

Hereditary Inclusion Body Myopathy: Single Patient Response to Intravenous Dosing of *GNE* Gene Lipoplex

Gregory Nemunaitis,^{1,2} Chris M. Jay,³ Phillip B. Maples,³ William A. Gahl,⁴ Marjan Huizing,⁴ Tal Yardeni,⁴ Alex W. Tong,³ Anagha P. Phadke,³ Beena O. Pappen,³ Cynthia Bedell,¹ Henry Allen,⁵ Cathy Hernandez,⁵ Nancy S. Templeton,³ Joseph Kuhn,⁶ Neil Senzer,^{1,3,7} and John Nemunaitis^{1,3,7}

Abstract

Hereditary inclusion body myopathy (HIBM) is an autosomal recessive adult-onset myopathy due to mutations in the *GNE* (UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase) gene. Affected patients have no therapeutic options. We have previously demonstrated in preclinical testing the ability to safely correct *GNE* gene function through liposomal delivery of the wild-type *GNE* gene. Results were verified in a single patient treated by intravenous infusion of *GNE* gene lipoplex. A single patient (patient 001) with severe HIBM treated with a compassionate investigational new drug received seven doses of *GNE* gene lipoplex via intravenous infusion at the following doses: 0.4, 0.4, 1.0, 4.0, 5.0, 6.0, and 7.0 mg of DNA. *GNE* transgene expression, downstream induction of sialic acid, safety, and muscle function were evaluated. Transient low-grade fever, myalgia, tachycardia, transaminase elevation, hyponatremia, and hypotension were observed after infusion of each dose of *GNE* gene lipoplex. Quadriceps muscle expression of the delivered *GNE*, plasmid, and RNA was observed 24 hr after the 5.0-mg dose and at significantly greater levels 72 hr after the 7.0-mg infusion in comparison with expression in quadriceps muscle immediately before infusion. Sialic acid-related proteins were increased and stabilization in the decline of muscle strength was observed. We conclude that clinical safety and activity have been demonstrated with intravenous infusion of *GNE* gene lipoplex. Further assessment will involve a phase I trial of intravenous administration of *GNE* gene lipoplex in individuals with less advanced HIBM with more muscle function.

Introduction

THE AUTOSOMAL RECESSIVE FORM of hereditary inclusion body myopathy (HIBM) is an early adult-onset progressive disabling myopathy transmitted through mutations of the *GNE* (UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase) gene (Huizing and Krasnewich, 2009; Jay *et al.*, 2009). *GNE* encodes the bifunctional enzyme UDP-*N*-acetylglucosamine (GlcNAc) 2-epimerase/*N*-acetylmannosamine (ManNAc) kinase (GNE/MNK), the rate-limiting enzyme in the formation of sialic acid (Hinderlich *et al.*, 1997; Huizing, 2005). Proper folding, stabilization, and function of skeletal muscle glycoproteins require sialy-

lation within muscle fibers (Langer, 1978; Keppler *et al.*, 1999; Huizing *et al.*, 2004; Ricci *et al.*, 2006). *GNE* mutations resulting in hyposialylation of muscle glycoproteins appear to contribute to myofibrillar degeneration and loss of normal muscle function, thereby leading to the myopathy of HIBM (Jay *et al.*, 2009).

We constructed a *GNE*-wt-DNA vector, using human *GNE* cDNA and the pUMVC3 expression vector. Transgene expression of *GNE* mRNA and GNE/MNK protein, in correlation with subsequent increased production of sialic acid, was demonstrated *in vitro* (Jay *et al.*, 2008). We also complexed the *GNE* expression vector with a cationic liposome, composed of 1,2-dioleoyl-3-trimethylammonium-propane

¹Mary Crowley Cancer Research Centers, Dallas, TX 75201.

²MetroHealth Medical Center, Cleveland, OH 44109.

³Gradalis, Inc., Dallas, TX 75201.

⁴Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892.

⁵Hospitalist Internists Associates, Dallas, TX 75251.

⁶WLS Surgical Associates, Dallas, TX 75246.

⁷Texas Oncology, Dallas, TX 75251.

(DOTAP) and cholesterol (*GNE* gene lipoplex) and demonstrated dose-related safety in BALB/c mice (Phadke *et al.*, 2009). Moreover, we demonstrated safety and a durable improvement in local regional skeletal muscle function of a single severely affected patient with HIBM (patient 001) in correlation with *GNE* transgene upregulation and local induction of sialic acid production after a series of local regional intramuscular injections of the *GNE* gene lipoplex (Nemunaitis *et al.*, 2010). We now report safety and response after a series of dose-escalating intravenous infusions of *GNE* gene lipoplex into patient 001.

Materials and Methods

Study design

The subject received *GNE* gene lipoplex mixed with 250 ml of D5W (5% dextrose in water) every 60 days, administered via 30-min intravenous infusions for seven doses (0.4, 0.4, 1.0, 4.0, 5.0, 6.0, and 7.0 mg). The subject was premedicated with oral dexamethasone and a combination of intravenous histamine H₁ and H₂ blockers, oral indocin, and oral acetaminophen. After injection of the *GNE* gene lipoplex, the subject was observed for 4 hr. During the observation period, vital signs were taken every 30 min for the first hour, and then every hour until hour 4. Vital signs during this period included blood pressure, temperature, pulse, respiration, and oxygen saturation. Toxicities were graded and reported according to the National Cancer Institute (NCI, Bethesda, MD) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. The subject was assessed for *GNE* gene expression, sialic acid production, and muscle strength before treatment with *GNE* gene lipoplex.

The histopathology and quantitative assessment of *GNE* transgene expression and sialic acid levels of the quadriceps muscle were assessed through sequential biopsy done at baseline (right quadriceps) before injection of the 5- and 7-mg doses of *GNE* gene lipoplex and at 24 hr (for the 5-mg dose) and 72 hr (for the 7-mg dose), both involving the right quadriceps after intravenous infusion.

Muscle strength was measured by force generation of selected muscles, using a break test technique (Bohannon, 1988, 1992; Stratford and Balsor, 1994; Burns *et al.*, 2005) with a handheld dynamometer (HHD). Isokinetic muscle function testing with an HHD has previously been done to assess quantitative changes in muscle strength (Andres *et al.*, 1986; Mendell *et al.*, 1989; Florence *et al.*, 1992; Personius *et al.*, 1994; Roy and Doherty, 2004; Paternostro-Sluga *et al.*, 2008). In our study, muscle force generation was measured with a Chatillon FCE-200 digital HHD (AMETEK, Largo, FL). It uses a digital ordinal scale for the measurement of muscle force in pounds, kilograms, or newtons. This dynamometer displays force measurements to the nearest 0.2 lb to a maximum of 200 lb for a total of 1000 potential muscle force readings.

Muscle strength was tested preinjection and on days 1, 2, and 30 after each injection. Thirty muscle groups were tested for muscle strength over time with an HHD, before the first intramuscular injection and subsequently thereafter: 4 muscle groups in the neck, 2 in the shoulders, 10 in the legs, and 14 in the arms (including the injected muscles). The muscle groups tested included the right lateral neck flexors, left lateral neck flexors, forward neck flexors, neck extensors, shoulder elevators, elbow flexors, wrist extensors, elbow extensors, finger

flexors, finger extensors, finger abductors, thumb extensors, hip flexors, knee extensors, ankle dorsiflexors, long toe extensors, and ankle plantar flexors. Before the first intravenous injection, four additional muscle groups were added for testing: right and left sides for wrist flexion and forefoot flexion.

