A phase 1/2 trial of Tauroursodeoxycholic acid supplementation in progressive MS patients

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JHM IRB - eForm A – Protocol

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1. Abstract

People with multiple sclerosis have abnormalities in multiple metabolic pathways that can be identified using metabolomics. The **aberrant pathways identified in people with MS, in our preliminary studies using untargeted metabolomics**, include **primary and secondary bile acid metabolism**. Bile acids have several biological functions beyond aiding in lipid absorption in the gut, and can modulate the function of cells in the peripheral immune system and the CNS. Bile acid metabolism is also closely linked to the gut microbiota. **Bile acid supplementation reduced neuroinflammation and was neuroprotective** in models of various neurodegenerative and neuroinflammatory disorders. Bile acid supplementation has been tested in early trials in neurodegenerative disease and has been well tolerated. Whether bile acid supplementation is beneficial in MS is not known.

The objective of this study is to test the **safety and tolerability of supplementation with a bile acid – tauroursodeoxycholic acid (TUDCA) in people with progressive MS**. We will also assess whether this intervention normalizes the abnormal bile acid levels in circulation and impacts the composition of the gut microbiota and peripheral immune cell function.

Aims:

1. Establish the safety of bile acid supplementation and determine whether this intervention leads to normalization of the bile acid profile in people with MS with abnormal bile acid metabolism

2. Assess the effects of bile acid supplementation on the gut microbiota and the peripheral immune system in people with MS

There is a need for adjunctive treatments that carry low risk and address the underlying pathophysiology of MS. This project aims to show that a bile acid – TUDCA is safe and well-tolerated in MS patients and could result in beneficial effects on bile acid metabolism, gut microbiome and peripheral immune cell function in people with MS. If successful, this project could yield a new treatment strategy with both immunomodulatory and neuroprotective effects.

2. Objectives

Primary Objectives

<u>Aim 1.</u> Establish the safety and tolerability of bile acid supplementation and determine whether this intervention leads to normalization of the bile acid profile in people with progressive MS with abnormal bile acid metabolism

Hypothesis: Bile acid supplementation is safe and tolerable and will help normalize the bile acid profile in people with MS.

Method: In a randomized, double-blind, placebo-controlled, phase 1/2 study of bile acid supplementation in people with progressive MS with abnormal bile acid metabolism, we will establish the safety and tolerability of this intervention and evaluate its effects on bile acid profiles as determined by targeted metabolomics.

Aim 2. Assess the effects of bile acid supplementation on the gut microbiota and the peripheral immune system in people with MS

Hypothesis: Bile acid supplementation leads to alterations in the gut microbiota and peripheral immune system in people with MS.

Method: We will determine the change in gut microbiota composition following bile acid supplementation and determine changes in peripheral immune cell function in participants enrolled in the phase 1/2 trial of bile acid supplementation mentioned in Aim 1.

3. Background

Multiple Sclerosis is associated with alterations in multiple metabolic pathways including bile acid metabolism. Multiple sclerosis (MS) is a chronic autoimmune disorder that leads to inflammation and neurodegeneration involving the central nervous system. Abnormalities in metabolic pathways have been identified in multiple sclerosis, both in plasma and cerebrospinal fluid[1]–[3]. The majority of reports identified alterations in energy metabolism or oxidative stress; however, technological limitations prevented a large-scale evaluation of the metabolome in MS until recently. Now, recent advances allow for the use of global untargeted metabolomics to identify hundreds of small molecules in biological matrices using gas/liquid chromatography coupled with mass spectrometry[4]. We utilized untargeted global metabolomics to understand alterations in the metabolome of people with MS and identified alterations in multiple metabolic pathways. Both primary and secondary bile acid metabolism were identified as metabolic pathways that were altered in the MS population (see preliminary data). Intriguingly, we noted dysregulation of these pathways in both relapsing remitting MS (RRMS) and progressive MS (PMS). While the precise mechanism of this alteration is unknown, it may be linked to alterations in the gut microbiota or intestinal motility.

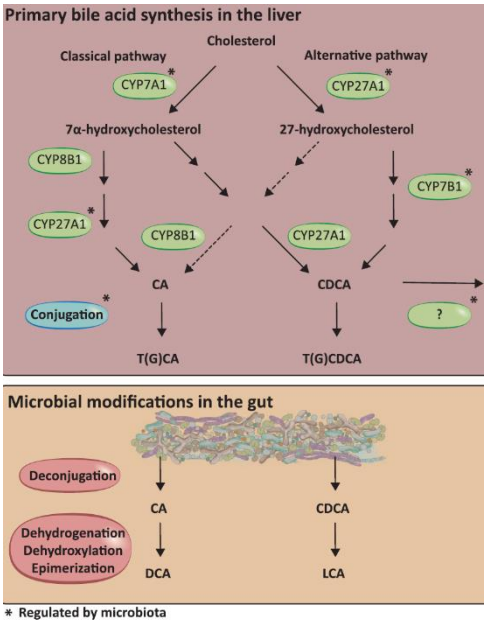


Figure 1. Bile acid synthesis and metabolism. The two pathways of bile acid production and associated enzymes are depicted. The role of gut microbiota in secondary bile acid production and bile acid metabolism is also highlighted (From Wahlstrom et al[38]).

