 18, 24 and 30 months). Safety lab evaluation will be also be performed at 456, 638, and 820 days (15, 21 and 27 months) either at the NIH clinical center or subjects' home laboratory or physician's office. Safety will be evaluated by adverse events (AEs), clinical laboratory tests, vital signs, and physical examinations. PK will be assessed for plasma ManNAc and Neu5Ac. Biochemical efficacy will be measured by change in the sialylation of proteins and clinical efficacy will be assessed using a battery of clinical assessments deemed to be relevant based on disease natural history.

Number of subjects (planned):

12 subjects

Diagnosis and main criteria for inclusion:

Inclusion criteria:

1. Subject is age 18-60 years, inclusive, and of either gender.
2. Subject has a diagnosis of GNE myopathy based upon a consistent clinical course and identification of two *GNE* gene mutations.
3. Subject must be willing to stop any treatment with ManNAc, sialic acid, intravenous immunoglobulin (IVIG), and/or other supplements containing sialic acid (e.g. St. John's wort, sialyllactose) 90 days prior to dosing and remain off such treatment for the duration of the trial.
4. Subjects must have a body mass index (BMI) between 18 and 30 $kg/m^{2,}$ with a bodyweight of >50 kg
5. Subjects must meet one of the criteria below on at least one of the following: 1) ankle dorsiflexion, 2) knee flexion, 3) hip extension, 4) grip, 5) elbow flexion, 6) shoulder abduction:
    a. 20-75% of predicted strength measured by QMA at baseline, or
    b. If predicted muscle strength above 75%, a documented change of at least 10% per year.
6. Subject has the ability to travel to the NIH Clinical Center for admissions.
7. Subject has an INR ≤1.5 and must have stopped warfarin and other anticoagulants 2 weeks prior and after muscle biopsy procedures. Aspirin and clopidogrel should be stopped 3 days and 5 days before the procedure, respectively.
8. Subject must be able to comply with requirements of the protocol, including blood collection, drug administration, muscle MRI/MRS, muscle biopsy and muscle strength assessments.
9. If a woman of reproductive age, subject must be willing to use an effective method of contraception for the duration of the trial.
10. Subject must be able to provide informed consent.

Exclusion Criteria:

1. Subject had a clinical significant infection or medical illness 30 days prior to the first protocol visit.
2. Subject has a psychiatric illness or neurological disease that would interfere with the ability to comply with the requirements of this protocol. This includes, but is not limited to, uncontrolled/untreated psychotic depression, bipolar disorder, schizophrenia, substance abuse or

dependence, antisocial personality disorder, panic disorder, or behavioral problems, which interfere with effective communication.

3. Subject has hepatic laboratory parameters (AST, ALT, GGTP) or renal laboratory parameters (creatinine, BUN) greater than 3 times the upper limit of normal.
4. Subject has known adverse reactions to anesthetic or sedatives utilized for muscle biopsy.
5. Subject is anemic (defined as Hematocrit <30%) or has platelets <100,000 or white blood cell count less than 3,000.
6. Subject shows evidence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematological, metabolic, or gastrointestinal disease, or has a condition that requires immediate surgical intervention.
7. Subject is pregnant or breastfeeding at any time during the study.
8. Subject has received treatment with another investigational drug, investigational device, or approved therapy for investigational use less than 90 days prior to the first protocol visit.
9. Subject has hypersensitivity to ManNAc/ManNAc or in the judgment of the investigator, has a condition that places the subject at increased risk for adverse effects.
10. The presence of persistent diarrhea or malabsorption that could interfere with the subject's ability to absorb drugs or to tolerate ManNAc therapy.

Investigational product, dosage, and mode of administration:

ManNAc (ManNAc) administered orally at either 3,000, or 6,000 mg twice daily (total daily dose of 6,000 or 12,000 mg, respectively).

Duration of treatment:

Study drug will be administered for 912 days.

