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Rationale and Design for a Phase 1 Study of *N*-Acetylmannosamine for Primary Glomerular Diseases

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Introduction: Sialic acids are important contributors to the polyanionic component of the glomerular filtration barrier, which regulates permeability selectivity. Pathologic glomerular hyposialylation, associated with podocyte effacement, has been implicated in human and mouse glomerulopathies. Oral treatment with *N*-acetylmannosamine (ManNAc), the uncharged precursor of sialic acid, ameliorates glomerular pathology in different models of glomerular disease.

Methods: Here we explore the sialylation status of kidney biopsies obtained from 27 subjects with various glomerular diseases using lectin histochemistry.

Results: We identified severe glomerular hyposialylation in 26% of the biopsies. These preliminary findings suggest that this condition may occur relatively frequently and may be a novel target for therapy. We describe the background, rationale, and design of a phase 1 study to test safety, tolerability, and pharmacokinetics of ManNAc in subjects with primary podocyte diseases.

Conclusion: We recently demonstrated that ManNAc was safe and well tolerated in a first-in-human phase 1 study in subjects with UDP-*N*-acetylglucosamine (GlcNAc) 2-epimerase/ManNAc kinase (GNE) myopathy, a disorder of impaired sialic acid synthesis. Using previous preclinical and clinical data, we propose to test ManNAc therapy for subjects with primary glomerular diseases. Even though the exact mechanisms, affected cell types, and pathologic consequences of glomerular hyposialylation need further study, treatment with this physiological monosaccharide could potentially replace or supplement existing glomerular diseases therapies.

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Sialic acids are negatively charged, terminal monosaccharides of carbohydrate chains that are attached to glycoproteins and glycolipids (glycans). Most glycans serve cellular signaling functions, and they frequently appear on the cell surface or are secreted into the circulation.[1–3] Altered glycan sialylation has been associated with diverse disease processes, including certain types of cancer, autoimmune disorders, myopathy, and monoclonal gammopathy.[2–5]

N-acetylneuraminic acid (Neu5Ac) is the most abundant mammalian sialic acid and the precursor of all other sialic acids; it is often referred to as simply 'sialic acid'.[1,2] Sialic acid synthesis occurs intracellularly (Figure 1) and is initiated and regulated by a ubiquitously expressed, bifunctional enzyme: GNE, encoded by the *GNE* gene.[3,6,7] Regulation of intracellular sialic acid synthesis occurs through feedback inhibition of UDP-GlcNAc 2-epimerase activity by the downstream product cytidine monophosphate-Neu5Ac

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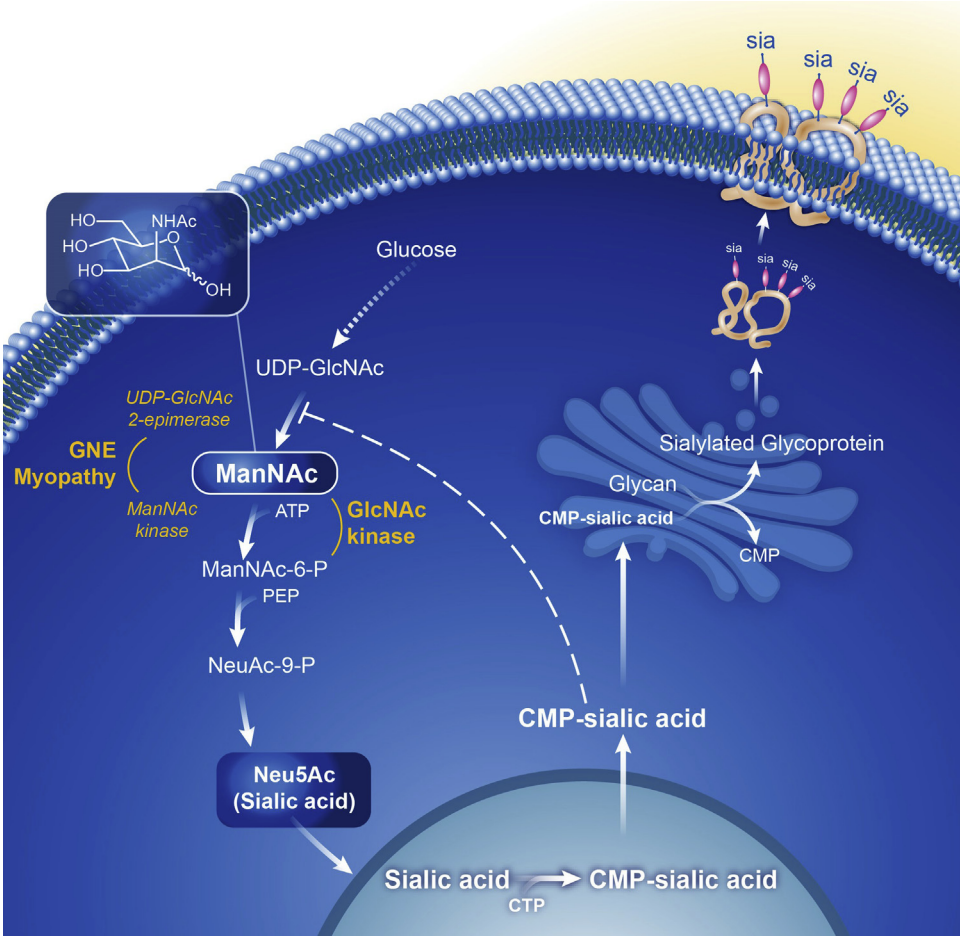