The break technique requires the examiner to overpower a maximal effort by the subject, thereby producing a measurement of eccentric muscle strength. Ten repeat measurements were taken for each muscle at each time point.

A symptom-directed physical examination was performed at baseline and on days 3, 8, 15, and 30 of each cycle. Blood studies (complete blood count [CBC], chemistry, creatine phosphokinase [CPK], aldolase, erythrocyte sedimentation rate [ESR], and C-reactive protein [CRP]) were done at baseline and on days 3, 8, 15, and 30 of each cycle. Long-term follow-up involved physical examination, blood studies (CBC and chemistry) and muscle strength testing on days 60, 90, 120, 150, and 180. Pulmonary function studies were performed in an independent laboratory by a certified technician, in accordance with American Thoracic Society guidelines, with an ongoing quality control program (American Thoracic Society, 1991, 1995). Pulmonary function studies were done every 2 months (at baseline and before each subsequent injection).

Study population

A single patient with HIBM was enrolled. The following inclusion criteria were met: (1) the ability to understand and the willingness to sign a written informed consent; (2) histological and genotypic confirmation of HIBM; (3) no acceptable effective treatment options; (4) age >18 years; (5) life expectancy greater than 3 months; and (6) normal organ and marrow function defined as leukocytes (>3000/ μ l), absolute neutrophil count (>1500/ μ l), platelets (>100,000/ μ l), total bilirubin (within normal institutional limits), aspartate transaminase (AST; serum glutamic oxaloacetic transaminase [SGOT])/alanine transaminase (ALT; serum glutamic pyruvic transaminase [SGPT]) (<2.5 \times institutional upper limit of normal), creatinine (within normal institutional limits), and creatinine clearance (>60 ml/min/1.73 m² for patients with creatinine levels above institutional normal limits).

The patient was not receiving any other investigational agents, had no history of allergic reactions attributed to compounds of similar chemical or biological composition, had no uncontrolled intercurrent illness, was not pregnant or nursing, and was not HIV positive.

GNE gene lipoplex

The DNA vector is composed of a 2169-base pair wild-type human *GNE1* isoform (NCBI reference sequence NM_005476.3) driven by the cytomegalovirus (CMV) promoter. The plasmid backbone is the pUMVC3 vector from Aldevron (Fargo, ND). Further details about the vector construction can be found in our previous publication (Jay *et al.*, 2008). The liposome delivery vehicle is composed of a 10:9 molar ratio of two lipids, DOTAP and cholesterol (Templeton *et al.*, 1997). The *GNE* gene lipoplex size ranged from 400 to 500 nm and the ζ potential ranged from 50 to 59 mV. The final product was suspended in D5W USP for a total volume of 100 μ l/injection. Manufacturing details are provided in prior work (Nemunaitis *et al.*, 2010).

The final product consisted of 4 mM DOTAP:cholesterol and *GNE* DNA (0.5 $\mu\text{g}/\mu\text{l}$).

Real-time quantitative PCR analysis

We used a method of reverse transcription to convert total RNA into cDNA. Extracted total genomic DNA and converted cDNA were analyzed via quantitative PCR (qPCR) relative to a known standard to quantitate the unknown samples. This method has been previously described (Jay *et al.*, 2008; Phadke *et al.*, 2009). Briefly, tissues were homogenized with a TissueLyser (Qiagen, Valencia, CA), and total nucleic acid was extracted with RNeasy and DNeasy kits (Qiagen). One microgram of total RNA was used as starting material for cDNA synthesis, using a Bio-Rad (Hercules, CA) iScript cDNA kit. *GNE*-specific primers were designed so that only the plasmid (recombinant *GNE* transcript, r*GNE*) would be amplified without cross-reacting with the endogenous *GNE* mRNA.

Histological analysis

Single needle core biopsies taken from the right quadriceps muscle at baseline and 24 hr after the fifth intravenous infusion (5 mg) were formalin fixed and paraffin embedded (FFPE). Slides were prepared, stained with hematoxylin and eosin (H&E), and analyzed by a certified pathologist (Pro-path, Dallas, TX).

Immunohistochemical analysis

FFPE sections were deparaffinized and rehydrated. For lectin stains, sections were blocked with 0.1% bovine serum albumin (BSA) and labeled with fluorescein isothiocyanate-conjugated wheat germ agglutinin (FITC-WGA), FITC-conjugated peanut agglutinin (FITC-PNA), or FITC-Jacalin (jackfruit agglutinin) (EY Laboratories, San Mateo, CA) for 1 hr at room temperature. For polysialylated neural cell adhesion molecule (PSA-NCAM), antigen retrieval was performed with 0.1 M sodium citrate, and sections were blocked with 1% BSA and labeled with mouse anti-PSA-NCAM (Chemicon International/Millipore, Temecula, CA) overnight at 4°C followed by Alexa 555-conjugated goat anti-mouse (Molecular Probes/Invitrogen, Carlsbad, CA). Imaging was done with an LSM 510 Meta confocal microscope (Carl Zeiss, Thornwood, NY).

Statistical analysis

Statistical analysis was performed on the HHD data generated for each muscle group. Separate comparison of the mean muscle strength at each time point was made with the baseline mean for each muscle, using the Student *t* test.

Results

Patient description

Patient 001 is a 41-year-old female who developed muscle weakness at age 18. She has experienced typical disease progression and gradually lost muscle mass and function over the last 23 years. Diagnosis of HIBM on the basis of molecular criteria revealed compound heterozygosity for *GNE* gene mutations in exon 7 (c.1132G>T; p.D378Y) and in exon 11 (c.1892C>T; p.A631V). Both mutations were previ-

ously reported in HIBM (Eisenberg *et al.*, 2003; Huizing and Krasnewich, 2009). The disease-specific family history was negative.

The *GNE* gene lipoplex investigational new drug (IND) was initially approved by the U.S. Food and Drug Administration (FDA) in July 2008 for a single patient intramuscular injection trial and later amended for administration of intravenous injections. Patient 001 received four intramuscular doses of *GNE* gene lipoplex (0.4 mg of DNA for first intramuscular injection and 0.2 mg of DNA for each of the next three intramuscular injections) and demonstrated local injected muscle strength improvement in correlation with *GNE* transgene and sialic acid increase on day 3 after injection compared with baseline before the first injection. Transient grade 1/2 flulike symptoms and transaminase elevation were observed within 24 hr of each dose (Nemunaitis *et al.*, 2010). Deterioration of diaphragm and intercostal muscle strength justified the protocol amendment to explore intravenous testing.

Safety

Patient 001 treatment with intravenous *GNE* gene lipoplex was well tolerated. Within 24 hr of each cycle, a low-grade fever of 100°F developed (Fig. 1A–C). This was associated with transient grade 1/2 myalgias, tachycardia, hypotension, and liver enzyme elevation (from grade 1 pretreatment baseline) after each dose (Fig. 1A–C). At 48 hr, myalgias diminished and tachycardia, hypotension, and fever resolved. No evidence of myalgia was detected 1 week after the administration of each dose. Slightly greater duration of myalgias, tachycardia, and hypotension was observed at the 7.0-mg dose (Fig. 1B), although no grade 3 or greater toxic effect was observed. Consistent grade 1–2 hyponatremia, transiently associated with each dose greater than or equal to 1.0 mg, was also observed (Fig. 2).