Reference therapy, dosage, and mode of administration:

None

Criteria for evaluation:

Safety will be assessed by adverse events, clinical laboratory tests, vital signs and physical exams.

PK/PD:

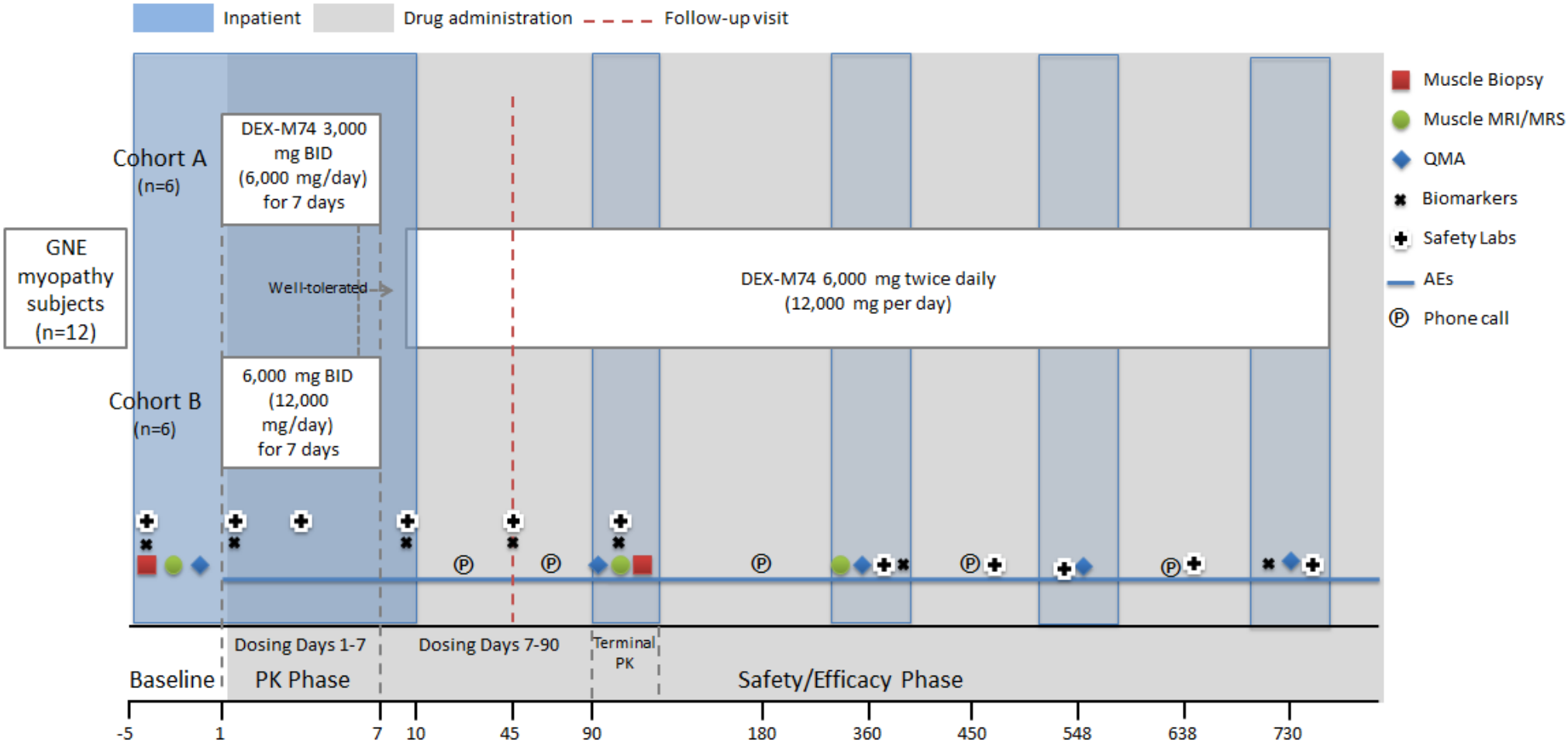
The following parameters will be assessed for plasma ManNAc and free Neu5Ac:

- Maximum observed plasma concentration (C_{max}),
- Time to C_{max} (T_{max}),
- Area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$),
- Area under the plasma concentration-time curve from time 0 to time of last measurable plasma concentration (AUC_{last}),
- Area under the plasma concentration-time curve within the dosing interval (AUC_t),
- Plasma elimination half-life ($t_{½}$).