Bile acids have multiple biological functions, and their metabolism is closely related to the gut microbiota. Bile acids are primarily produced in the liver through the action of multiple enzymes on cholesterol[5]. Primary bile acids are further modified by conjugation in the liver (with glycine or taurine) and are then stored in the gall bladder. They are secreted into the intestine and the majority of bile acids are converted to secondary bile acids and reabsorbed in the ileum and colon and transported back to the liver by the portal circulation (enterohepatic circulation)[5],[6]. In the gut, bile acids can undergo deconjugation (removal of glycine or taurine) through the action of bile salt hydrolases (BSH) found in several bacteria. The process of deconjugation allows further conversion

of primary to secondary bile acids through 7-dehydroxylation. While BSH activity is found in all major bacterial and archaeal divisions, The 7-dehydroxylation ability is limited to *Clostridium* (Cluster XIVa & XI) and *Eubacterium*, both belonging to *Firmicuites* phylum[7]. A previous study found a significant reduction in species belonging to Clostridial clusters XIVa & XI in people with MS[8]. Such an alteration could lead to a decrease in the secondary bile acid pool.

Bile salts have differing affinities for the various classes of bile acid receptors, and hence alterations in the composition of the bile acid pool may have a significant impact even when the overall bile acid pool is unchanged. Table 1 lists some of the receptors at which bile acids are active and the affinities of individual bile acids at these receptors. The action of bile acids at these receptors have been linked to control of various metabolic functions including – lipid metabolism, glucose homeostasis and development of obesity[6].

Receptor	Abbreviation	Principal ligand	Additional ligands
Farneosid X receptor	FXR (NR1H4)	CDCA>DCA>LCA	farnesol
Pregnane X receptor	PXR (NR1I2)	LCA>6-ketoLCA	pregnenolone
Constitutive androstane receptor	CAR (NR1I3)	CA>6-ketoLCA>7-ketoDCA	androstanes
Vitamin D receptor	VDR (NR1I1)	vitamin D	LCA>GLCA>CDCA
Bile acid-activated GPCR	GP-BAR1/M-BAR/TGR5	TLCA>LCA>DCA>CDCA>CA>UDCA	linolenic acid and oleanolic acid

GPCR = G-protein-coupled transmembrane receptors; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; LCA = lithocholic acid; CA = cholic acid; GLCA = glycol-conjugated lithocholic acid; TLCA = taurine-conjugated lithocholic acid; UDCA = ursodeoxycholic acid. From Fiorucci et al. [11].

Table 1. Classes of bile acid receptors with a listing of bioactive endogenous ligands

Bile acid supplementation can alter the gut microbiota in humans and animal models of autoimmune disease. A recent study in patients with primary biliary cholangitis noted alterations in the gut microbiota in this population. Treatment with ursodeoxycholic acid (UDCA) led to a partial restoration of the gut microbial profile to a normal state, suggesting that bile acid supplementation can lead to alterations in the composition of the gut microbiome[9]. Another recent study utilizing an animal model of inflammatory bowel disease, demonstrated the presence of an altered gut microbiota in mice with experimental colitis. UDCA treatment normalized the increased ratio of Firmicutes to Bacteroidetes[10]. In this study bile acid supplementation also prevented a decrease in Clostridium cluster XIVa which has been noted to be decreased in people with MS[10].

Bile acids have immunological functions and can modulate both innate and adaptive immunity. The gut is the largest immune organ of the body and alterations in the gut microbiome have a profound effect on neuroinflammation[11]. Recent studies in MS have noted alterations in gut microbiota, however the metabolic consequences of these alterations have not been elucidated[12],[13]. Metabolites produced by gut microbes, including bile acids, can have significant effects on immune cells[14]. Bile acids activate receptors on immune cells including the nuclear Farnesoid X receptor (FXR) and cell-surface G-protein coupled receptors (TGR5). FXR receptors are found predominantly on myeloid cells, but are also present on lymphocytes and NK cells[15]. Treatment of mice with experimental autoimmune encephalomyelitis (EAE) with a FXR agonist resulted in amelioration of disease, through increased production of interleukin-10 (IL-10) from myeloid cells[15]. The TGR5 receptor is also found on microglia and myeloid cells and in animal models of neuroinflammation a TGR5 agonist led to a reduction in severity and inhibition of myeloid cell activation[16],[17].

Bile acids are bioactive in the CNS and have neuroprotective effects. Bile acids and their precursors have been detected in brain tissue and cerebrospinal fluid[18]. Besides microglia, TGR5 receptors have also been described on astrocytes and neurons[19]. The activation of this receptor is associated with increased adenylate cyclase activity, increased intracellular Ca^{2+} and production of reactive oxygen species. Bile acids have also been postulated to have effects on mitochondrial function, as well as the unfolded protein response. Several studies have demonstrated that secondary bile acids such as UDCA, glycoursodeoxycholic acid (GUDCA) and TUDCA may have neuroprotective properties. In a mouse

model of Parkinson's disease (PD) utilizing MPTP toxicity – TUDCA supplementation mediated neuroprotection through modulation of c-Jun N-terminal kinase (JNK) phosphorylation and reactive oxygen species production[20]. UDCA supplementation reversed mitochondrial dysfunction noted in the fibroblasts from patients with *LRRK2* mutations linked to familial PD[21]. Neuroprotection in the retina, in various models (retinal detachment, optic nerve injury), has also been demonstrated with bile salt treatment[22]–[24]. This effect is thought to be mediated through a reduction in apoptosis and endoplasmic reticulum (ER) stress.