Muscle function

Muscle strength testing with a dynamometer revealed a variable distribution of skeletal muscle functional changes manifested by a loss of force generated in some muscles (e.g., upper trapezius and quadriceps; Fig. 3A), stabilization in others (extensor carpi radialis longus, and wrist flexion; Fig. 3B), and rare improvement in others (forefoot flexion; Fig. 3C).

However, manual muscle testing on patient 001 suggested overall stabilization of muscle function. The following muscle groups were tested on each side of the body, using the Medical Research Council (MRC) Scale (Medical Research Council, 1981): elbow flexors, wrist extensors, elbow extensors, finger flexors, finger abductors, hip flexors, knee extensors, ankle dorsiflexors, long toe extensors, and ankle plantar flexors. The sum of the motor scores from the 20 muscles was defined as the total motor score.

Review of past records revealed several incomplete and complete manual muscle examination records on the 20 muscles that were used for this study. The first recorded examination was on February 5, 1992, which revealed a total motor score of 61 and subsequently a total motor score of 55 on April 9, 1998.

Regular manual muscle testing by a single examiner on a monthly basis started on March 16, 2007 and continues to date (Fig. 4). The total motor score on her initial

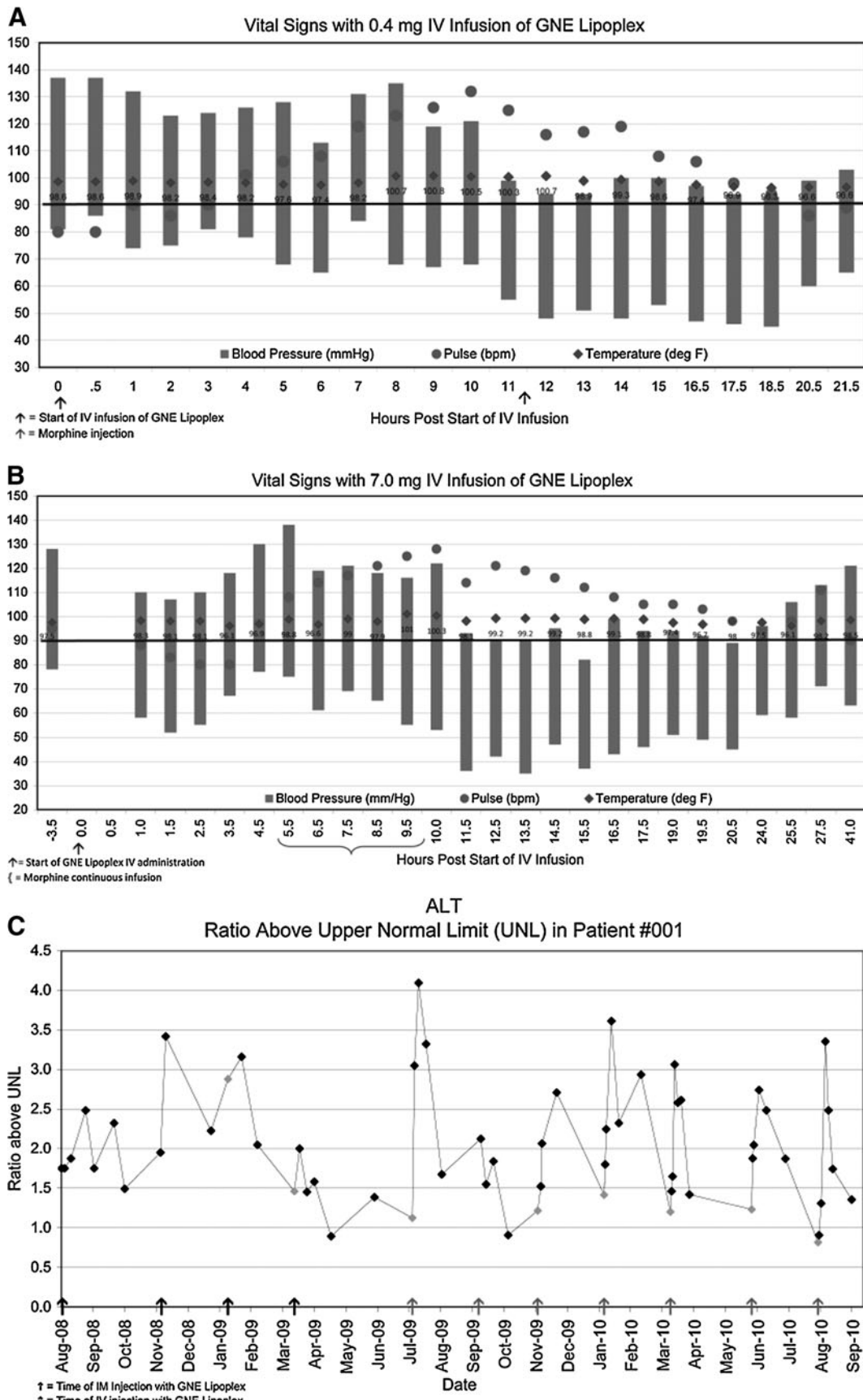


FIG. 1. (A) Characterization of vital signs for patient 001: Cycle 1 of intravenous infusion of *GNE* gene lipoplex. Results were similar with each infusion. (B) Characterization of vital signs: Cycle 11 of intravenous infusion of *GNE* gene lipoplex. Results were similar with each infusion. (C) Characterization of alanine transaminase (ALT) response after each injection of *GNE* gene lipoplex. Results were similar with each infusion. Light gray symbols indicate the value before treatment. IV, intravenous; IM, intramuscular.

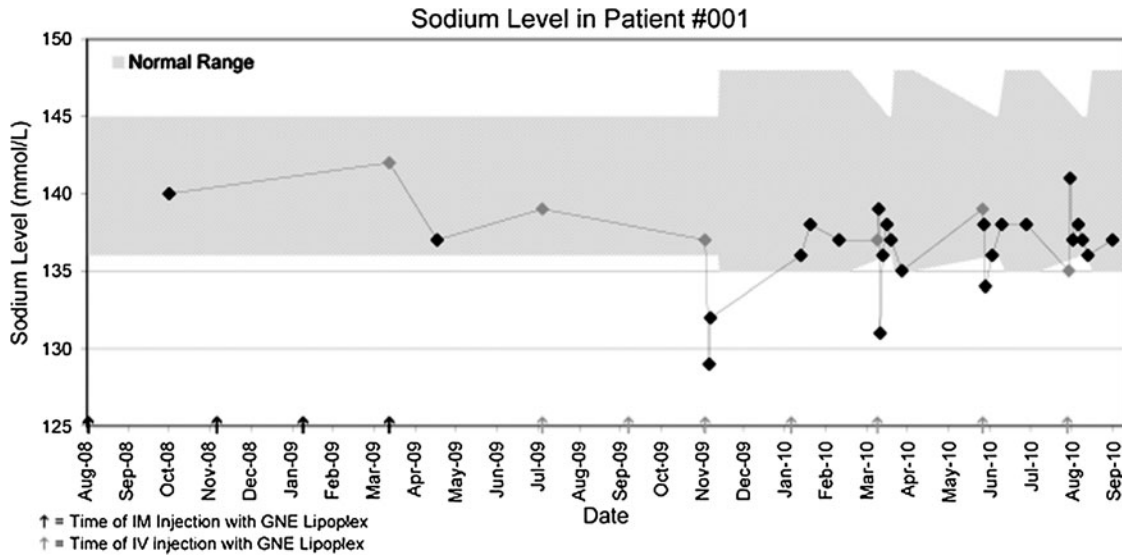


FIG. 2. Relationship of blood sodium level in patient 001 to time of dose with *GNE* gene lipoplex. Gray shading represents the normal value range used by the laboratory. Light gray symbols indicate the value before treatment.