Other:

Biochemical efficacy will be measured by change in the sialylation of proteins and clinical efficacy will be assessed using a battery of clinical assessments deemed to be relevant based on disease natural history.

APPENDIX B. STUDY DESIGN



APPENDIX C. SCHEDULE OF EVENTS

Event	Baseline	PK Phase			Safety/Efficacy Phase						PK	Safety/ Efficacy					Phone Follow-up
	Day -30 to 0	Day 1	Day 2-6	Day 7	Day 8-9	Day 10 (±3)	Day 28 (±7)	Day 45 (±7)	Day 63 (±7)	Day 85 (±5)	Day 85-90 (±5)	Day 180 (±14)	Day 365 (±14)	Day 450, 638, 820 (±30)	Day 548, 912 (-30)	Day 730 (-30)	Day 120, 240, 300, 400, 638, 820 (±30)
Admission	X							X		X		X	X		X	X	
Informed consent	X																
Eligibility	X	X						X		X		X	X		X	X	
Medical history	X							X		X		X	X		X	X	
Physical exam[1]	X	X	X	X	X	X		X		X	X	X	X		X	X	
Vital signs[2]	X	X	X	X	X	X		X		X	X	X	X		X	X	
Safety Labs[3]	X		X		X	X		X		X	X	X	X	X[11]	X	X	
Pregnancy test[4]	X							X		X		X	X		X	X	
12-lead ECG	X			X						X			X			X	
Muscle MRI/MRS	X									X			X			X	
QMA[5]	X									X		X	X		X	X	
Rehab Measures[6]	X									X		X	X		X	X	
Muscle Biopsy[7]	X									X							
Activity Monitor	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Study drug administration		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Blood for PK [8]		X	X	X				X		X	X	X	X		X	X	
Blood for PD [9]	X	X	X	X	X	X		X		X	X	X	X		X	X	
Research samples	X		X	X		X		X		X	X	X	X			X	
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
PRO[10]	X							X		X		X	X		X	X	
Medications	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Discharge						X		X			X	X	X		X	X	
Phone call							X		X								X

[1] The initial physical exam may be conducted at any time between Screening and up to and including Baseline.
[2] Collect vital signs: temperature, respiration, blood pressure and heart rate at Baseline, pre-dose and at post-dose per unit's nursing schedule. Weight must be obtained at baseline to verify eligibility (required weight > 50 kg)
[3] Blood for clinical laboratory tests and urine for urinalysis. See 5.2.2 for a list of clinical laboratory tests.
[4] Pregnancy test is required in female subjects with childbearing potential within 7 days prior to starting administration of study drug
[5] Quantitative Muscle Strength at Baseline and follow up visits
[6] Other Rehab measures include MMT-28, AMAT, 6 Min walk (or 10 meter run), timed up-go test, forward/ functional reach test will be performed at baseline and every follow-up visit. Jebsen hand test to be performed only at baseline, 12, and 24 months.
[7] Muscle biopsy at Baseline and at 3 months
[8] Collect blood for PK analysis. See Appendix D
[9] Collect blood for PD. See Appendix D.
[10] Patient-Reported Outcomes to include Global Rate of Change, HAP, IBMFRS, PROMIS and ABC scale.
[11] Performed at NIH clinical center, or home physician office or local lab; only safety blood draws to be performed.