Trials of bile acid supplementation have been conducted in other neurodegenerative conditions and have shown good safety profiles and CNS penetration. A recent trial of TUDCA in 34 ALS patients randomized to either TUDCA 1gm twice daily or placebo for 54 weeks revealed a higher rate of response (defined as slowing in the slope of decline on a standardized rating scale) in the TUDCA arm[25]. TUDCA was well tolerated and there were no serious adverse effects in this trial. Two patients in the TUDCA arm of the trial had mild diarrhea and none of the patients had any changes in safety laboratory parameters that were monitored. A previous trial of UDCA in ALS employed a cross-over design and utilized 3.5 gm/day of UDCA for 3 months[26]. This trial found a reduction in the slope of decline in one of the three outcomes utilized. There was a high attrition rate related to GI adverse effects of UDCA. Another trial of UDCA supplementation in ALS revealed a dose-dependent increase in CSF UDCA concentrations, demonstrating good CNS penetration[27]. The European Medicines Agency (EMA) recently accorded orphan drug status to TUDCA for the treatment of ALS in Europe. Trials are also ongoing in Huntington's disease and PD (NCT02967250, NCT00514774).

PRELIMINARY DATA

People with MS have alterations in their metabolome compared to healthy controls. We performed untargeted global metabolomic profiling of plasma from people with RRMS and healthy controls (n=27 in each group) utilizing gas or liquid chromatography coupled with tandem mass spectrometry. The mass spectra obtained were compared to a reference library to identify individual compounds and the relative abundance was calculated using the area under the curve of the mass spectra. Using this method, we identified over 600 metabolites in the plasma of study participants and were able to differentiate the two groups based on their metabolic profiles using a statistical model optimized for high-dimensional datasets - orthogonal partial least squares -discriminant analysis (OPLS-DA) (Figure 2).

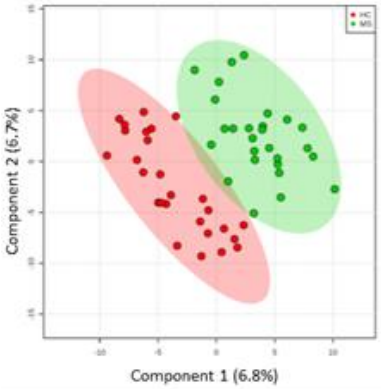


Figure 2. Metabolomics differentiates people with MS from healthy controls. The plot depicts the first two components from an orthogonal partial least squares -discriminant analysis (OPLS-DA) model. The two groups can be clearly distinguished in this plot.

People with MS have alterations in both primary and secondary bile acid metabolism. In the first discovery cohort described above we noted reductions in the levels of multiple primary and secondary bile acids in people with MS. To follow-up on our findings, we performed untargeted metabolomics in a validation cohort of 50 RRMS, 50 progressive MS and 50 healthy controls. In this study we again noted a reduction in multiple bile acids in RRMS and PMS groups (Figure 3A). We also investigated alterations in individual metabolic pathways using pathway dysregulation scores (calculated as described in Drier et al)[28]. This method generates a score for a metabolic pathway for each individual based on their distance from a principal components curve constructed based on healthy control data. These scores range from 0 to 1 with higher scores representing greater abnormality in the pathway. We found significant alterations in both primary and secondary bile acid metabolism in RRMS and PMS groups (Figure 3B & 3C). In this cohort, we also noted a correlation of PDS scores for secondary bile acid metabolism with those for xenobiotic

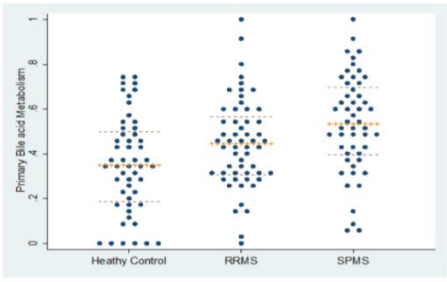
metabolism (Xanthine and Benzoate, p<0.01 for both) indicative of a link between the gut microbiota and bile acid metabolism.

Since pathway analysis utilizes *a priori* specification of metabolic pathways, we also used an agnostic method - weighted correlation network analysis (WGCNA) that identifies modules of highly correlated metabolites. We identified a module that contained bile acids and the eigen-metabolite values for this module differed significantly between RRMS, PMS and control groups with a reduction noted in bile acids in both RRMS and PMS groups (p<0.001 for both).

A

Pathway	Biochemical Name	Cohort HC	
		RRMS	PMS
Primary Bile Acid Metabolism	cholate	1.24	1.04
	glycocholate	0.71	0.53
	taurocholate	0.45	0.50
	chenodeoxycholate	1.18	0.84
	glycochenodeoxycholate	0.79	0.45
	taurochenodeoxycholate	0.69	0.46
	tauro-beta-murichолate	0.76	0.72
	glycochenodeoxycholate glucuronide (	0.60	0.48
	glycochenodeoxycholate sulfate	1.07	0.74
Secondary Bile Acid Metabolism	deoxycholate	0.79	0.73
	glycodeoxycholate	0.58	0.59
	taurodeoxycholate	0.53	0.65
	glycolithocholate	0.39	0.43
	glycolithocholate sulfate*	0.57	0.67
	taurolithocholate 3-sulfate	0.68	0.76
	ursodeoxycholate	1.01	0.89
	glycoursodeoxycholate	1.24	0.93
	tauroursodeoxycholate	1.19	1.01
	hyocholate	1.35	1.01
	glycohyocholate	1.05	0.64
	glycocholenate sulfate*	1.00	0.77
	taurocholenate sulfate	0.96	0.84
	3b-hydroxy-5-cholenoic acid	0.82	1.12
	glycodeoxycholate sulfate	0.71	0.72
	glycodeoxycholate glucuronide (1)	0.49	0.40

