Microbiota Transfer Therapy for Children with both Pitt Hopkins Syndrome and Gastrointestinal Disorders

NCT04132427

Clinical Study Protocol Version 3 – March 28, 2022

Previous versions: September 8, 2020 version6.i Objectives and purpose of the study

This will be a Phase 2 Trial to determine the safety, tolerability, and efficacy of Microbiota Transfer Therapy (MTT) for treating patients with Pitt Hopkins Syndrome and gastrointestinal disorders (chronic constipation and/or diarrhea).

6.ii Name and qualifications of sponsor, investigators and sub-investigators

Sponsor and Investigator: James B. Adams, Ph.D.
Arizona State University
PO Box 876106
Tempe, AZ 85287-6106

Prof. Adams is the director of the Autism/Asperger's Research Program at Arizona State University, which focuses on medical causes of autism, how to treat it, and how to prevent it. He has published over 150 papers in peer-reviewed journals, including over 40 on autism-related research. He has led several clinical trials for ASD and assisted with several others. He led the Phase 1 study on MTT, and is currently leading a study on "Microbiota Transfer Therapy (MTT) for Treating Gastrointestinal Problems in Adults with ASD."

Co-Investigator: Rosa Krajmalnik-Brown, Ph.D.
Biodesign Institute at Arizona State University
P.O. Box 875701
Tempe, AZ 85287-5701

Prof. Krajmalnik-Brown is a Professor in the School of Sustainable Engineering and the Built Environment, and in the Swette Center for Environmental Biotechnology in the Biodesign Institute at Arizona State University. She has extensive expertise in microbial ecology with more than 60 papers in peer-reviewed journals. She has led many studies determining the gut microflora structure and function. She has worked with Prof. Adams on 4 previous autism microbiome studies, including the Phase 1 study on MTT for children with ASD and a current study on MTT for adults with ASD.

Lead Physician: .

Dr. Robert Hellmers

Arizona Allergy Associates
705 S. Chandler Blvd.
Chandler, AZ85224

Robert Hellmers, MD, is board-certified in pediatrics and board certified in allergy and immunology. He has over 40 years of clinical experience, including evaluating and treating many children and adults with autism. He previously was the senior study physician for a randomized clinical trial of comprehensive nutritional intervention for children and adults with autism, in collaboration with Prof. Adams. He is now semi-retired, and this study will be one of his major projects.

Research Facilities at Arizona State University
Autism Research Program
Arizona State University
ECG 302
501 E Tyler Mall
Tempe, AZ 85287-6106

Biodesign Institute
Arizona State University
727 East Tyler Road
Tempe, AZ 85287-5701 U.S.A.

Institutional Review Board
The IRB at Arizona State University will be the IRB for this study.

6.iii Patient Inclusion, Exclusion, and Number

Inclusion Criteria
1) Children ages 7-17 years with Pitt Hopkins Syndrome (verified by genetic testing)
2) GI disorder as defined below that has lasted for at least 2 years.
3) No changes in medications, supplements, diet, or therapies in last 2 months, and no intention to change them during the Parts 1 and 2 of the clinical trial.
4) Ability to swallow pills (without chewing) - this will be verified by the study staff during the first eligibility visit by administering a placebo capsule and ensuring that the participant can swallow it without chewing.
5) Review of last two years of medical records by the study physician.

Exclusion Criteria
1) Antibiotics in last 3 months (topical antibiotics are allowed)
2) Probiotics in last 2 months, or fecal transplant in last 12 months
3) Tube feeding
4) Severe gastrointestinal problems that require immediate treatment (life-threatening)

5) Ulcerative Colitis, Crohn's Disease, diagnosed Celiac Disease, Eosinophilic Gastroenteritis, or similar conditions
6) Unstable, poor health (based on study physician's opinion)
7) Recent or scheduled surgeries
8) Current participation in other clinical trials
9) Females who are pregnant or who are at risk of pregnancy and sexually active without effective birth control. We will conduct a pregnancy test on all female participants as part of the screening and at each clinical visit.
10) Allergy or intolerance to vancomycin or magnesium citrate
11) Clinically significant abnormalities at baseline on two blood safety tests: Comprehensive Metabolic Panel, and Complete Blood Count with Differential. Note that some abnormalities may occur due to PTHS, so only those likely to significantly increase risk in this study would be grounds for exclusion, at the discretion of the study physician. See detailed discussion at end of this section on Interpreting Laboratory Results. re. Eligibility for Admission to Study.
12) Evidence of significant impairment of immune system, or taking medications that can compromise the immune system, and thus increase risk if exposed to multiple-drug resistant bacteria.