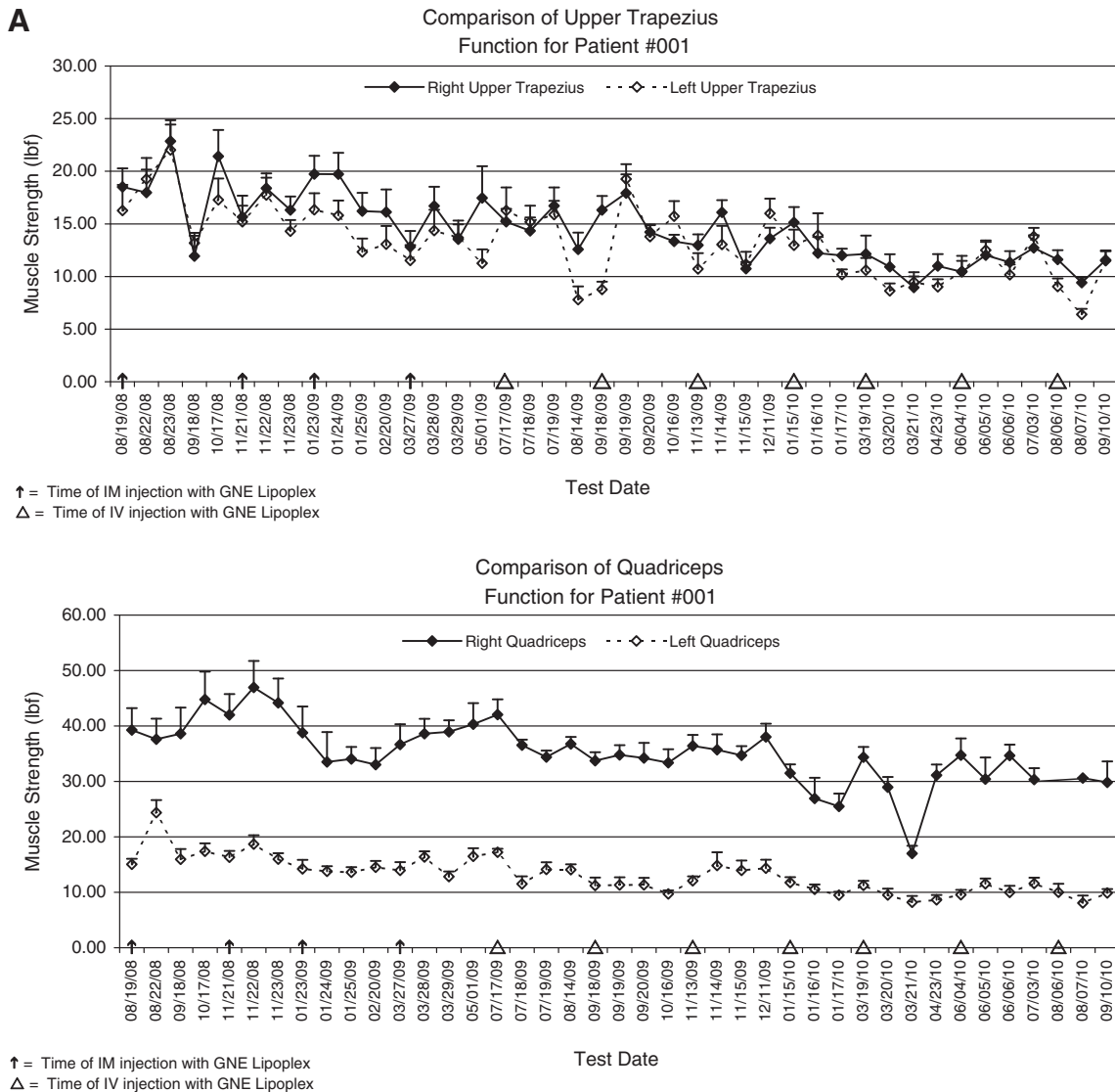


FIG. 3. (A) Deteriorating muscle response in patient 001 by dynamometer testing. (B) Stable muscle response by dynamometer testing. (C) Improving muscle response by dynamometer testing (Figure continued →)

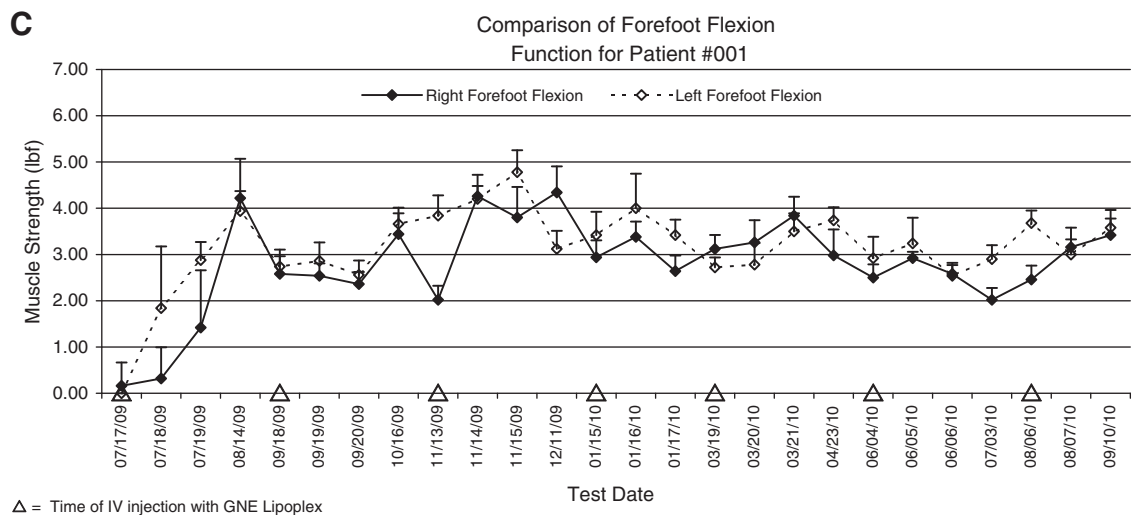
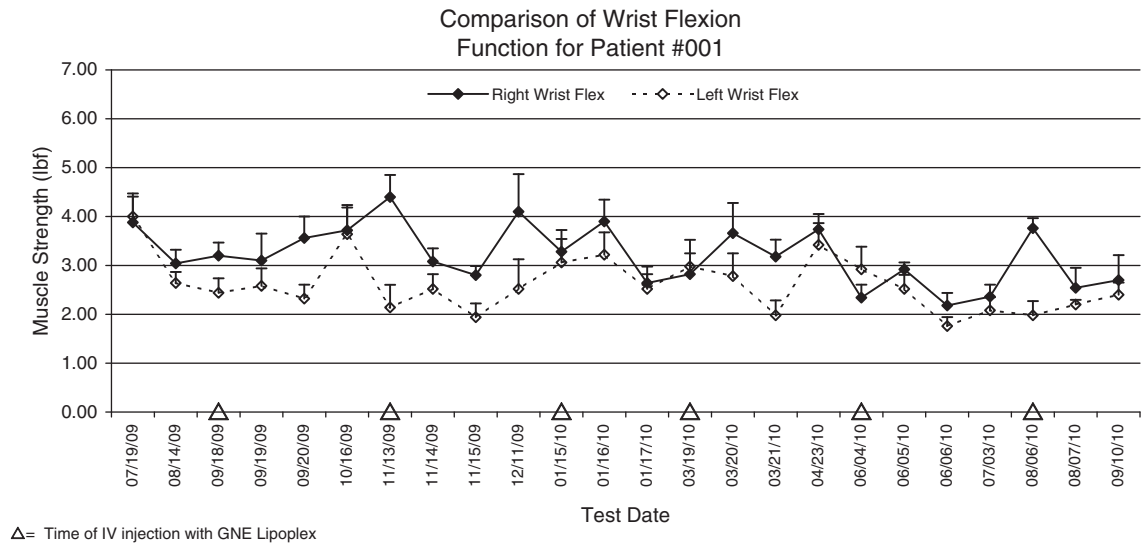
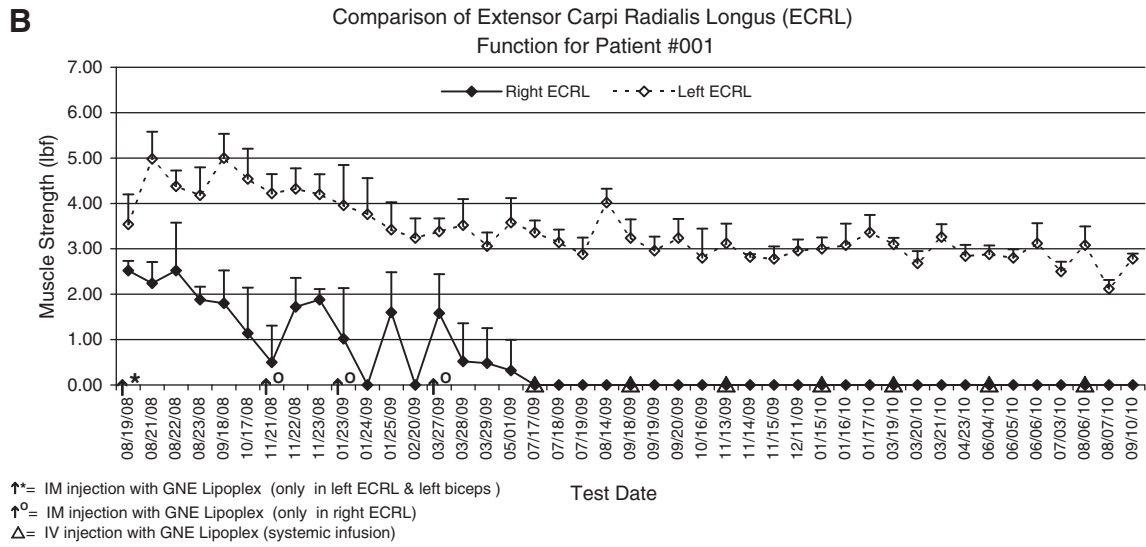