Figure 1. Intracellular sialic acid biosynthesis pathway. The biosynthesis of sialic acid (Neu5Ac) is initiated in the cytosol where the substrate uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc; derived from glucose) is converted to 2-epimerase/N-acetylmannosamine (ManNAc) and, subsequently, ManNAc-6P by the bifunctional enzyme UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE). ManNAc-6P undergoes 2 additional committed conversions to become cytosolic-free sialic acid. A nuclear step mediated by cytidine monophosphate (CMP)–sialic acid synthase (CMAS) activates free sialic acid to CMP-sialic acid, which is translocated back into the cytosol. CMP-sialic acid acts as a sialic acid donor to sialylate glycans on nascent glycoproteins ("sia") and glycolipids in the Golgi complex; it also acts to regulate cytosolic sialic acid–synthesis by feedback inhibition of UDP-GlcNAc 2-epimerase activity through binding to its allosteric site (dashed line). Proposed sialylation-increasing therapies (ManNAc and sialic acid) are boxed in white. Reprinted from Xu X, Wang AQ, Latham LL, et al. Safety, pharmacokinetics and sialic acid production after oral administration of *N*-acetylmannosamine (ManNAc) to subjects with GNE myopathy. *Mol Genet Metab.* 2017;122:126–134.[7] Published by Elsevier Inc. ATP, adenosine triphosphate; CTP, cytidine triphosphate; ManNAc-6-P, N-acetylmannosamine-6-phosphate; PEP, phosphoenolpyruvate; NeuAc-9-P, N-acetylneuraminic acid-9 phosphate.

(CMP-sialic acid).[8,9] CMP-sialic acid is the substrate for sialyltransferases in the Golgi-complex to sialylate nascent glycans.[10,11]

Even though sialic acids are important components of the glomerular filtration barrier, studies of sialylation of glomerular glycans in relation to human kidney diseases have been conducted only sporadically. Reduced sialic acid staining of podocyte membranes was shown in kidney biopsies from subjects with minimal change disease and membranous nephropathy,[12,13] and hyposialylated circulating IgA1 is present in serum from subjects with IgA nephropathy, resulting in the loss of nephrin, a structural podocyte protein.[14,15]

A mechanistic association between hyposialylation and podocytopathy was demonstrated by injection of wild type mice with sialidase, which removes sialic acids from glycans, and resulted primarily in glomerular disease, with proteinuria, podocytopathy, and renal failure in a dose-dependent manner.[16] The authors noted that this animal model, in addition to providing an opportunity to study basic mechanisms of renal physiology, mimicked minimal change disease. Three additional independent mouse studies, reported by the present authors and others, involved mice with genetic alterations in the sialic acid–synthesis pathway, including 2 models with point mutations in GNE,[17–19] and one with point mutations in CMP-sialic acid synthetase (*Cmas*).[20] All 3 models exhibited early death due to severe renal podocytopathy, whereas other tissues appeared unaffected. Hyposialylation of glomerular glycans in these mouse models was demonstrated by immunoblotting and

lectin histochemistry, and it showed that the structural podocyte sialoproteins podocalyxin and nephrin were hyposialylated.