The rational for exclusions 1-2 is that they interfere with gut flora.
The rationale for exclusion 3 is that those individuals are probably less likely to respond to the proposed treatment.
The rationale for exclusions 4-7 is that those individuals are at higher risk of safety problems with MTT.
The rational for exclusion 8 is that participation in other trials would interfere with the results of this one.
The rationale for exclusion 9 is to avoid risk to fetuses.
The rationale for exclusion 10 is to avoid known allergic reactions to study medications.
The rationale for exclusions 11 is to ensure that participants are in adequate health (aside from chronic GI and PTHS-related problems) at the start of the study.

Definition of a GI Disorder (for this study):
On the Daily Stool and Symptom Record, five or more abnormal days out of 14 days, where an abnormal day is defined as a day which involves one or more of the following symptoms:

1) Abnormal stool: type 1-2 (hard) or type 6-7 (soft/liquid) on the Bristol Stool Scale
2) Three or more bowel movements in 1 day
3) No bowel movement that day.
4) Significant abdominal/gastrointestinal pain.
5) GI medication or treatment is required (such as laxative, enema, etc.)

Interpreting Laboratory Results re. Eligibility for Admission to Study

Each participant will have a fasting blood draw to assess safety criteria for admission to the study. Those tests include a comprehensive metabolic panel (including liver/kidney function tests) and complete blood count with differential (CBC). The tests will be conducted by LabCorp or an equivalent CLIA-certified laboratory.

Normal Values: Participants who have values for all tests within the reference ranges will be deemed eligible to participate.

Borderline Values (at or slightly outside the reference range limits): Those whose values are in the borderline range and whose values are isolated and not consistent with a potential clinical concern, will also be deemed eligible. For example, if ALT were slightly above the reference range, but AST was normal, then we would assume liver function is acceptable and the participant would be eligible for enrollment.

Abnormal Values: Participants who have a value clearly outside of the reference range, but for a known reason, will be considered for eligibility based on the study physician's clinical judgement following review of prior medical records, history from parent, and a determination that medical care is being provided for the cause of the abnormality and likely the lab value will remain stable during the study. Examples of acceptable reasons for an abnormal value include:

1) Participant is taking a psychiatric or anticonvulsant medication known to increase liver enzymes (ALT and AST)
2) Altered CO_2 due to altered respiratory function typical in PTHS and is under medical care

For any applicant that has abnormal laboratory test values we will document the abnormality and the physician's interpretation of the test result and why it is or is not an exclusion criteria for admission to the study.

In some marginal cases the study physician may request a second blood draw before making a decision on eligibility, or if there is a suspicion of a laboratory error.

Below is a list of the LabCorp Reference Ranges for each test.

Lab Test	***Age***	***Reference Range (males)***	***Reference Range (females)***	***Reference Range (all)***
Comprehensive Metabolic Panel				
BUN (mg/dL)	1-17 years	5-18	same	
Creatinine, Serum (mg/dl)	1-2 years	.19-.42	same	
	3-4 years	.26-.51	same	
	5-6 years	.30-.59	same	
	7-8 years	.37-.62	same	
	9-10years	.39-.70	same	
	11-12 years	.42-.75	same	
	13-14 years	.49-.90	same	
	>14 years	.76-1.27	.57-1.00	
Glucose, Serum (mg/dl)	all			65-99