FIG. 3. (Continued).

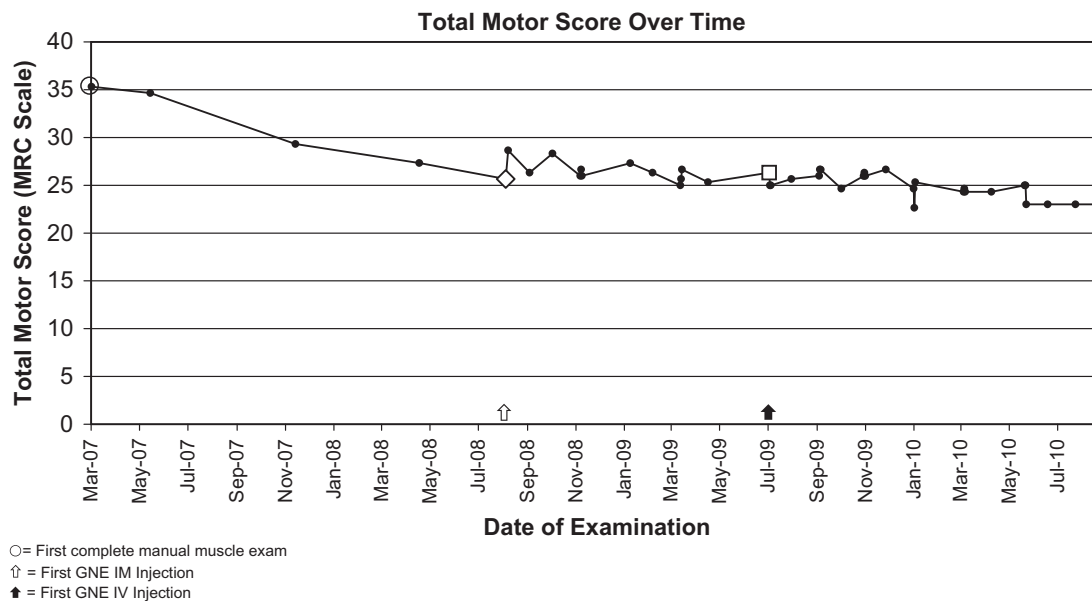


FIG. 4. Progression of muscle weakness (Total Motor Score) over time (years) in patient 001. The diamond indicates the value before the first intramuscular injection. The square indicates the value before the first intravenous injection.

examination on March 16, 2007 was 34. She had her first intramuscular *GNE* gene lipoplex injection on August 19, 2008 and before the injection a total motor score of 25 was recorded. The first intravenous *GNE* gene lipoplex infusion was on July 17, 2009 and her total motor score before the injection was still 25. The last examination, on September 10, 2010, 1 month after her most recent intravenous *GNE* injection, revealed a total motor score of 23. The recorded total motor score dropped 27 points from 1992 to 2007, and then dropped 9 points during the first 5 months of the study period. After *GNE* gene lipoplex administrations her total motor score dropped only 2 points over the next 24 months from 2008 to 2010 (Fig. 4). These results are suggestive of stabilization of muscle function during *GNE* gene lipoplex treatment.

In addition, the May 29, 2007 (first examination by dynamometer) results revealed 18 of 30 muscles producing force. Seventeen months later, at the time of first intramuscular injection of *GNE* gene lipoplex, seven more muscles had lost the ability to produce force. However, during the 24 months of treatment with intramuscular and intravenous *GNE* gene lipoplex, no additional muscles lost significant detectable force by dynamometer measurement. In fact, one additional muscle recorded generated force. Pulmonary function as an assessment of diaphragm and intercostal muscle strength was determined by monitoring forced vital capacity (FVC). Pulmonary function appeared to stabilize as well, as highlighted by FVC in comparison with baseline and prior pulmonary function tests (PFTs) in correlation with improved ability to sleep in a flat position (Fig. 5).

Results suggest overall stabilization in the loss of muscle strength via manual muscle testing.

Histological analysis

Histological analysis of the quadriceps tissue showed skeletal muscle tissue with a small amount of connective

tissue. Ringed inclusion bodies consistent with HIBM were also observed.