APPENDIX D. BLOOD/URINE SAMPLING SCHEDULE

Baseline and Pharmacokinetic Phase

Sample	Timepoints																						Sample	Blood Volume (mL)	Lab
	Baseline	PK Phase																				Safety Phase			
		Day 1 (hours post-dose)										Day 2-6	Day 7 (hours after last dose)										Day 8-10			
		Pre[2]	0.5	1	2	3	4	6	8	10	12[2]	Pre[2]	Pre[2]	0.5	1	2	3	4	6	8	10	12				
Blood PK[1]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		Blood	3	Alliance
Clinical labs[3]	X											X	X										X	Blood/ Urine	14.5	NIH DLM
PD[4]	X							X		X	X	X	X										X	Blood	20	Alliance
Research labs[5]	X											X	X										X	Blood/ Urine	10-30	Gahl/ TRND
Urine PK[6]		X		X		X		X			X	X	X													

[1]Blood for plasma PK analysis will be collected on Day 1 at pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 hours after the first administration of study drug. Note that 12-hour timepoint should be collected immediately before administering the evening dose of study drug (trough level).
On Days 2-6 (weekdays only), samples will be collected immediately before administering the morning dose of the study drug.
On Day 7, blood will be collected before the morning dose and at 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours after morning administration of study drug. Note that 12-hour timepoint should be collected immediately before administering the evening dose of study drug (trough level) (Section 5.2.3)
[2]Collection of trough blood samples (immediately before drug administration).
[3]Clinical laboratory test will be collected to perform safety evaluation at baseline, on Day 2, 5 and 8 (±1). Subjects in Cohort A will have an additional test on Day 10 (±1). Clinical laboratory tests will include CBC with differential, platelet count, reticulocyte count, ESR, C-reactive protein, PT, and PTT, acute care panel (sodium, potassium, chloride, carbon dioxide, glucose, creatinine, blood urea nitrogen (BUN)), mineral panel (albumin, calcium, magnesium, phosphorus), hepatic panel (alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin), GGT, lipid panel, creatine kinase, and cystatin-C, urinalysis, and pregnancy test (Section 5.2.2)
[4]Blood for PD to include plasma and white blood cell pellets and will be collected at baseline, on Day 1 at pre-dose and at 6, 10 and 12 hours after administration of the first dose, and on Day 3 (±1) and Day 7. Subjects in Cohort A will have an additional test on Day 9 (±1).
[5]Research labs to include DNA/RNA, blood plasma, serum, red blood cells, and urine (first morning void) will be collected at baseline and on Day 3 (±2) and Day 7. Subjects in Cohort A will have an additional test on Day 9 (±1) (Section 5.3.2).
[6] Collect urine on Day 1 at Pre-dose (first morning void), 0-1 h, 1-3h, 3-6h, 6-12h and 12-24h. On Days 2-7 (weekdays only) collect first morning void.

Blood volume collected shall not exceed 10.5 mL/kg or 550 mL whichever is smaller, over any eight-week period.

Safety/Efficacy Phase and Final Pharmacokinetic Phase

Sample	Timepoints																				Sample	Blood Volume (mL)	Lab
	Safety/Efficacy Phase		Terminal PK												Safety/Efficacy Phase								
	Day 45 (±5 days)	Day 85 (±5 days)	Day 90 (-5 days)												Day 180 (±5 days)	Day 365 (±5 days)	Day 450 (±30 days)	Day 548, 730 (±30 days)	Day 638, 820 (±30 days)	Day 912 (±30 days)			
			Pre[2]	0.5	1	2	3	4	6	8	10	12	24	48	72								
Blood PK[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	Blood	3	Alliance
Clinical labs[3]	X	X												X		X	X	X	X	X	Blood/ Urine	14.5	Various
Research labs[4]	X	X												X		X	X		X		Blood/ Urine	10-30	Gahl
PD[5]	X	X	X											X		X	X		X			Blood	20	Various

[1]Blood for plasma PK analysis will be collected on Day 45, 85, 180, 365, 548 and 730 (±5 days) immediately before administering the morning dose of the study drug (though levels). On Day 90 (-5 days), blood will be collected for terminal PK at pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hours after administration of study drug. Drug will be withheld for 72 hours (Section 5.2.3). On Day 912, PK will be collected at 2, 4, 6, 8, 24 and 48 hours after the first study drug administration on TID dosing. Plasma will be collected right before ManNAc dose (trough) at the 8, 24 and 48 hour timepoints. Patient will avoid ingestion of ManNAc for 5-7 days prior to pre-dose PK draw on TID dosing.