BUN/Creatine Ratio	7mths-1year	20-71	same	
	2-5years	19-51	19-49	
	6-12years	14-34	13-32	
	13 -17 years	10-22	same	
Sodium, Serum (MMOL/L)	all			134-144
Potassium, Serum (MMOL/L)	all			3.5-5.2
Chloride, Serum (MMOL/L)	all			96-106
Carbon Dioxide, Total	all			17-26
Calcium, Serum(mg/dL)	11 days- 1 year	9.2-11.0	same	
	2-11 years	9.1-10.5	same	
	12-17 years	8.9-10.4	same	
Protein, Total, Serum (g/dl)	all			6.0-8.5
Albumin, Serum (g/dl)	1-2years	3.4-4.2	same	
	3-17 years	3.5-5.5	same	
Globulin, Total (g/dl)	all			1.5-4.5
A/G Ratio (No unit)	all			1.5-2.6
Bilirubin, Total (mg/dL)	all			0.0-1.2
Alkaline Phosphatase, S (IU/L)	all			133-309
AST (SGOT) (IU/L)	all			0-60
ALT (SGOT) (IU/L)	1-11 years	0-29	0-28	
	12-17 years	0-30	0-24	
Complete Blood Count with Differential				
WBC (X10E3/UL)	all			4.3-12.4

RBC (X10E6/UL)	1-7 years	3.96-5.30	same	
	8-12 years	3.91-5.45	same	
	>12 years	4.14-5.80	3.77-5.28	
Hemoglobin (g/dl)	1-7 years	10.9-14.8	Same	
	8-12 years	11.7-15.7	Same	
	13-15 years	12.6-17.7	11.1-15.9	
	>15	13.0-17.7	11.1-15.9	
Hematocrit (%)	1-7 years	32.4-43.3	Same	
	8-12 years	34.8-45.8	Same	
	>12 years	37.5-51.0	34.0-46.6	
MCV (fL)	1-7 years	75-89	Same	
	8-12 years	77-91	Same	
	>12 years	79-97	Same	
MCH (pg)	1-7 years	24.6-30.7	Same	
	8-12 years	25.7-31.5	Same	
	>12 years	26.6-33.0	Same	
MCHC (g/dl)	1-12 years	31.7-36.0	Same	
	>12 years	31.5-35.7	Same	
RDW (%)	1-7 years	12.3-15.8	Same	
	8-12 years	12.3-15.1	Same	
	>12 years	12.3-15.4	Same	
Platelets (X10E3/UL)	all			150-450
Neutrophils (Absolute) (X10E3/UL)	1-7 years	0.9-5.4	Same	
	8-12 years	1.2-6.0	Same	
	>12years	1.4-7.0	Same	
Lymphs (Absolute) (X10E3/UL)	1-7 years	1.6-5.9	Same	
	8-12 years	1.3-3.7	Same	
	>12years	0.7-3.1	Same	
Monocytes (Absolute) (X10E3/UL)	1-7 years	0.2-1.0	Same	
	8-12 years	0.1-0.8	Same	
	>12years	0.1-0.9	Same	

Eos (Absolute) (X10E3/UL)	1-7 years	0.0-0.3	Same
	>7 years	0.0-0.4	Same
Baso (Absolute) (X10E3/UL)	1-17 years	0.0-0.2	Same
Immature Grans (Abs) (X10E3/UL)	> 30 days	0.0-0.1	Same

6.iii Study Design

Summary Table of Study Design

	Part 1	Part 2	Part 3
Group A	Treatment	Observation	Observation
Group B	Placebo	Treatment	Observation

Part 1: Treatment

The three treatments (oral vancomycin, magnesium citrate, and Fecal Microbiota) will be given sequentially.

- Oral Vancomycin (an oral antibiotic) for 10 days to reduce the amount of pathogenic bacteria, followed by
- Magnesium citrate (a laxative/bowel cleanse) for one day to remove the vancomycin and further reduce intestinal bacteria, followed by
- Fecal Microbiota (purified intestinal bacteria from healthy, carefully-screened donors) for 2 weeks. The dosage for the first four days will be approximately 5×10^{11} cells/day. It can be administered as a single dose or b.i.d. The maintenance dose for the next 12 weeks will be approximately 1×10^{11} cells every four days.