B



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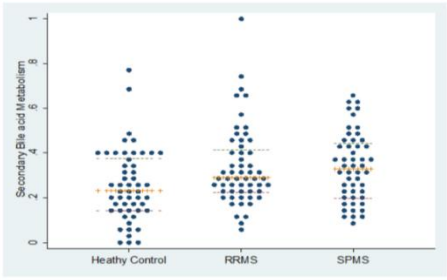
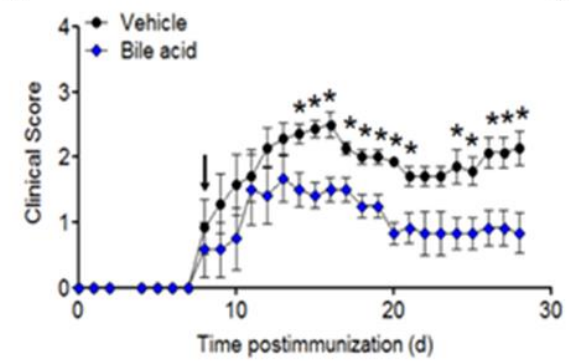


Figure 3. Bile acid metabolism is altered in MS. Panel A shows a heat-map of ratios of concentrations of various bile acids in RRMS and PMS groups compared to healthy control. The green color denotes levels of statistical significance with dark green (p<0.05) and light green (p<0.10). Panel B and C are dot plots of pathway deregulation scores for Primary (panel B) and Secondary (panel C) bile acid metabolism. In both these plots there is a similar trend for higher scores (greater dysfunction) in both MS groups compared to healthy controls.

In animal-models of MS supplementation with bile acids leads to improvement of disease. We then tested the hypothesis that supplementation with a bile acid (TUDCA) would alter the course of disease in a mouse model of MS - EAE. Seven-week old C57/BL6 mice were immunized with MOG_{35-55} and CFA subcutaneously along with injection of pertussis toxin intraperitoneally (IP). Mice were then monitored for development of disease, and at disease onset they were randomized to either TUDCA 500 mg/kg or placebo (PBS) IP for 3 weeks. At the end of the experiment we noted a significant reduction in the severity of disease in the TUDCA treated group (Figure 4A) and a reduction in spinal cord demyelination (based on Black-gold staining) and macrophage infiltration (Iba1+ cells) on histology (Figure 4B). Recent studies from other groups corroborate our findings; Ho & Steinman demonstrated that supplementation with a synthetic bile acid resulted in reduction in severity of EAE[29]. Additionally, another independent group demonstrated the ability of a FXR agonist to reduce CNS inflammation through an IL-10 mediated pathway[15].

A



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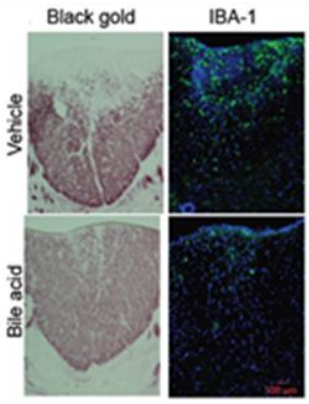


Figure 4. Bile acid supplementation reduced severity of EAE. Female C57/BL6 mice with EAE were randomized to receive either TUDCA or vehicle daily from disease-onset to day 28 (n=6 in each group). Panel A shows behavioral scores of the two groups and a reduction in disease severity is noted in the TUDCA group. At the end of the experiment mice were euthanized and tissue obtained for histological analysis. Black gold staining revealed reduced demyelination in the TUDCA treated mice in addition to reduced infiltration of Iba-1+ cells noted on immunohistochemistry (Panel B).

Metabolomics can be used to monitor the effects of therapeutic interventions on the metabolome. We utilized metabolomics to assess the effect of vitamin D supplementation on the metabolome in people with MS and healthy controls. In this study, 24 people with MS and 27 controls received 5000 IU of vitamin D daily for 3 months. We obtained plasma at beginning and end of the study and performed metabolomic profiling. We utilized WGCNA to identify metabolite modules and then utilized generalized estimating equations (GEE) to determine the change in metabolite modules following vitamin D treatment. We found a reduction in markers of oxidative stress and alterations in lipid metabolism in healthy controls following vitamin D supplementation but noted a lack of a similar effect in people with MS (Figure 5). This study demonstrated the ability of metabolomics to track changes in metabolic pathways following a therapeutic intervention.

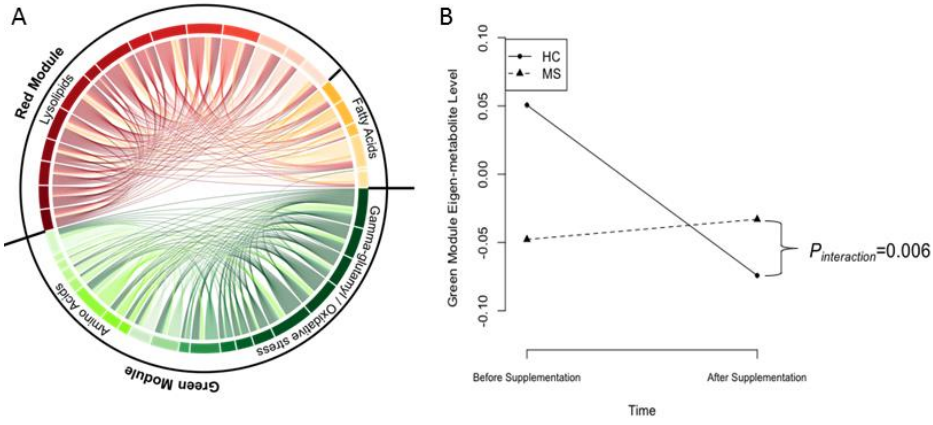


Figure 5. Metabolomics identifies the effects of vitamin D supplementation of oxidative stress and lipid metabolism. Panel A is a circos plot displaying contents of two metabolite modules that were altered by vitamin D supplementation – red module containing lipids and green module containing oxidative stress related metabolites. Panel B shows change in the green module (redox homeostasis) derived from a GEE model with a change noted in healthy control group but not in the MS group (p=0.006 for interaction).