N-ACETYLMANNOSAMINE (MANNAC) THERAPY AMELIORATES GLOMERULAR HYPOSIALYLATION IN ANIMAL MODELS

As a therapy for our gene-targeted knock-in GNE-deficient mouse model ($Gne^{M712T/M712T}$), we demonstrated therapeutic efficacy of oral supplementation of the sialic acid precursor ManNAc.[17,18,21] ManNAc is an uncharged monosaccharide (molecular weight: 221 Daltons) and is the first committed precursor in the intracellular sialic acid biosynthetic pathway (Figure 1). ManNAc administration bypasses the rate-limiting feedback inhibition enzymatic step catalyzed by UDP-GlcNAc 2-epimerase. In contrast to sialic acid, which is a negatively charged molecule, ManNAc is the only neutral molecule in the sialic acid biosynthesis pathway. As such, it crosses membranes more easily than other pathway intermediates and is thought to reach the intracellular space through passive diffusion or a plasma membrane transporter, as shown by *in vitro* studies.[22–24] In cell culture systems, ManNAc is added to culture medium to allow for full sialylation and/or growth of the cells in culture (and products produced by these cells) and for production of recombinant sialylated proteins.[25–28] Similarly, in cell studies, labeled ManNAc is commonly used to identify sialoglycoproteins.[29,30]

We have previously demonstrated that ManNAc supplementation given to GNE-deficient mice could ameliorate neonatal death, proteinuria, podocytopathy, and hyposialylation of glomerular glycans. Glomerular pathology and podocytopathy were demonstrated by ultrastructural studies, tissue hyposialylation was demonstrated by lectin histochemistry, and hyposialylation of particular proteins, podocalyxin, and nephrin, was shown by immunoblotting.[17,18,21] Subsequently, other studies also demonstrated therapeutic effects of ManNAc in models of glomerulopathy. ManNAc supplementation reduced proteinuria associated with sialylation deficiency in puromycin aminonucleoside (PAN)–induced nephrosis in rats,[31] as well as in angiopoeitin-like 4 overexpressing rats, an experimental model for minimal change disease.[32]

ManNAc is currently in development (Investigational New Drug 78091) as a therapy for the rare muscle disease GNE myopathy, previously known as hereditary inclusion body myopathy (HIBM) or distal myopathy with rimmed vacuoles (DMRV), caused by deficiency of GNE, the key enzyme in sialic acid biosynthesis.[33,34] Muscle fibers of GNE myopathy subjects exhibit hyposialylated glycans, which is believed to contribute to disease pathogenesis.[34,35] A first-in-human, placebo-controlled, escalating single-dose phase 1 study to evaluate the safety, tolerability, and pharmacokinetics (PK) of ManNAc in subjects with GNE myopathy was carried out at the National Institutes of Health (NIH) Clinical Center, involving 22 subjects with GNE myopathy (clinicaltrials.gov: NCT 01634750).[7] Following administration of a single oral dose of ManNAc, there was a significant and sustained increase in plasma-free sialic acid levels up to 48 hours postdose. Given that sialic acid has a short plasma half-life,[36] the prolonged elevation of plasma sialic acid after a single dose of ManNAc strongly suggested that intracellular biosynthesis of sialic acid was restored in subjects with GNE myopathy.[7] This was followed by a phase 2 open-label study of 12 GNE myopathy subjects receiving oral ManNAc in a dose of 6000 mg twice daily for a duration of 30 months (clinicaltrials.gov: NCT 02346461). This trial was recently completed. No major safety concerns emerged from this study.

Of note, subjects with GNE myopathy do not develop renal disease, a remarkable difference from the GNE myopathy mouse models, which all exhibit podocytopathy.[17–19] Similarly, no genetic variants in the *GNE* gene have been reported in subjects with glomerular disease. This discrepancy may be attributed to interspecies differences in the type of sialic acid present and in the relative importance of sialic acid to the kidney. Most mammalian species, including rodents, utilize the sialic acid Neu5Gc (*N*-glycolylneuraminic acid), but humans have evolutionarily lost the ability to synthesize Neu5Gc and instead rely on Neu5Ac as their main sialic acid.[37] Protein glycosylation patterns also vary; the glomerular sialoprotein podocalyxin itself differs widely among species in the contingent of *O*- and *N*-linked glycosylation sites.[38] Future studies may further elucidate species-specific sialylation issues.

HYPOSIALYLATION IN HUMAN GLOMERULOPATHIES

The murine models described above, manifesting sialylation-associated glomerular disease, raise the question of whether glomerular hyposialylation could be a common feature in some human glomerulopathies. Therefore, we performed an exploratory study in which we assessed renal biopsies of 27 deidentified subjects with different glomerular diseases and 18 control subjects (Table 1 and Figure 2) for the sialylation status of glomerular glycans using lectin histochemistry. Lectins are carbohydrate (glycan)-binding proteins with ligand specificities for defined carbohydrate sequences.[39] Due to limited availability of kidney biopsy slides, we used only 2 informative