Group A: vancomycin, magnesium citrate, FM

Group B: placebo vancomycin, magnesium citrate, placebo FM (note that this group receives the real magnesium citrate because the bowel-emptying effects are obvious)

Part 2: Open-Label Observation and Cross-Over

Group A: observation only (no treatment) for 14 weeks

Group B: real treatment given in Part 1 to Group A; i.e, vancomycin, magnesium citrate, FM

Part 3: Observation: Follow-up at 14 weeks after the end of Part 2

Participant duration will be 28 weeks for treatment/observation (part 1 and 2), and 14 weeks for follow-up after treatment ends (Part 3).

Study duration will likely be 2-3 years, depending on the enrollment rate of participants.

Tables describing schedule for Parts 1 and 2 (note that dates might vary by a few days due to patient scheduling issues, but treatment duration is fixed)

Part 1: Treatment for Groups A and B

		Part 1			
	Baseline	**Days 1-10**	**Day 11**	**Days 12-15**	**Days 16-99**
Group A		Vancomycin	Magnesium Citrate	High-dose FM	Maintenance FM
Group B		Placebo vancomycin	Magnesium Citrate (not placebo)	Placebo FM	Placebo FM
Physical Exam, test for pill swallowing, and CGI. OT evaluation	Day 0				Day 98
AE monitoring by physician					Day 98
Contact with Study Coordinator and AE monitoring		Day 10		Day 12	Day 16, 45
DSR	Daily for 14 days prior to Day 0	Daily		Daily	Daily
Vancomycin adverse effect		Days 1-10			
MT adverse effect				Days 12-15	Days 16-18
GSRS and PGI-PTHS and FLACC	Day 0	Day 10			Days 24, 38, 52, 66, 80, 94
Diet Evaluation	Day 0				

Blood, buccal swab	Day 0				Day 99
Urine, Stool	Day 0	Day 8			Day 24, 94

Part 2: Group A: Observation

	Days 99-198
Treatments	none
Physical Exam, AE monitoring, and CGI. OT evaluation	Day 198
Contact with Study Coordinator and AE monitoring	Day 150
DSR	Days 134-148, 180-194
GSRS and PGI-PTHS and FLACC	Days 148,194
Blood, buccal swab	Day 198
Urine, Stool	Day 198

Part 2: Group B: Open-label cross-over

	Part 2			
	Days 101-110	Day 111	Days 112-115	Days 116-199
Treatments	Vancomycin	Magnesium Citrate	High-dose FM	Maintenance FM
Physical Exam, AE monitoring, and CGI. OT evaluation				Day 199
Contact with Study Coordinator and AE monitoring	Day 110		Day 112	Day 116, Day 145
DSR	Daily		Daily	Daily
Vancomycin adverse effect	Days 101-110			

MT adverse effect			Days 112-115	Days 116-119
GSRS and PGI-PTHS and FLACC	Day 110			Days 124, 138, 152, 166, 180, 194
Blood, buccal swab				Day 199
Urine, Stool	Day 108			Day 124, 194

Part 3: Follow-up at 14 weeks after end of Part 2 for Groups A and B

	Day 296
Phone call with study physician and AE monitoring. OT evaluation	Day 296
Contact with study coordinator and AE monitoring	Day 245
DSR	Daily days 280-294
GSRS and PGI-PTHS; Diet form	Day 296
Urine, Stool	Day 296
Diet Evaluation	Day 296

There will also be contact (phone, email, or text) with the study coordinator on approximately the following dates to check on compliance with the study protocol and to screen for possible adverse events.

Part 1: Both Groups	Days 10, 12, 16, 45
Part 2, Group A:	Day 150
Part 2, Group B:	Day 110, 112, 116, 145
Part 3: Both Groups	Day 245

Participants will be required to complete a medication checklist to determine their compliance with the study medication. Also, they will be required to return medication containers with any unused medication at their clinic appointments.