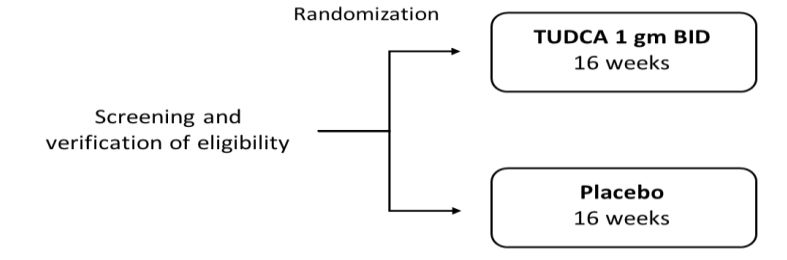
These preliminary data clearly demonstrate that people with MS have altered bile acid metabolism and that supplementation of a bile acid (TUDCA) had a beneficial effect in an animal model of MS. Additionally, we demonstrate that metabolomics can be used to monitor the effects of a therapeutic intervention in MS.

We hypothesize that bile acid supplementation in people with MS will be safe, well tolerated and lead to normalization of the bile acid profile and favorable immune and neuroprotective effects

4. Study Procedures

a. Study design, including the sequence and timing of study procedures

This will be a randomized, double-blind, placebo controlled trial of TUDCA 1gm twice daily versus placebo in patients with progressive MS who satisfy the inclusion and exclusion criteria.
A pictorial representation of the trial design is shown here.



Week	-2	0	8	16
Study visit	*	*	*	*
Safety assessments	*	*	*	*
Stool and blood collection		*	*	*

Study personnel:

Treating physician will assess eligibility criteria, obtain informed consent, obtain and update medical history and concomitant medication record, monitor patient safety, conduct physical examination, evaluate and report adverse events.

Examining physician will perform EDSS evaluations.

Study coordinator will be responsible for the study's administrative duties, obtaining vital signs, conducting the MSFC, administering the suicidality questionnaire, performing the phone check-in, drawing and processing blood samples, obtaining and storing stool samples and performing urine pregnancy testing when indicated.

Research technologist will isolate PBMCs from blood samples and will perform the immunophenotyping, prepare stool samples for metagenomic sequencing and prepare plasma samples for targeted metabolomics analyses.

Study statistician from the Johns Hopkins School of Public Health will create the randomization schedule, monitor randomization assignments, generate the data dictionary and perform final data analysis.

All personnel except the study statistician will be blinded to treatment assignment.

Study visits and procedures:

Study procedures that can be done remotely will be conducted via video visit to minimize patient contact and provide scheduling flexibility to participants.

1. **Screening visit (-2 weeks)**:** At the screening visit participants will undergo the following procedures.
   a. Informed consent
   b. Collection of demographic and disease characteristics
   c. Collection of concomitant medication information
   d. Vital signs, physical and neurological examination*
   e. Verification of eligibility based on inclusion/ exclusion criteria
   f. Venipuncture for baseline safety laboratory testing*
   g. MSFC* and MSQOL-54 assessments
   h. Urine pregnancy test*
   i. Suicidality evaluation (C-SSRS)

If the participant is eligible for the trial, they will then return for the baseline visit at which time they will be randomized to a treatment group and begin the trial.

2. **Baseline visit (0 weeks)**:**
   Following procedures will occur at this visit:
   a. Screening laboratory tests will be reviewed to assess whether there is any contraindication for continuation in trial
   b. Update concomitant medication information

c. Update medical and disease history
d. Vital signs, physical and neurological information*
e. Venipuncture for PBMCs and safety laboratory tests*
f. Stool specimen collection
g. MSFC*, MSQOL-54 and Food frequency questionnaire assessments
h. EDSS evaluation*
i. Urine pregnancy test*
j. Suicidality evaluation

3. Mid-study visit (8 weeks)

The following procedures will occur at this study visit.

a. Assessment of adverse effects
b. Review safety labs
c. Update medical and disease history
d. Update concomitant medication information
e. Vital signs, physical and neurological examination*
f. Venipuncture for PBMCs and safety labs*
g. Stool specimen collection
h. MSFC*, MSQOL-54 and Food frequency questionnaire assessments
i. EDSS evaluation*
j. Urine pregnancy test*
k. Suicidality evaluation

4. End-of-study visit (16 weeks)

The following procedures will occur at this study visit.

a. Assessment of adverse effects
b. Review of safety labs
c. Update medical and disease history
d. Update concomitant medication information
e. Vital signs, physical and neurological examination*
f. Venipuncture for PBMCs and safety labs*
g. Stool specimen collection
h. MSFC*, MSQOL-54 and Food frequency questionnaire assessments
i. EDSS evaluation*
j. Urine pregnancy test*
k. Suicidality evaluation

*Must be done at in-person portion of visit

** Due to the COVID-19 pandemic and to make it more convenient for the patients to participate, we decided to offer the option of three visits instead of four as an alternative. In this case, we will perform the research blood draw and stool sample at the screening visit, along with providing patients with medicine. We will ask patients not to start taking medicine until we receive the screening lab results and let them know whether they are eligible and can start the medication.