Table 1. Human kidney biopsies tested by lectin histochemistry

Human renal disorder	Number tested	Normal sialylation	Moderately hyposialylated	Severely hyposialylated
Minimal change disease (MCD)	5	2	1	2
Focal segmental glomerulosclerosis (FSGS)	5	4	0	1
Membranous nephropathy (MN)	4	1	2	1
IgA nephropathy (IgA)	4	0	3	1
IgM nephropathy (IgM)	2	1	1	0
Immune complex glomerulonephritis (ICGN)	4	2	2	0
Lupus nephritis (LN)	3	1	0	2
Total glomerulopathies, n (%)	27	11 (41)	9 (33)	7 (26)
Normal kidney tissue, n (%)	18	16 (89)	2 (11)	0 (0)

lectins to distinguish normally sialylated from hyposialylated glomerular glycans, as previously described in mice.[18] SNA (*Sambucus nigra agglutinin*, binding terminal α[2,6]-linked sialic acid [Neu5Ac] endgroups on glycans) was used to demonstrate the presence of glomerular sialylation, and HPA (*Helix pomatia agglutinin*, predominantly binding terminal O-linked N-acetylgalactosamine [GalNAc]—without sialic

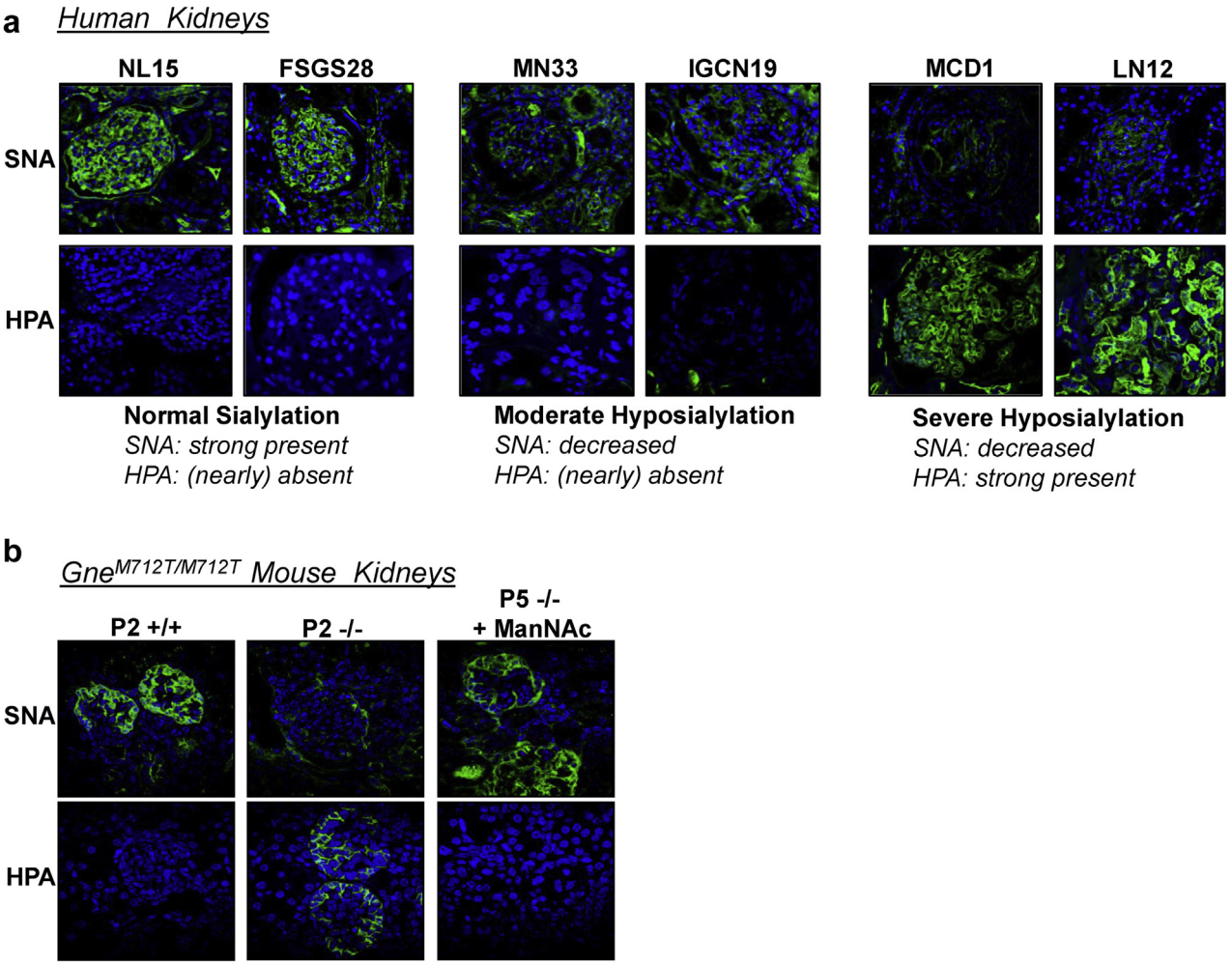

Figure 2. Lectin staining to determine the glomerular sialylation status of kidney tissue. Representative glomerular images stained with fluorescein isothiocyanate–labeled lectins HPA (*Helix pomatia*) or SNA (*Sambucus nigra*), both green, and 4′,6-diamidino-2-phenylindole nuclear dye (blue). See Supplementary Figure S2 for lectin specificity. Lectin staining of kidney biopsy slides of 27 subjects with glomerular disorders, and 18 human controls were scored in 3 groups: normal sialylation, moderate hyposialylation, and severe hyposialylation of glomeruli (a). See Table 1 and Supplementary Figures S1 and S2 for additional details. Glomerular lectin staining of UDP-*N*-acetylglucosamine (GlcNAc) 2-epimerase/*N*-acetylmannosamine (ManNAc) kinase $(Gne)^{M712T/M712T}$ mutant mice (b). Mutant mice at postnatal day 2 (P2 –/–) showed severe hyposialylation by decreased SNA and increased HPA staining compared to wild-type littermates (+/+). ManNAc treatment rescued mutant mouse glomeruli from hyposialylation (P5 –/– + ManNAc), as demonstrated by a normal lectin staining pattern similar to that in wild-type mice (P2 +/+).

acid attached—on glycans) to demonstrate glomerular hyposialylation.[40] For kidney tissue and staining details, see Supplementary Methods.