Physical Examinations: The initial physical exam will be conducted by the lead study physicianin Arizona. Since PTHS is a rare disorder, we think it is possible that some participants will be from out-of-state. Due to the severe physical and mental challenges of the participants, we wish to minimize the burden of travel on out-of-state participants. Therefore, out-of-state participants will have the option of having the subsequent blood draws and physical examinations done by their local physician, with a report being sent to the lead study physician for review. The local physician will be offered a copy of the Investigator Brochure. In those cases the lead study physician will also talk with the participants by Skype or similar video-conferencing as part of the physical examination process, and to conduct the CGI evaluations. The physical exam will include an assessment of the characteristic signs and symptoms of Pitt Hopkins as listed in the international consensus statement.
All physical examinations and discussions with the study physician will include monitoring for possible adverse events. Also, the study physician will review the Vancomycin Adverse Effect Form, the Microbiota Adverse Effect form, the GSRS form, and the PGI-PTHS form. At the 3month and 6 month post treatment visits the study physician will also check for serious adverse events and adverse events of special interest including new onset diagnosis of obesity, glucose intolerance, an autoimmune condition, or metabolic syndrome.

Sample Size Justification

PTHS is a very rare condition, affecting approximately 1000 people (primarily children) in the US, and approximately 70% of those have chronic GI disorders. This significantly limits our recruiting ability, even with the help of the PTHS Foundation which is funding the study and offering to help recruit for it. Also, this is the first treatment study of any type for PTHS, so we hope to learn a lot from it. Therefore, we plan to initially enroll 10 participants to obtain initial estimates of safety, tolerability, dosage, and efficacy. We may enroll an additional 10 if results are promising but inconclusive for the first 10.

If this study is successful and demonstrates good safety and efficacy with minimal adverse effects, then we will discuss with the FDA what are the next steps required to seek drug approval of MTT for individuals with PTHS. If the study has limited success, we may need to enroll more participants, possibly with a modified study protocol based on the results of this initial study.

Analysis of Safety

The primary safety analysis will be based on the following:

- Rate and severity of adverse effects and serious adverse effects.
- Average changes in any blood safety tests (blood chemistry panel and complete blood count with differential), and number of test results in abnormal reference range.

Analysis of Tolerability

The primary estimate of tolerability will be the percentage of consumption of each study medication, and number of dropouts from the study.

Analysis of Efficacy

The primary estimate of efficacy will be a comparison of changes in the Daily Stool Record (DSR). The secondary measures of efficacy include changes in the Clinical Global Impressions (CGI), Parent Global Impressions – Pitt Hopkins (PGI-PTHS), and Gastrointestinal Symptom Rating Scale (GSRS).

Informed Consent

After applications are screened and approved, the study coordinator will contact participants and explain the study to the participant's parent/guardian. If the child has a developmental age of approximately 7 years of age or older, then the study will be explained to them in a simplified manner, and given the opportunity to ask questions. The study coordinator will provide an opportunity to ask questions, and then invite the parent/guardian (and child if possible) to participate in the study.

Compensation for Participation

Medications will be provided at no charge. No compensation will be provided for participation in the study.

6.v Dosage of Medications

Proposed dosage for this study, and Justification:

Vancomycin (oral): 40 mg/kg P.O. per day, maximum of 2 gram P.O. (oral) per day, divided into three doses. It is the same dosage we used for the Phase 1 study of MTT for ASD study, except for that study we treated for 14 days, and here we propose treatment for 10 days as we think that will be sufficient.

Magnesium Citrate (oral): The standard concentration is 1.75 g/30 mL solution. The standard dose will be:

5-8 yr: 4 ml/kg-bodyweight
8-12 yr: 150 ml
12-15 yr: 200 ml
15-18 yr: 300 ml
Half the standard dose (above) will be given in the early afternoon, and followed by another ½ dose within 12 hours if sufficient clean out does not occur after 3-4 hours.

The dosage may be modified based on the clinician's assessment of the child's history of GI problems and response to initial mag citrate dose. Evening meal to be clear liquids.

Initial FM Oral Dose: The dosage for the first four days will be approximately 5×10^{11} cells/day. It will be administered as two capsules in the morning and 3 capsules in the evening.

Maintenance FM Oral Dose: The maintenance dose for the next 12 weeks will be approximately $1x\ 10^{11}$ cells (1 capsule) every four days.

All FM doses will be given 2 hours away from meals.