Study procedure	Screening	Baseline Week 0	Week 8	Week 16
Written consent	X			
Verify eligibility	X			
Medical history, relapse assessment	X	X	X	X
Medication review (also antibiotic and probiotic use)	X	X	X	X
Vital signs, physical, neurologic (EDSS) exams*	X	X	X	X
MS Functional Composite* & MSQOL-54	X	X	X	X
Adverse event assessment		X	X	X
Food frequency questionnaire		X	X	X
Laboratory testing (CBC, CMP, HbA1c, Lipid profile, pregnancy test)*	X	X	X	X
Blood for PBMC isolation* & Stool specimen collection		X	X	X
Suicidality evaluation (C-SSRS)	X	X	X	X

*Must be done at in-person portion of visit

b. Study duration and number of study visits required of research participants.

Study duration: 16 weeks.

Number of study visits: 4

Study visits
1. Screening
2. Baseline
3. Mid-study visit
4. End-of-study visit
5. Unscheduled visit will occur if participant develops adverse events or new symptoms

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

In this trial all study personnel and patients will be blinded to reduce any biased assessment of study outcomes including adverse effects related to the treatment as well as the included efficacy end-points (EDSS, MSFC, MSQOL-54), some of which are subjective and could thus be influenced by prior knowledge of treatment assignment.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

Participants will continue to receive routine care for their multiple sclerosis.

e. Justification for inclusion of a placebo or non-treatment group.

While this trial is primarily focused on establishing the safety and tolerability of TUDCA in patients with progressive MS, the inclusion of a placebo group will enable us to determine whether changes observed in serum bile acid profiles, gut microbiota, immunophenotype and clinical/ quality of life measures are related to TUDCA treatment. This would provide information regarding the utility of pursuing larger trials focused on clinical outcomes in this patient population.

f. Definition of treatment failure or participant removal criteria.

Patients selected for the study will be removed if they:
1) They develop abnormal safety labs as defined below
2) Develop a serious adverse event
3) Inform us that they became pregnant during the study
4) Are unable to follow the requirement of the study
5) Request to be removed

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

The subject's medical care will continue unaltered when the study ends or if a subject is removed from the study.

5. Inclusion/Exclusion Criteria

The inclusion and exclusion criteria for participants in this trial are listed in the table below.

Eligibility criterion	Rationale
Diagnosis of Progressive MS based on Lublin criteria	We are focusing on people with PMS since they are more likely to have low bile acid levels
Age ≥ 18 years	To include only adults in the trial
On the same therapy for the past 6 months and not expected to switch therapy in the next 6 months	To avoid confounding by changes in immunomodulatory therapy during the trial
No relapse in the past 3 months	To avoid any confounding introduced by relapse
Exclusion criterion	Rationale
No previous history of liver disease	Confounding due to decreased bile acid production or altered bile acid metabolism
No stage IV/V chronic kidney disease or other severe metabolic derangements	To reduce risk of adverse effects or confounding due to other systemic disorders
BMI < 15 kg/m^2 and BMI > 40 kg/m^2	Reduce confounding due to extremes of BMI
Female patients who are pregnant or nursing, or not	Prevent potential teratogenicity

willing to use contraception	
Chronic antibiotic use	Prevent confounding of microbiome analyses
Corticosteroid treatment within the past 30 days	Prevent confounding of treatment effect
Known history of other neuroinflammatory, neurodegenerative or systemic autoimmune disease	These diseases could require other treatments or be affected by the trial medication.
No current or prior history of the following – • Bariatric surgery • Small intestinal surgery resulting in loss of length of the jejunum or ileum • Chronic diseases of the small intestine (e.g., Crohn's Disease, gluten sensitive enteropathy, or other disorders that could lead to malabsorption) • Abnormal bile duct anatomy • Sclerosing cholangitis • Anti-mitochondrial antibodies	These conditions could alter bile acid handling in the gut or bile acid levels in circulation

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Tauroursodeoxycholic acid (TUDCA) is a hydrophilic bile acid that is normally produced endogenously in humans in the liver, by conjugation of taurine to UDCA. It is used in Europe to treat cholestatic liver disease and gallstones. TUDCA has been used in trials for liver cirrhosis, primary biliary cirrhosis, transthyretin related amyloidosis, cholesterol gallstones, diabetes, and ALS[30]–[33], with a good safety profile. Diarrhea was the most common side effect noted in these studies, which utilized varied doses of TUDCA.

As noted in the background section, we chose TUDCA as an intervention since it has been previously demonstrated to be safe and have several beneficial effects (on ER stress, mitochondrial dysfunction, immunological effects and neuroprotection) that could potentially lead to benefits in patients with MS. Additionally since TUDCA can change the circulating bile acid pool this could potentially help correct this metabolic abnormality that we have identified in our preliminary studies.

We will obtain TUDCA from Bruschettini S.r.l (Genoa, Italy). This medication is manufactured as 250 mg capsules and hence participants will take 4 capsules two times a day of either TUDCA or placebo. The study medication will be provided through the Johns Hopkins investigational drug services and will appear identical to maintain blinding. TUDCA is approved in Italy for the treatment of cholestasis and gallstones. It was recently accorded orphan drug status by the European Medicines Agency for the treatment of Amyotrophic lateral sclerosis.

Dose: We chose a dose of 1 gm BID based on the trial of TUDCA in ALS that was recently completed across three centers in Italy[25]. Participants in this trial had no significant adverse

events on TUDCA, and there appeared to be some evidence for a benefit in this early phase study. This dose is higher than the dose utilized in treatment of cholestatic disease and is close to the 30 mg/kg dose utilized in a trial of UDCA in ALS that was well tolerated and showed no significant difference in serum and CSF concentrations compared to a higher dose of 50 mg/kg[27],[30].