Severe glomerular hyposialylation, manifested as reduced SNA binding and increased HPA binding similar to that observed in the mutant mouse model,[18] was found in 7 (26%) of the glomerular disease biopsies and none of the 18 control tissues. The severely hyposialylated biopsies included 2 with minimal change disease (MCD; n = 5), 1 with focal segmental glomerulosclerosis (FSGS; n = 5), 1 with membranous nephropathy (MN; n = 4), 1 with IgA nephropathy (IgA; n = 4), and 2 with lupus nephritis (LN; n = 3; Table 1 and Figure 2). In these 7 kidney biopsies manifesting severe hyposialylation, the extent and intensity of the staining was consistent in nearly all glomeruli present in each section. We observed moderate glomerular hyposialylation, defined by significant decreased SNA binding compared to normal and (nearly) absent HPA binding, in 9 (33%) of the glomerular disease biopsies but also in 2 of 18 control tissues. Determination of the etiology of this "moderate" condition (i.e., whether an isolated condition, linked to certain clinical or pathologic manifestations, a temporary physiological state, or a prelude to severe hyposialylation) will require further investigation with a larger number of biopsies. Normal sialylation, defined by normal, intense SNA binding and absent HPA binding, was found in 11 (41%) of the glomerular disease biopsies and 16 normal biopsies (Figure 2, Table 1, and Supplementary Figure S1).

These preliminary findings indicated that human glomerular hyposialylation may occur relatively frequently in diverse glomerular diseases; the underlying pathology and consequences will require further study. Given that this was a pilot experiment and we had access to only limited unstained biopsy slides (2 per subject), we were unable to perform additional histology studies to distinguish affected glomerular cell-types, perform co-localization, or quantify staining intensities. Moreover, given that the samples were deidentified, we were unable to link glomerular hyposialylation to clinical features such proteinuria, estimated glomerular filtration rate (eGFR), hyperlipidemia, and steroid therapy responsiveness. Such studies are in the planning stage.

RATIONALE FOR EXPLORING MANNAC THERAPY IN HUMAN GLOMERULOPATHIES

Previously described murine models of glomerular disease indicated that glomeruli may be susceptible to subtle systemic deficits in sialic acid concentrations.[16–20] These findings were supported by detection of variable degrees of hyposialylation in various human glomerulopathies (Table 1 and Figure 2).[12,14,15,41] The frequency of this condition in human kidney biopsies and the similarity of lectin staining patterns to the GNE mouse model prompted us to explore ManNAc as a therapy for human glomerulopathies.