Justification of FM Dosing: Our Phase 1 study of MTT for ASD (IND # 15886) used a dose of oral FM for 2 days of $8.3x10^{11}$, t.i.d, for a total daily dose of 2.5×10^{12} /day, and a maintenance FM dose of $2.5x10^{9}$ CFU/day, administered 1x/day orally, for 8 weeks. That study found that GI improvements were near maximal at about 5-6 weeks, and ASD improvements were initially maximal at 10 weeks, with greater improvement at the 2-year follow-up. Children with PTHS have worse phylogenetic diversity than children with ASD (see section 4.i), so we want to treat them for longer, and with a higher maintenance dose, to attempt to maximize their chances of clinical improvement.

It is important to point out that the dosing of vancomycin and FM proposed here is our best estimate, and that the results of the proposed study will provide important guidance on dosing for future studies.

Shipping, Handling, and Administration of Medications

The study medications will be provided to participants by Pure Compounding Pharmacy (Naperville, IL). They will individually compound vancomycin capsules for each participant. They will also put the FM capsules from University of Minnesota into individual bottles for the participants. They will also send magnesium citrate to the participants. The pharmacist will provide the medications to each participant individually after receiving the prescription from the study physician, using a randomization key which only they will have direct access to.

The vancomycin, magnesium citrate, and FM will be prescribed by the study physician after reviewing their baseline lab safety tests (CBC and metabolic panel).

The FM will be shipped overnight from the University of Minnesota to the pharmacy in an insulated container with dry ice and a temperature monitor. The pharmacy will store it in a temperature-monitored freezer. The pharmacy will give the FM to individual participants in an insulated container with freezer packs and/or dry ice to keep it frozen (below zero ºC) with a temperature monitor. During storage and shipping, temperatures of 8-21 ºC for up to 48 hours will be tolerated, but longer time periods, or temperatures above 21 ºC, will result in discarding of the FM and replacing it with new material. Participants will maintain the FM in their personal refrigerators. Participants are given the following instructions, and any dosages which are unrefrigerated at room temperature for up to 24 hours, or any dosages which exceed room temperature (23ºC) at any time, will be discarded and replaced.

Instructions for Participants:

DO NOT OPEN THE CAPSULE – it must be swallowed intact, to protect the bacteria from stomach acid

Refrigeration: Keep the microbiota in the refrigerator at all times, except to open the bottle and briefly remove dosage. Take dose within 15 minutes of removing from refrigerator. Do not expose to any heat.

Keep Dry: Keep all capsules in the container with the desiccant. Seal the container tightly after each use.

Avoid Heat: If microbiota capsule is exposed to any heat above room temperature, or is left at room temperature for more than 60 minutes – CONTACT YOUR STUDY COORDINATOR

Avoid placing microbiota capsules near hot spots (oven/stove/toaster, or leaving in hot cars), and keep refrigerated. Even a brief dose of heat above room temperature may destroy the bacteria. Request a replacement dose if there are any concerns.

The above procedures are based on new stability data from the University of Minnesota:

Summary of Stability Data:

-80 ºC: at 12 months, 6.5% loss

-20 ºC: at 12 months, 1% loss

4 ºC: at 6 months, 4% loss

Room temperature: at 4 weeks, 0% loss

Here is the raw data on stability testing of FM samples by University of Minnesota.