[2]Collection of trough blood samples (immediately before drug administration)

[3]Clinical laboratory evaluations will be collected at baseline and on Days 45, 85, 90, 180 , 365, 548, 730, 820, 912 (±5 days), and 24-48 hours after last administration of study drug and will include CBC with differential, platelet count, reticulocyte count, ESR, C-reactive protein, PT, and PTT, acute care panel (sodium, potassium, chloride, carbon dioxide, glucose, creatinine, blood urea nitrogen (BUN), mineral panel (albumin, calcium, magnesium, phosphorus), hepatic panel (alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin), lipid panel, creatine kinase, and cystatin-C, urinalysis, and pregnancy test (Section 5.2.2). Day 450 and 638 will include all clinical labs except pregnancy test and lipid profile.

[4]Research labs to include plasma, serum, red blood cells, and urine (first morning void) and will be collected on Days 45, 85, 180, 365, 548, 730 (±5 days), and 48 hours during terminal PK evaluation. (See Section 5.3.2).

[5] Blood for PD will be collected on Days 45, 85, 90, 180 and 365 and 548, 730 and 912 (±5 days), and post-48 hours during terminal PK evaluation.

Blood volume collected shall not exceed 10.5 mL/kg or 550 mL whichever is smaller, over any eight-week period.

APPENDIX E. INVESTIGATIONAL AGENT(S) INFORMATION FOR PHARMACY

Investigational Agent	**Active compound**: ManNAc/ManNAc (N-acetyl-D-mannosamine monohydrate; ManNAc)
Storage requirements:	Store refrigerated at 2 to 8°C.
Stability:	Shelf life of 5 years when ManNAc powder is stored at 2 to 8°C.
Inpatient Administration	
Product description:	ManNAc will be provided as powder-in-bottle at doses of 3,000 mg, 4,000 mg and 6,000 mg.
Solution preparation:	ManNAc will be reconstituted as follows: Add 200 mL of sterile water for irrigation Shake to dissolve the drug Re-label the bottles appropriately for delivery to the subjects.
Route of administration:	ManNAc will be administered as a liquid for ORAL administration. Subjects will drink the dose directly from the prescription bottle. After ingestion, the bottle will be rinsed with 100 mL water for irrigation that will also be ingested by the subjects. Attach an appropriate investigational label including the Food and Drug Administration (FDA) caution statement "New drug- Limited by Federal Law to Investigational Use."
Self-Administration	
Product description:	ManNAc will be provided as bulk powder in a canister (300 g/container) to be measured by subjects with calibrated Spatula Balance™.
Self-administration:	Measure ManNAc using a Spatula Balance™. Add exact amount to a glass of water. Stir to dissolve the drug and drink all contents immediately. Rinse glass with water, stir to dissolve leftover drug and drink all contents.

APPENDIX F. 90-DAY TOXICOLOGY STUDIES

The doses to be investigated (3,000, 4,500 and 6,000 mg ManNAc twice daily for a total daily dose of 6,000, 9,000 and 12,000 mg, respectively) are based on the safety and PK data collected in NIH Study 12-HG-0207 (See Section 3.5.3).

Effects on the gastrointestinal tract were the most prominent in the 90-day toxicology studies performed in rats and dogs, and included loose and discolored feces, mild liver enlargement and mucosal hypertrophy of the colon. Given these findings, the No Observed Adverse Effect Level NOAEL was determined to be 12,000 mg/kg/day in rats and 6,000 mg/kg/day in dogs as described in the table below. It was determined that the human equivalent dose (HED) is 1,714 mg/kg/day, or approximately 12,000 mg/day for a 70 kg subject based on the NOAEL observed in rats, the most sensitive species, with a 10-fold safety factor.