Even though the exact mechanism of glomerular hyposialylation is under investigation, we chose to initiate a phase 1 clinical trial of ManNAc treatment in subjects with proteinuria and normal or reduced glomerular filtration rate. Such a trial would gather safety, tolerability, pharmacokinetic, and possibly early pharmacodynamic data for ManNAc supplementation in human glomerular diseases/podocytopathies; such data could then be used in more focused, disease-specific, future phase 2 trials. This approach is especially attractive, as current therapies for glomerular diseases are nonspecific, are associated with adverse side effects, and often are ineffective, whereas ManNAc treatment is simple, apparently safe, and physiologic. In addition, we were in a unique position to use (repurpose) regulatory preclinical and clinical data, necessary for an Investigational New Drug application, from our previous phase 1 trial in 22 subjects with GNE myopathy.[7] Below, we outline the design of a phase 1 clinical trial to examine the safety, tolerability, and PK of ManNAc in subjects with primary podocyte diseases (ClinicalTrials.gov: NCT02639260).

PHASE 1 STUDY DESIGN: MANNAC FOR PRIMARY PODOCYTE DISEASES

Study Protocol and Human Subjects Issues

This NIH protocol 16-DK-0036, entitled "Phase 1 Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of ManNAc in Subjects with Primary Podocyte Diseases" was approved by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)/National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) Institutional Review Board (ClinicalTrials.gov: NCT 02639260) and will be conducted under Investigational New Drug (IND) application 125,192. The study was designed as a phase 1, single-site, single and multiple ascending dose study of ManNAc in subjects with primary podocyte diseases, including MCD, FSGS, and MN. The primary objectives for this study are to evaluate the safety, tolerability, and PK of single and multiple doses of orally administered ManNAc to subjects with primary podocytopathy. The secondary objective is to explore the effect of ManNAc on potential pharmacodynamic markers of primary podocyte diseases. All subjects will provide informed consent prior to study participation.

Study Overview

The study will enroll up to 12 adult subjects (≥18 years of age) of either sex and of all racial backgrounds,

weighing more than 40 kg, with a biopsy-proven diagnosis of MCD, FSGS, (including collapsing glomerulopathy), or MN. Inclusion and exclusion criteria are summarized in Table 2. Subjects under 18 years of age will be excluded, considering that there might be an increase over minimal risk, without expectation of benefit, associated with participation in this trial. Subjects must have an eGFR ≥15 ml/min per 1.73 m^2 according to the Chronic Kidney Disease Epidemiology Collaboration creatinine/cystatin equation,[42] and a random void urine protein/creatinine ratio >1 g/g. Subjects must either be on no immunosuppressive therapies (e.g., glucocorticoids, calcineurin inhibitors, and mycophenolate mofetil) or be able to tolerate a stable dose of such therapies for 30 days prior to the ManNAc dosing and 30 days afterward (day −30 to day +31). Subjects at risk of initiating pregnancy will be required to use at least one effective contraception method for the duration of the trial. We will attempt to obtain unstained formalin-fixed, paraffin-embedded sections from each kidney biopsy that the subjects have had prior to this study, and glomerular sialylation status will be assessed by staining with fluorescent lectins. Subjects whose slides cannot be obtained will not be excluded from the study.

Enrolled subjects will be randomly assigned to 1 of 2 cohorts of 6 subjects each, to receive total daily doses of ManNAc of 3000 mg (cohort A) or 6000 mg (cohort B). Each cohort will include subjects in 2 eGFR groups (>30 and 15–29 mg/min per 1.73 m^2; Table 3). We will not attempt to control the distribution of nephrotic/non-nephrotic subjects or glomerular sialylation status of subjects among these groups.

Drug Exposure and Study Schedule

ManNAc (*N*-acetyl-D-mannosamine monohydrate) will be supplied by New Zealand Pharmaceuticals, Ltd. as a bulk powder. On the day of the dosing, ManNAc will be prepared as powder-in-bottle by the NIH Clinical Center Pharmacy by diluting the appropriate ManNAc powder dose in 200 mL of sterile water.

The study visit schedule is summarized in Supplementary Table S1, with a more detailed description of the schedule of events and testing given in Supplementary Table S2. The study will last 31 days, starting with an 11-day inpatient stay at the NIH Clinical Center. During that admission, subjects will receive ManNAc as a single dose on day 2 and twice daily on days 5 to 9. After the first single dose on day 2 (3000 mg for cohort A and 6000 mg for cohort B), safety and PK will be monitored for 72 hours. Subjects will then receive ManNAc in 2 daily doses (1500 mg every 12 hours for cohort A and 3000 mg every 12 hours for cohort B) for 5 subsequent days (days 5 to 9). Safety will be monitored, and PK data will be collected during this period and for 36 hours following the last administration of the study drug (days 10 and 11). Each subject will be discharged on day 11 upon successful completion of the study drug administration.