Immunohistochemical analysis

Three different lectins (WGA, PNA, and Jacalin) and antibodies to one protein (polysialylated neural cell adhesion molecule, PSA-NCAM) were used for staining (Fig. 6). Results of Jacalin and PSA-NCAM staining are consistent with modest improvement in sialic acid expression 24 hr after infusion of *GNE* gene lipoplex. However, no change in PNA was observed. WGA binds the terminal sialic acid on all glycans and provides a general overview of all sialylation. Its intensity was expected to remain constant. PNA labels terminal O-linked and N-linked galactose groups and is a marker for hyposialylation. The day 0 quadriceps sample was brightly stained, indicating hyposialylation, but there was no improvement (reduction in staining) at 24 hr. Jacalin predominantly labels terminal galactose groups on O-linked glycans (indicating hyposialylation) and the staining results illustrated a reduction in staining (increased sialylation) intensity at 24 hr. The PSA-NCAM antibody binds to polysialylated NCAM protein and the results demonstrate increased staining, indicating increased sialic acid on NCAM 24 hr after *GNE* gene lipoplex infusion.

qPCR analysis

Total DNA and RNA were extracted and qPCR analysis confirmed significant human wild-type *GNE* plasmid in the quadriceps muscle sample 24 hr after infusion of 5 mg of *GNE* gene lipoplex ($p=0.00003$). In addition, modest expression of *GNE* RNA was demonstrated by RT-qPCR ($p=0.05$) from the same tissue. However, a substantial increase in expression of RNA ($p=0.005$) and DNA ($p=0.0005$) was detected in a 72-hr posttreatment biopsy from the same quadriceps muscle treated with a 7-mg dose, in comparison with pretreatment baseline (Fig. 7A–C).

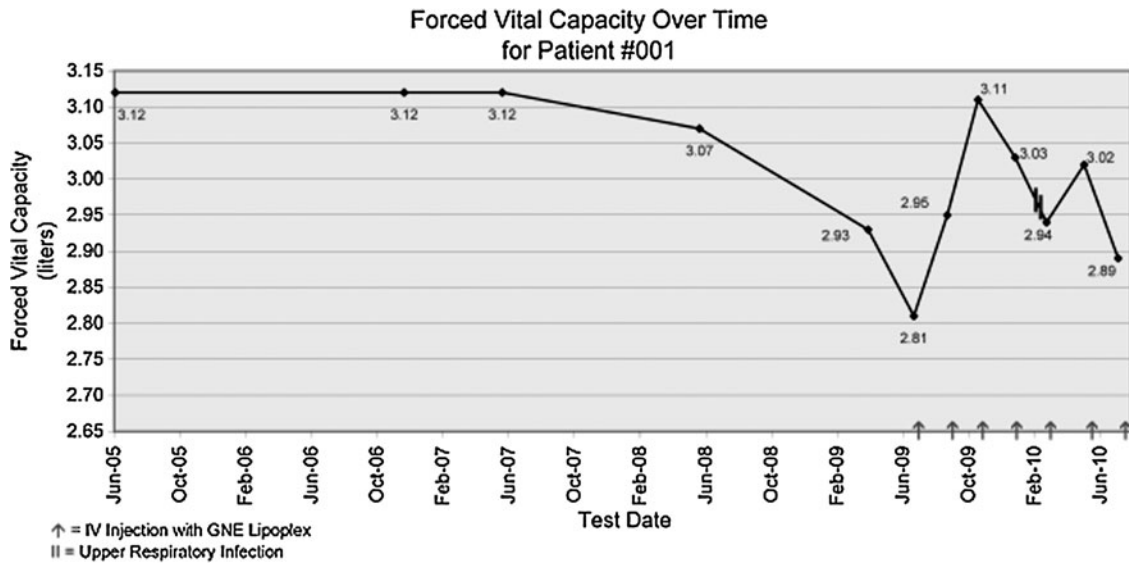


FIG. 5. Forced vital capacity (FVC) over time for patient 001.

Discussion

We have demonstrated acceptable safety after a series of seven intravenous infusions of *GNE* gene lipoplex up to a

dose of 7 mg of *GNE* gene lipoplex over a 13-month span in a single patient with late-stage HIBM. Variable stabilization and limited transient improvement in muscle strength, correlating with significant detection of plasmid, r*GNE* trans-

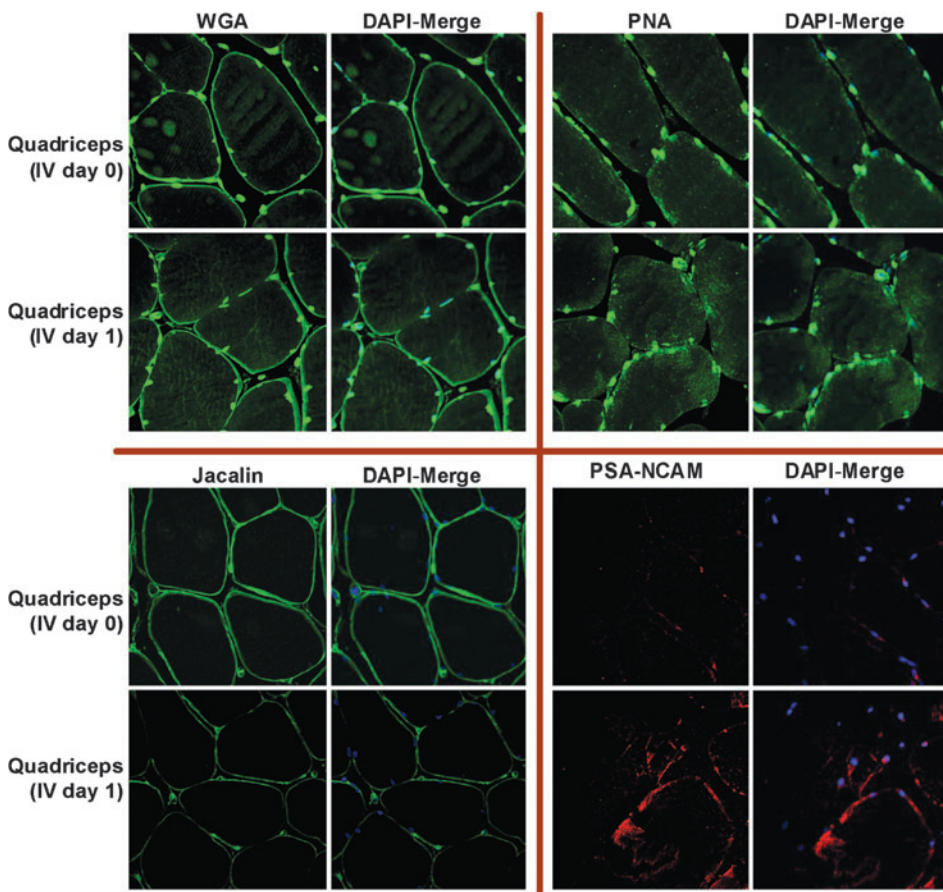


FIG. 6. Immunohistochemical staining of quadriceps biopsies from patient 001 before and after *GNE* gene lipoplex infusion. Wheat germ agglutinin (WGA): Predominantly binds terminal sialic acid on all glycans. This stain is a general “overview” of how much sialic acid is present in the tissue; it is nonspecific. For patient 001, there was no difference in WGA stain before and after treatment. This was as expected, and as previously described for human HIBM muscle. Peanut agglutinin (PNA): Predominantly labels terminal galactose groups, and therefore is a marker for hyposialylated glycans. Patient 001 showed bright PNA staining, indicating hyposialylation. There appeared to be no difference in intensity of the PNA stain before and after treatment. Jackfruit agglutinin (jacalin): Predominantly labels terminal galactose groups on O-linked glycans, and therefore is a marker for hyposialylated O-linked glycans. Jacalin staining showed a slight decrease in intensity after treatment, suggestive of

improvement in hyposialylation on O-linked glycans. Polysialylated neural cell adhesion molecule (PSA-NCAM) antibody: Binds PSA-NCAM. PSA-NCAM showed a modest increase in intensity after treatment, consistent with improvement in hyposialylation.