Pharmacokinetics of TUDCA: Previous studies have demonstrated that about 65% of the oral dose of TUDCA is absorbed and then undergoes first pass metabolism in the liver followed by extensive enterohepatic circulation[34]. Administration of bile acids such as UDCA and TUDCA results in a change in the overall serum bile acid concentration – between 1.9 – 8 fold and alters the composition of the bile acid pool[35],[36]. A trial of UDCA in ALS also demonstrated dose-dependent increased in CSF concentrations of the bile acid[27].

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

N/A

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

We are using a non-FDA approved drug, however this will be utilized either with an IND or with an IND waiver from the FDA.

7. Study Statistics

a. Primary outcome variable.
The primary outcome of this study is the safety and tolerability of TUDCA supplementation. This will be based on number of adverse events occurring in the two treatment groups and the rate of drop-out in each study arm. This data will be documented at each study visit.

b. Secondary outcome variables.

1. Metabolomics – We will store plasma before and after the study intervention to assess the effect of the study medication on fasting bile acid levels. This will enable us to determine whether the study drug raised the levels of bile acids that were deficient in people with MS. Targeted metabolomics analyses will be performed through Metabolon Inc.
2. Gut microbiota –The composition of the gut microbiota is closely related to bile acid metabolism, and alterations in bile acid receptor (FXR) signaling can significantly affect composition of the gut microbiota[37],[38]. We will collect first morning stool specimens prior to and following study treatment to determine the effect of bile acid supplementation on the gut microbiota in people with MS.
3. Immunophenotyping – We will store PBMCs from before and after the study intervention to assess the effect of TUDCA supplementation on the peripheral immune system. Our laboratory has expertise in immunophenotyping people with MS and assessing the effect of

therapeutic interventions on peripheral immune cell function. These assays will be performed at the end of the study.

4. Other outcomes – We will also collect information regarding quality of life using the MSQOL-54 instrument[39] administered at the visits noted in the table above. We will also measure lipid profile and hemoglobin A1c at baseline and end-of-study, since bile acid metabolism is linked to obesity and metabolic syndrome.

Assessment of important covariates: We will collect demographic information including age, gender, cigarette smoking, waist circumference, and BMI which can affect circulating bile acid levels. Another critical factor that can affect bile acid metabolism and the gut microbiota is diet. Since TUDCA could potentially lead to GI side effects, this may result in a change in diet. To monitor changes in diet we will perform the Block Food frequency questionnaire before and after the study intervention[48]. Since antibiotics and probiotics can lead to changes in the gut microbiome and could also affect bile acid metabolism, we will collect detailed information on the use of both these covariates so they can be adjusted for in our final analyses.

c. Statistical plan including sample size justification and interim data analysis.

Safety data will be analyzed as they are captured in real time. Assessments will focus on AEs (including study treatment tolerability assessments, laboratory evaluations, vital signs and physical examination). The incidence rate of AEs will be recorded by system organ class, severity and by relationship to study treatment. Tolerability analysis will be based on the number (%) of participants who failed to complete the study due to adverse events. Lab values for each parameter will be summarized by shift tables. For quantitative parameters, we will present summary statistics for actual values and change from baseline. We will compare changes over the 16-week treatment period between the groups using mixed effects regression analyses to account for repeated measures using random subject-specific intercepts and slopes.

Metabolomics data will be analyzed at the end of the study following quantitation of the 15 bile acids included in our panel. Summary statistics will be used to characterize these outcomes. We will then assess normality of these measures and perform appropriate univariate testing to determine which bile acids are significantly altered by TUDCA supplementation. A mixed effects longitudinal model will also be used to test which bile acids are altered between visits across the two groups. Models will also adjust for important covariates including BMI, age, gender and measures derived from the food frequency questionnaire.

Gut microbiota data, *a priori* analyses will assess changes in relative abundances of different gut bacteria as well as microbial genes that encode bile acid metabolizing enzymes (identified from the literature and NCBI Gene database) which occur as a result of TUDCA treatment. Exploratory analysis will assess the effects of TUDCA on the metagenome using a combination of pathway and set-based approaches previously applied in metagenomics analysis of MS and other diseases to identify other biologically relevant changes[41]–[43].

Immunophenotyping data, we will summarize data using summary statistics (mean and standard deviation), before and after study treatment. We will utilize paired *t* tests to compare proportions of various immune cell sub-populations at different time points. For both sets of data we will utilize mixed-effects regression models to assess the change in bacterial taxa/ metagenomics pathways/ immune cell subsets over time, and determine their relationship to changes in bile acid concentrations with TUDCA supplementation.

Sample size justification
Since this is primarily a safety study we based our sample size on the ability to detect changes in our secondary outcomes, especially metabolomics. Previous studies of supplementation of UDCA and TUDCA have resulted in alterations in the total serum bile acid levels (2-8 fold change) and the composition of the bile acid pool. Based on our preliminary data, to detect a 50 - 70% change in levels of an individual bile acid, with a power of 85% and two-tailed alpha of 0.05 the required sample size would be between 14 – 27 in each arm. Accounting for a 10% drop-out rate, we will aim to recruit 30 participants in each arm of the trial. We have previously noted significant alterations in metabolic profiles in MS patients treated with vitamin D and dimethyl fumarate in studies utilizing between 18-24 patients (as shown in preliminary data).
From a safety stand-point, a sample size of 30 would enable us to conclude with 80% confidence that the rate of occurrence of serious adverse events with TUDCA treatment was less than 5% if none of the patients experienced a serious adverse event during the study[40].

d. **Early stopping rules**.