The first 2 subjects in cohort A and cohort B will be discharged at study day 4, so that the safety and tolerability of their single dose can be evaluated by a Safety Review Committee. These subjects will be readmitted later for the multiple-dose phase, proceeding from study day 5. The other 4 subjects per cohort will be admitted for 11 consecutive days (days 1–11). Subjects will return to the NIH Clinical Center for an outpatient visit on day 17 (±1 day), and they also will be contacted twice by phone on days 24 and 31 (±2 days). On those days, subjects will send urine samples to the NIH laboratory for clinical lab tests as well as PK and pharmacodynamic assays.

Table 2. ManNAc phase 1 study design

eGFR (ml/min per 1.73 m^2)	Cohort A[a] ManNAc, 3000 mg/d	Cohort B[a] ManNAc, 6000 mg/d
≥30	n = 4	n = 4
15–29	n = 2	n = 2
Total	6 subjects	6 subjects

eGFR, estimated glomerular filtration rate; ManNAc, *N*-acetylmannosamine.
[a]Subjects weighing between 40 and 50 kg will be enrolled in only cohort A. Subjects weighing >50 kg will be enrolled in either cohort A or cohort B.

Table 3. ManNAc phase 1 clinical trial inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
• Age >18 yr	• Pregnant or breastfeeding subject
• Biopsy-proven MCD, FSGS, or MN	• Vulnerable subjects, e.g., unable to provide consent
• Weight >40 kg	• Diabetes mellitus (any type)
• Random void urine protein/creatinine >1 g/g	• Hematocrit ≤30%
• eGFR >15 ml/min per 1.73 m^2	• HIV seropositive
• No immunosuppressive therapy or able to maintain a stable dose during the trial	• Positive HBV surface antigen or HCV antibody
	• AST, ALT, or γGTP > 3× upper limit of normal
	• Psychiatric, cognitive, or neurologic disorder impairing adherence
	• Received another investigational drug <60 d prior to ManNAc dosing
	• Received ManNAc, sialic acid, i.v. Igs, and/or other supplements containing abundant sialic acid <60 d prior to ManNAc dosing

ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; HBV, hepatitis B virus; HCV, hepatitis C virus; ManNAc, N-acetylmannosamine; MCD, minimal change disease; MN, membranous nephropathy; γGTP, gamma-glutamyltransferase.

Plasma PK

During the single-dose phase, plasma samples for pharmacokinetic analysis will be drawn at day 1, day 2 pre-dose, and at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, and 72 hours post–single dose administration, to obtain a full PK profile. Plasma samples will be obtained twice daily (before dosing) during the multiple-dose phase (days 5–9), as well as at 12, 24, and 36 hours after the last dose on day 9. To assess trough levels (C_{trough}), plasma samples will be obtained immediately before administering the ManNAc dose each morning on days 5 to 9. Plasma samples will also be obtained on day 5 and day 9, at 2 hours after the morning dosing, to assess peak ManNAc levels during the multiple-dose phase. During the follow-up period, blood will be collected on day 17 at the in-person follow-up visit. Plasma samples for PK analysis will be placed in potassium ethylenediamine tetraacetic acid tubes and kept on ice until processed into aliquots, which will be stored at −80 °C. Frozen plasma samples will be analyzed by Alliance Pharma (Malvern, PA) to determine ManNAc and free Neu5Ac (sialic acid) concentrations by a validated liquid chromatography–mass spectrometry (LC-MS/MS) method.[43] Detailed blood and urine collection schedules are summarized in Supplementary Tables S3 and S4. Some collected samples will be used for research purposes to assess pharmacodynamic parameters, including markers of sialylation and of podocyte diseases.

Urine Studies

Pooled urine (assembly of serial voids) will be collected during specific time frames in the single-dose phase for PK analysis and safety tests. Those intervals will be 0–1 hours, 1–3 hours, 3–6 hours, 6–12 hours, 12–24 hours, and 24–48 hours. First morning void samples will be collected on days 4–11. During the follow-up period, a single random void urine sample will be collected at day 17 (±1 day) at the in-person consult, and subjects will ship a first morning void urine sample to NIH on days 24 and 31 (±2 days; Supplementary Table S4). Urine parameters, determined by the NIH Clinical Laboratory Service, will include urinalysis, protein/creatinine ratio (PCR), and urine albumin/creatinine ratio (ACR). Other urine studies (including ManNAc and Neu5Ac assessment) will be performed to determine PK and pharmacodynamic parameters.