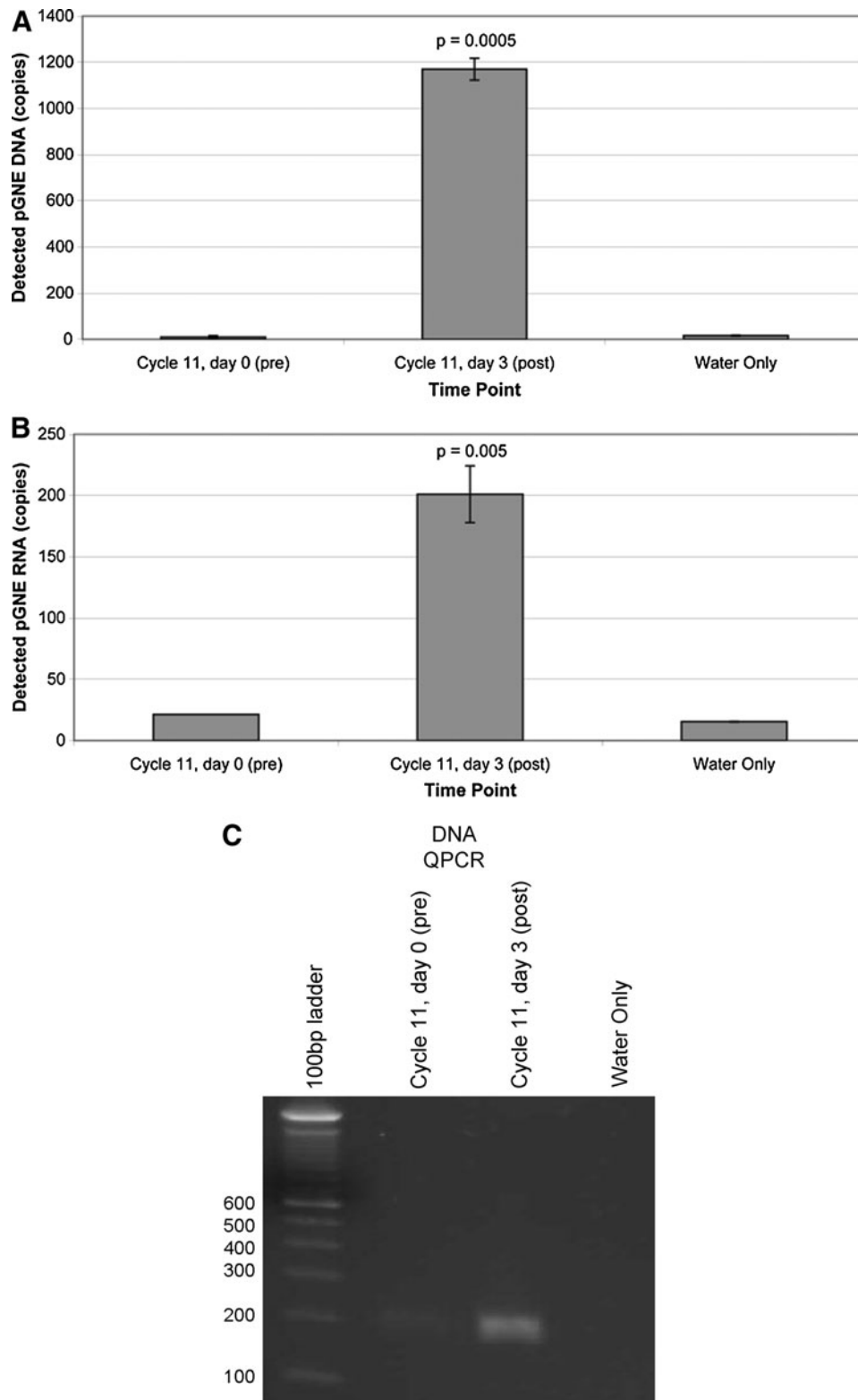


FIG. 7. (A) Expression of plasmid DNA harvested from quadriceps by qPCR at baseline and 72 hr after *GNE* gene lipoplex (7-mg dose) infusion. The DNA expression illustrates the results for real-time PCR. The bar graphs represent the amount of detected *GNE* relative to a known standard curve. (B) Expression of mRNA harvested from quadriceps by RT-qPCR at baseline and 72 hr after *GNE* gene lipoplex (7-mg dose) infusion. The RNA expression illustrates the results for real-time PCR. The bar graphs represent the amount of detected *GNE* relative to a known standard curve. (C) qPCR results of DNA at baseline and 72 hr after *GNE* gene lipoplex (7-mg dose) infusion.

gene, and consequent increase of cell surface sialylation (via Jacalin and PSA-NCAM staining), were observed in the biopsied quadriceps muscle 24 hr after intravenous infusion in comparison with preinfusion levels, verifying successful vector delivery to target organ. Moreover, stabilization of pulmonary function associated with quality of life improvement (reclining to supine sleeping position) are encouraging observations; however, additional HIBM patients with extensive functional monitoring will be required for further study before conclusions on *GNE* gene lipoplex effect are made. Nevertheless, these results are encouraging and justify further phase I trial investigation.

GNE/*MNK* enzymatic activity in HIBM may impair sialic acid production and interfere with proper sialylation of glycoconjugates (Huizing and Krasnewich, 2009; Jay *et al.*, 2009). Data using the D176V-Tg mouse model demonstrated that muscle atrophy and weakness could be prevented with prophylactic treatment of sialic acid metabolites (Malicdan *et al.*, 2009). Untreated mutant mice showed hyposialylation in multiple organs, including skeletal muscle, whereas oral treatment with NeuAc or ManNAc improved sialylation and reduced the number of rimmed vacuoles and amyloid deposits. Sialic acid is the only sugar component of glycoproteins that bears a net negative charge. This charged sugar provides the terminal carbohydrate on a variety of N-linked and O-linked glycans of proteins, as well as glycolipids that mediate cell-cell and protein-protein interactions (Varki, 1997, 2008).

Hypoglycosylation of α -dystroglycan, a central protein of the skeletal muscle dystrophin-glycoprotein complex, could be one mechanism that may provide an explanation for the muscle weakness of patients with *GNE* mutations. α -Dystroglycan helps to anchor the extracellular matrix to the cytoskeleton of the sarcolemma. If hypoglycosylated α -dystroglycan is unable to properly bind to the extracellular matrix, the connection between the extracellular matrix and the cytoskeleton provided by dystroglycan is lost and cannot stabilize the sarcolemma during contraction-induced stress. Previous examinations of the glycosylation status of α -dystroglycan in muscle biopsies of four individuals with HIBM (Huizing *et al.*, 2004) showed absent or markedly reduced immunolabeling with two different antibodies (VIA4 and I1H6) to glycosylated epitopes of α -dystroglycan. These findings resemble those found for other congenital muscular dystrophies, suggesting that HIBM may be a “dystroglycanopathy,” and providing an explanation for the muscle weakness of patients with HIBM.