Findings on Toxicology studies: 90-Day Rat Study

Doses (mg/kg/day)	**Type of Observation**	**Dose (mg/kg/day)**	**Findings and Comments**	**Monitoring in Current protocol**
0, 400, 1200, 4000, 12000	Gastrointestinal effects	≥4,000*	- Discolored feces (Days 63 and 77) - Minimal to mild mucosal hyperplasia in the cecum and/or colon in males and females at all doses	Clinical Evaluation
		12,000	- Discolored, loose feces (daily from Day 42 to the end of dosing)	
	Liver effect	12,000	- Mild increase in mean ALT in males (60.5 U/L) compared to controls (34.4 U/L). - Increased liver weight in males at 4,000 and 12,000 mg/kg/day - Minimal to mild hepatocellular hypertrophy in 6 of 10 males.	Liver function tests
	Kidney effects	12,000	- Increased kidney weight in males and females. - Blood in the urine 6 of 10 males and 2 of 10 females at 1, mpkd compared with 1 of 10 males and no females in the control group at the end of the dosing period. - Turbid urine in 5 of 10 males and 2 of 10 females. - No correlative kidney-related clinical chemistry or histopathological changes.	Cystatin C Urinalysis

Recovery: complete or trend towards recovery observed for all changes
*Monitorable and reversible effects
References: 90-day study in rats – IND 78091 vol08 11.2.4.3A 2012 06 27.pdf and 90-day study in rats bionalytical– IND 78091 vol09 11.2.4.3B 2012 06 27.pdf

Findings on 90-Day Dog Toxicology Study

Doses (mg/kg/day)	**Type of Observation**	**Dose (mg/kg/day)**	**Findings and Comments**	**Monitoring in current protocol**
0, 200, 600, 2,000, 6,000	GI effects	600	Loose discolored feces (Days 21-70)	
		2,000	Loose discolored feces (Days 11 onward)	
		6,000	Loose discolored feces almost daily for all males and females.	
	Hematology	≥2,000*	Minimally decreased mean RBC/HGB/HCT in females at 2,000 mg/kg/day and in males and females at 6,000 mg/kg/day.	Complete blood count
	Liver effects	≥2,000*	- Minimally reduced mean albumin concentrations - Mild diffuse vacuolations of panlobular hepatocytes (all doses but at less severity)	Serum albumin Liver function tests
	Kidney effects	≥2000*	Minimally elevated mean BUN.	BUN
*Monitorable and reversible effects References: 90-day study in dogs – IND 78091 vol11 11.2.4.6A 2012 06 27.pdf and 90-day study in dogs bioanalytical - IND 78091 vol 12 11.2.4.6B 2012 06 27.pdf				

APPENDIX G. LONG-TERM TOXICOLOGY STUDIES

Table 9. Summary table of findings from 26-Week Rat oral toxicity studies of ManNAc.

Type of Observations	Noteworthy Test Article-Related Observations	
	Dose (mg/kg/day)	Findings and Comments
Mortality	≥ 2,400	2 females and one male from Gr-4 and one male from Gr-3 dose groups died prematurely during week 20-26 of treatment but there were no consistent or adverse macroscopic or microscopic abnormalities that were considered to have had a contributing factor to these deaths
GI effects	12,000	Caecum distended at 26 weeks: 5 males and 2 females in Gr-4. Minimal mucosal hyperplasia 26 weeks: 13 males and 7 females in Gr-4.
Liver effects	12,000	Alkaline phosphatase levels were slightly elevated (1.45X) at weeks 13 and 26 in Gr-4, however they seem comparable to historical control range. No treatment-related liver weight abnormalities noticed. Minimal centrilobular hypertrophy was seen in 12 males and 10 females in Gr-4.
Adrenal effects	≥ 400	Analysis of urine: Weeks 13 and 26 revealed test article-related effects (predominantly at 12000 mg/kg/dayGr-4) including abnormalities in urinary volume, slightly high specific gravity and slightly low total sodium (0.62X) and potassium (0.38X) in Weeks 13 and 26 in both sexes in Gr-4. Histopathological changes included minimal hypertrophy of the zona glomerulosa in 10 males and 6 females in Gr-4;,1 male and 3 females in Gr-3 and 1 male and 2 females in Gr-2. No treatment related adrenal weight changes noticed.
Kidney effects	≥ 2,400	Slightly high plasma urea concentration (1.46X) in weeks 13 and 26 in Gr-4. A higher than control incidence of trace amounts of blood pigment were detected in the urine in Weeks 13 and 26 in males in Gr-4 and, to a lesser extent, in males at Gr-3, but this finding showed complete recovery. No correlative kidney-related histopathological changes. No treatment related kidney weight changes noticed.