Statistical Analysis

Standard descriptive statistics will include number (n), mean and SD, median, minimum, and maximum values. Analyses will be generated using SAS version 9.2 or higher (SAS Analytics, Cary, NC). Two ManNAc dose levels (3000 mg/d and 6000 mg/d) will be evaluated by a statistical dose–response model of the relationship between dose and the rate at which plasma ManNAc and free Neu5Ac (sialic acid) levels return to baseline. Other parameters, such as proteinuria, eGFR/nephrotic syndrome, hyperlipidemia, and primary podocyte disease type, will also be evaluated, if appropriate, using the same statistical model. With respect to plasma concentrations of ManNAc and free Neu5Ac (sialic acid), certain PK parameters will be estimated using noncompartmental analyses to assess the concentration–time data, including maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration time curve (AUC), AUC from time 0 to time of last measurable plasma concentration (AUC_{last}), AUC within the dosing interval (AUC_t), AUC from time 0 to time infinity ($AUC_{0-\infty}$), terminal-phase half-life ($t_{1/2}$), and plasma concentration immediately before administering the next study dose (C_{trough}). Also, the C_{max} geometric mean will be determined. These data can be compared to subjects with GNE myopathy, who had normal renal function and who received ManNAc[7]; this will allow assessment of whether ManNAc and Neu5Ac are handled differently in subjects with compromised renal function.

DISCUSSION

The mechanisms and pathologic consequences of glomerular hyposialylation remain to be determined. All 3 layers of the glomerular filtration barrier, including the glomerular endothelial cells, glomerular basement membrane, and podocyte slit diaphragm, contain or express glycosylated/sialylated proteins and lipids. The specific glycoproteins or glycolipids affected in nephrotic diseases remain unknown. Further, where the abnormal sialylation reported in this study is located at the microscopic and ultrastructural levels remains to be determined. Hyposialylation was observed in both primary glomerulopathies (IgA nephropathy and the nephrotic disorders) and a secondary glomerulonephritis (lupus nephritis), suggesting that hyposialylation may represent a shared pathway of glomerular cell injury. Given that hyposialylation was not seen in all cases, it may represent an idiosyncratic, possibly genetic, response to glomerular injury, or it may be influenced by other factors, which might be specific to particular stages of disease. Alternatively, glomerular hyposialylation may be a characteristic feature of a distinctive subset of glomerular disease cases. Future studies are needed to confirm these findings systematically in a larger group of subjects, and relate the presence of hyposialylation to glomerular diagnosis,

disease stage, proteinuria, response to therapy, and long-term renal outcome.

These preliminary findings suggest the possibility of therapies that promote sialylation for selected glomerular diseases. Recent mouse and rat studies have demonstrated that oral supplementation with the uncharged sialic acid precursor ManNAc produces partial reversal of glomerular pathology, reduced proteinuria, and partially restored protein sialylation.[17,18,21,31,32] ManNAc (ClinicalTrials.gov: NCT 01634750) is under investigation for the treatment of a rare hyposialylation disorder, *GNE* myopathy.[7]

In parallel with further investigations into the pathogenic mechanisms of glomerular hyposialylation, we designed a phase 1 study to investigate safety, tolerability, and PK in 12 patients with different glomerular diseases, and variable eGFR and proteinuria levels. The information gathered in this phase 1 trial will inform future phase 2 trials of ManNAc for glomerular diseases. ManNAc therapy is given orally and appears to have minimal toxicity. If proven effective, it might replace or supplement current glomerular disease therapies. Future efforts to investigate ManNAc therapy for glomerular disease may contribute to efforts to reduce the burden and disparities in kidney diseases worldwide.[44]

DISCLOSURE

MH and WAG are co-inventors on Patent No. 8,410,063: "*N*-acetylmannosamine As a Therapeutic Agent." All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

MH and TY planned and performed the lectin histology experiments. AV, BD, and JBK supplied human kidney slides and contributed to manuscript writing. MH, TY, MCVM, PL, WAG, and YA conceived the lectin histology study, interpreted the data, and wrote the manuscript. MH, NC, WAG, and JBK contributed to phase 1 clinical trial design and (together with FF, EB, JB, AP, and HC) contributed to manuscript preparation. Each author contributed intellectual content during manuscript drafting or revision and accepts accountability for the work.

SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Methods: Lectin staining of kidney biopsies.

Figure S1. Exploratory lectin staining of human kidney biopsy slides.

Figure S2. Control panel for *Sambucus nigra agglutinin* (SNA) and *Helix pomatia agglutinin* (HPA) lectin specificity.

Table S1. Subject visit schedule.

Table S2. Schedule of events.

Table S3. Blood sampling schedule.

Table S4. Urine sampling schedule.

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