Despite demonstrated proof of principle that intravenous infusion of *GNE* gene lipoplex can be safely administered, subjective improvement in this single individual with severe HIBM has been limited (improved wheelchair control, bed-to-chair transfer, restful sleeping). Continuation of dose escalation may result in greater muscle functional effect. However, it is possible that the level of muscle deterioration in this patient has advanced beyond reversibility, thereby limiting efficacy to stabilization, as was observed. Previous biopsies in muscles other than the quadriceps (characteristically spared until late in the course of disease progression) revealed sparse muscle fiber residue. It is also encouraging that plasmid and RNA expression was demonstrated and verified with *GNE* gene lipoplex in the quadriceps muscle, suggesting appropriate dosing and functional capacity of the

GNE gene lipoplex. Results support continued dose escalation in the current patient and justify further phase I testing in patients with less advanced disease.

Acknowledgments

The authors thank Susan Mill for competent and knowledgeable assistance in the preparation of this manuscript. Samir Sliheet is also acknowledged for transport support. The authors thank Dr. Gregory Bevels, M.D., of Primary Care Specialists (Alexandria, LA) for assistance with weekly physical assessments during the study period. This work was partially supported by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health (Bethesda, MD). The authors are also grateful for the financial support of the Mary Crowley Cancer Research Centers (Dallas, TX) and the Neuromuscular Disease Foundation (Beverly Hills, CA).

Author Disclosure Statement

John Nemunaitis, Neil Senzer, Phillip Maples, and Nancy Templeton have direct affiliation with Gradalis, Inc., in which they own stock. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this paper.

References

- American Thoracic Society. (1991). Lung function testing: Selection of reference values and interpretative strategies. *Am. Rev. Respir. Dis.* 144, 1202–1218.
- American Thoracic Society. (1995). Standardization of spirometry: 1994 update. *Am. J. Respir. Crit. Care Med.* 152, 1107–1136.
- Andres, P.L., Hedlund, W., Finison, L., *et al.* (1986). Quantitative motor assessment in amyotrophic lateral sclerosis. *Neurology* 36, 937–941.
- Bohannon, R.W. (1988). Make tests and break tests of elbow flexor muscle strength. *Phys. Ther.* 68, 193–194.
- Bohannon, R.W. (1992). Hand-held myometry. *J. Neurol. Neurosurg. Psychiatry* 55, 982.
- Burns, S.P., Breuninger, A., Kaplan, C., and Marin, H. (2005). Hand-held dynamometry in persons with tetraplegia: Comparison of make- versus break-testing techniques. *Am. J. Phys. Med. Rehabil.* 84, 22–29.
- Eisenberg, I., Grabov-Nardini, G., Hochner, H., *et al.* (2003). Mutations spectrum of *GNE* in hereditary inclusion body myopathy sparing the quadriceps. *Hum. Mutat.* 21, 99.
- Florence, J.M., Pandya, S., King, W.M., *et al.* (1992). Intrarater reliability of manual muscle test (Medical Research Council Scale) grades in Duchenne’s muscular dystrophy. *Phys. Ther.* 72, 115–122; discussion 122–126.
- Hinderlich, S., Stäsche, R., Zeitler, R., and Reutter, W. (1997). A bifunctional enzyme catalyzes the first two steps in *N*-acetylneuraminic acid biosynthesis of rat liver: Purification and characterization of UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase. *J. Biol. Chem.* 272, 24313–24318.
- Huizing, M. (2005). Disease mechanisms associated with mutations of the *GNE* gene. *Drug Discov. Today Dis. Mech.* 2, 519–527.

- Huizing, M., and Krasnewich, D.M. (2009). Hereditary inclusion body myopathy: A decade of progress. *Biochim. Biophys. Acta* 1792, 881–887.
- Huizing, M., Rakocevic, G., *et al.* (2004). Hypoglycosylation of α -dystroglycan in patients with hereditary IBM due to GNE mutations. *Mol. Genet. Metab.* 81, 196–202.
- Jay, C., Nemunaitis, G., Nemunaitis, J., *et al.* (2008). Preclinical assessment of wt GNE gene plasmid for management of hereditary inclusion body myopathy 2 (HIBM2). *Gene Regul. Syst. Bio.* 2, 243–252.
- Jay, C.M., Levonyak, N., Nemunaitis, G., *et al.* (2009). Review: Hereditary inclusion body myopathy (HIBM2). *Gene Regul. Syst. Bio.* 3, 181–190.
- Keppeler, O.T., Hinderlich, S., Langner, J., *et al.* (1999). UDP-GlcNAc 2-epimerase: A regulator of cell surface sialylation. *Science* 284, 1372–1376.
- Langer, G.A. (1978). The structure and function of the myocardial cell surface. *Am. J. Physiol.* 235, H461–H468.
- Malicdan, M.C., Noguchi, S., Hayashi, Y.K., *et al.* (2009). Prophylactic treatment with sialic acid metabolites precludes the development of the myopathic phenotype in the DMRV-hIBM mouse model. *Nat. Med.* 15, 690–695.
- Medical Research Council. (1981). Aids to the examination of the peripheral nervous system. Memorandum no. 45. (Her Majesty's Stationery Office, London).
- Mendell, J.R., Moxley, R.T., Griggs, R.C., *et al.* (1989). Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. *N. Engl. J. Med.* 320, 1592–1597.
- Nemunaitis, G., Maples, P.B., Jay, C., *et al.* (2010). Hereditary inclusion body myopathy: Single patient response to GNE gene lipoplex therapy. *J. Gene Med.* 12, 403–412.
- Paternostro-Sluga, T., Grim-Stieger, M., Posch, M., *et al.* (2008). Reliability and validity of the Medical Research Council (MRC) Scale and a modified scale for testing muscle strength in patients with radial palsy. *J. Rehabil. Med.* 40, 665–671.
- Personius, K.E., Pandya, S., King, W.M., *et al.*; FSH DY Group. (1994). Facioscapulohumeral dystrophy natural history study: Standardization of testing procedures and reliability of measurements. *Phys. Ther.* 74, 253–263.
- Phadke, A.P., Jay, C., Chen, S.J., *et al.* (2009). Safety and *in vivo* expression of a GNE-transgene: A novel treatment approach for hereditary inclusion body myopathy-2). *Gene Regul. Syst. Bio.* 3, 89–101.
- Ricci, E., Broccolini, A., Gidaro, T., *et al.* (2006). NCAM is hyposialylated in hereditary inclusion body myopathy due to GNE mutations. *Neurology* 66, 755–758.
- Roy, M.A., and Doherty, T.J. (2004). Reliability of hand-held dynamometry in assessment of knee extensor strength after hip fracture. *Am. J. Phys. Med. Rehabil.* 83, 813–818.
- Stratford, P.W., and Balsor, B.E. (1994). A comparison of make and break tests using a hand-held dynamometer and the Kin-Com. *J. Orthop. Sports Phys. Ther.* 19, 28–32.
- Templeton, N.S., Lasic, D.D., Frederik, P.M., *et al.* (1997). Improved DNA:liposome complexes for increased systemic delivery and gene expression. *Nat. Biotechnol.* 15, 647–652.
- Varki, A. (1997). Sialic acids as ligands in recognition phenomena. *FASEB J.* 11, 248–255.
- Varki, A. (2008). Sialic acids in human health and disease. *Trends Mol. Med.* 14, 351–360.

Address correspondence to:
 Dr. John Nemunaitis
 1700 Pacific
 Suite 1100
 Dallas, TX 75201

E-mail: jnemunaitis@marycrowley.org

Received for publication September 24, 2010;
 accepted after revision February 14, 2011.

Published online: February 17, 2011.