Dose Groups: Gr-1: 0 mg/kg TID, Gr-2: 133.3 mg/kg TID (400 mg/kg/day); Gr-3: 800 mg/kg TID (2,400 mg/kg/day), Gr-4: 4000 mg/kg TID (12,000 mg/kg/day).
Recovery: complete or trend towards recovery observed for all changes.
Abbreviations. TID: three times daily
Reference: BEQ0005 Final Toxicology Report (09 Dec 14).pdf

Table 10. Summary table of findings from 39-Week Dog oral toxicity study of ManNAc

Type of Observations	Noteworthy Test Article-Related Observations	
	Dose (mg/kg/day)	**Findings and Comments**
GI effects	≥ 1,080	Liquid feces for all males and females on Gr-4 and within the first 4 weeks of treatment for a few animals on Gr-3. On Gr-4, the fecal disturbances occasionally consisted of mucoid or abnormal colored faces (red or green) during the early stages of the study. No correlative GI related histopathological changes noticed.
Liver effects	≥ 1,080	Week 26 Males: Higher ALT (40-41 U/L, Gr 4 and 3), cholesterol (3.86 mmol/L, Gr 4) and triglyceride concentrations (0.42-0.44 mmol/L, non-statistically higher in Gr 3 and 4). Week 39 Males: Higher ALT (42 U/L, Gr 4), cholesterol (3.86 mmol/L, Gr 4) and Triglycerides (0.41 mol/L, non-statistically higher in Gr 4). No accompanying liver histopathology changes noticed. No treatment-related liver weight changes noticed.
Kidney effects	4,500	Urinalysis : Wk 13- three females in Gr 4 with higher proteins and urinary blood. Histopath changes, Wk 40: one Gr 4 female with Kidney Infiltration, Inflammatory Cells, Interstitial, minimal; one Gr 3 female with urinary bladder mineralization. No treatment related kidney weight changes noticed.

Dose Groups: Gr-1: 0 mg/kg TID, Gr-2: 60 mg/kg TID (180 mg/kg/day); Gr-3: 365 mg/kg TID (1,080 mg/kg/day), Gr-4: 1,500 mg/kg TID (4,500 mg/kg/day).
Recovery: complete or trend towards recovery observed for all changes.
Abbreviations. TID: three times daily
Reference: BEQ0001 Final Report 17 Oct 2014.pdf

APPENDIX H. SIGNATURE OF INVESTIGATOR

Title:	An Open-Label Phase 2 Study to Evaluate the Pharmacokinetics, Safety and Efficacy of ManNAc in Subjects with GNE Myopathy
Protocol number:	15-HG-0068
Date:	December 7, 2017

By signing this page I attest that I have read and understand the contents of Clinical Protocol 15-HG-0068 and any subsequent amendments. I agree to adhere to the design, conduct, and reporting requirements of the study as stated in the clinical protocol and to my obligations to the Sponsor as described in the protocol and executed contracts between myself, my Institution, and the Sponsor.

Investigator's Signature: _____

Investigator's Name: _____

Institution: _____

Date: _____