We will discontinue treatment if a participant develops abnormal safety labs (serum AST or ALT > 3 x upper limit of normal, serum bilirubin > 3 x upper limit of normal, eGFR < 60ml/min/1.73m^2), becomes pregnant during the study, cannot tolerate the drug, develops an adverse event grade 3 or higher that is possibly related to study treatment or wants to discontinue treatment. Any death that is at least possibly related to the study will put the study on hold. The Data and Safety Monitoring Board will determine if it is safe to resume the study. Subjects who become suicidal during the study (codes 1-4, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM225130.pdf) will be referred to a psychiatrist or to a psychiatric emergency room for evaluation at the discretion of the site treating physician.

Data and Safety Monitoring Board (DSMB): An independent DSMB will be utilized for subject monitoring. The DSMB will consist of a neurologist, a hepatologist and a statistician. The DSMB will meet every 12 weeks and any time an SAE occurs. All SAEs will be reported to the DSMB delegates within 24 hours of the study staff becoming aware of the SAE. The DSMB will be required to review SAEs and respond with recommendations within two business days. If more than 3 subjects experience serious adverse events, the study will be put on hold and the DSMB will decide on whether the adverse events were secondary to study treatment. They will make a judgement as to whether the study should continue and, if so, whether a reduction in TUDCA dosing or other protocol modifications are recommended.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

TUDCA treatment: In previous studies of TUDCA and UDCA – adverse effects include – mainly GI related effects – diarrhea, constipation, gas, nausea, vomiting, abdominal pain and anorexia, other adverse effects which are less frequent (< 1% each) include – headache, rash and pruritus [25],[27],[30].

While no serious adverse effects have been note in previous studies, several of the described AEs could lead to issues with tolerability in MS patients especially those with bowel function abnormalities.

In a recent trial of TUDCA 1 gm twice daily in patients with Amyotrophic lateral sclerosis there were no serious adverse effects.

Venipuncture: The potential risks include discomfort, bleeding or bruising at site of blood draw, and in rare cases fainting or infection.

Clinical examination (including EDSS, MSFC): There are no significant risks of this procedure.

Stool specimen collection: There are no significant risks of this procedure.

b. Steps taken to minimize the risks.

TUDCA treatment: We are utilizing a dose of TUDCA that has recently been shown to be safe in a trial in another chronic neurological disorder. We are also monitoring safety labs and conducting neurological evaluations every 8 weeks to monitor for any adverse effects that may be related to the treatment.

Venipuncture: Blood will be drawn by a trained study team member.

c. Plan for reporting unanticipated problems or study deviations.

Unexpected Adverse Event: An adverse event is considered unexpected when its nature or severity is not consistent with the product information (e.g. package insert safety information, the investigational plan, the investigator's brochure, the protocol, or the informed consent form).

Serious Adverse Event: A serious adverse event (any adverse event that suggests a significant hazard, contraindication, side effect, or precaution) must be reported. This includes, but may not be limited to, death, a life-threatening event, inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability/ incapacity, congenital anomaly/ birth defect, other protocol-specified conditions, or an event that requires intervention to prevent permanent impairment or damage. Serious adverse events will be collected from informed consent signing until 30 days after study completion or until 30 days after a participant withdraws from the study.

Grading of Adverse Events
We will use the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 to report and grade all adverse events, whether or not they are related to disease progression or treatment. The relationship between an adverse event and the study drug will be determined by the site investigator and recorded on the appropriate form.

Serious Adverse Event Reporting The following process for reporting a serious adverse event will ensure compliance with the International Conference on Harmonisation guidelines:

1. The Institutional Review Board will be notified in one business day of a serious adverse event.
2. Standard reporting will occur if the event is serious, expected and drug-related, serious, expected and not drug-related, or serious, unexpected and not drug-related.
3. Expedited reporting is required if the event is serious, unexpected and drug-related. This type of event must be reported to the appropriate authorities within 15 days unless it is fatal or life-threatening; a fatal or life-threatening event must be reported within 7 days.

Pregnancy Reporting Requirements: Any pregnancy that occurs during a clinical study with an investigational drug will be reported to the IRB and DSMB, and pregnancies will be followed to their conclusion. Female participants should immediately inform the investigator of pregnancies and will stop taking the study medications. The investigator will report pregnancies to the Institutional Review Board and DSMB within one business day. The investigator will counsel the participant about the risks of continuing the pregnancy

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Each participant will be assigned a study identification number, and the data key will be kept in a separate, secure location. Electronic records will be stored on a secure, password-protected network. Any paper records will be kept in a locked file cabinet inside a locked office. These measures should prevent a breach of privacy. The consequences should be minimal, as the subjects with MS will have already established care and carried the diagnosis thereof. There should be no social stigmata for the participants.

e. Financial risks to the participants.

There are no significant financial risks to participants from this study.

9. Benefits

a. Description of the probable benefits for the participant and for society.

There are no individual medical benefits for participating in this study. As there is no curative treatment for MS, there is a glaring need for more knowledge and more effective treatment approaches for individuals with progressive MS. This study has the potential to benefit progressive MS patients in the future.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

No compensation will be provided to subjects for participating in this trial.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

The subjects will not be charged for any of the procedures in the study. They will only be responsible for the cost of any travel to and from the Hospital as well as any meals they eat while participating. The costs of parking at the Hospital garage will be covered.

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