Donor 42 - 3/7/2017												
Membrane integrity	**replicate 1**	**replicate 2**	**replicate 3**									
Initial	62.4	65.5	61.1									
Post lyo	58.4	62.7	61.4									
	-80C Storage			**-20C Storage**			**4C Storage**			**Room Temp**		
	replicate 1	**replicate 2**	**replicate 3**	**replicate 1**	**replicate 2**	**replicate 3**	**replicate 1**	**replicate 2**	**replicate 3**	**replicate 1**	**replicate 2**	**replicate 3**
1 week	56.2	62.2	57.2	55.4	51.2	58.3	61.3	55.3	59.4	51.4	49.3	57.5
2 Weeks	55.3	51.9	54.3	59.6	52.9	54.3	51	57.9	54	52.3	58.7	53.2
3 Weeks	61.2	57.4	58.3	54.3	55.2	60.4	59.8	62.1	51.7	59.3	49.4	48.4
4 Weeks	55.3	51.2	57.4	55.1	54.2	61.7	57.8	56	56.4	51.2	55.4	52.8
2 Months	49.2	55.8	59.3	51.5	60.4	62.3	53.8	52.6	53.8			
3 Months	51.2	58.8	61.2	47.2	49.6	57.2	58.9	60.9	61.4			
4 Months	58.4	59.3	51.3	62	50.9	47.8	49.3	57.7	58.9			
5 Months	57.3	58.8	51.9	57.9	62.5	60.9	61.8	57.8	50.9			
6 Months	61.3	54.3	49	50.6	60.7	47.7	52.7	51.2	57.5			
12 Months	51.2	54.4	53.2	51.2	54.9	52.6	NA	NA	NA			
% loss from week 1	91.1%	87.5%	93.0%	92.4%	107.2%	90.2%	86.0%	92.6%	96.8%	99.6%	112.4%	91.8%
average of 3 samples	90.5%			96.6%			91.8%			101.3%		
Donor 42 - 3/8/2017												
Membrane integrity	**replicate 1**	**replicate 2**	**replicate 3**									
Initial	72.4	76.5	74.3									
Post lyo	51.3	52.8	53.2									
	-80C Storage			**-20C Storage**			**4C Storage**			**Room Temp**		
	replicate 1	**replicate 2**	**replicate 3**	**replicate 1**	**replicate 2**	**replicate 3**	**replicate 1**	**replicate 2**	**replicate 3**	**replicate 1**	**replicate 2**	**replicate 3**
1 week	53.5	55.5	51.3	47.8	47.3	51.2	47.8	47.6	50.7	44.7	44.8	44.2
2 Weeks	53.8	47.6	53.9	46.7	47.8	49.4	47.8	48.4	43.1	41.2	42.8	43.1
3 Weeks	53.3	47.4	54.2	50.7	52	48.2	48.2	45.8	46	42.4	52.3	48.4
4 Weeks	51.3	47.5	54.1	51.1	46.9	50.9	48.8	48.7	48.3	44.6	47.2	42.3
2 Months	54.5	50.9	50.5	45.3	50	51.2	48.2	42.2	42.6			
3 Months	48.4	50.4	45.6	50.5	47.5	52	44.2	47.4	48.4			
4 Months	52.7	52.9	53.9	50.4	46.7	52.6	43.8	46.2	46.5			
5 Months	52.9	46.1	52.7	49.4	49	50.2	42.1	47.7	49.6			
6 Months	46.4	54.7	47.2	52.5	45.5	50.3	49	47	49.4			
12 Months	48.2	53.9	52.3	47.7	49.9	51.4	NA	NA	NA			
% loss from week 1	90.1%	97.1%	101.9%	99.8%	105.5%	100.4%	102.5%	98.7%	97.4%	99.8%	105.4%	95.7%
average of 3 samples	96.4%			101.9%			99.6%			100.3%		
average from both donors	93.5%			99.3%			95.7%			100.8%		

Labelling of Study Medications

The following are the proposed labels for this study for the study medications.

Primary Label that will be on FM bottle shipped from the University of Minnesota

Intestinal Microbiota for Transplant

LOT # _____

DOM _____

Donor ID _____

# of Capsules _____

Store in Freezer at 0 to – 35° C

Caution: New Drug--Limited by Federal Law to Investigational Use

James B Adams
Arizona State University
ECG 302, 501 E Tyler Mall
Tempe, AZ 85287-6106
Phone: (480) 818-0741

The following are the labels for the Pure Compounding Pharmacy

Pure Compounding Pharmacy label for Initial FM/placebo for each patient

Fecal Microbiota (FM) or Placebo **Qty: 20 caps**

Initial dose: Take 2 capsules by mouth on an empty stomach in early morning and 3 capsules by mouth in evening.

No caloric intake (solid or liquid) for 1-2 hours prior and 1 hour after dosing.

Take capsules with at least 1.5 ounces (3 tablespoons) of water.

For oral consumption only. Swallow capsules whole – do not open.

Keep refrigerated (36-46°F) – very important.

Prescribed by _____ and compounded by Pure Compounding Pharmacy (630-995-4300).

Caution: New Drug - Limited by Federal Law to Investigational Use

Produced for:
James B. Adams
Arizona State University
ECG 302, 501 E. Tyler Mall
Tempe, AZ 85287
Phone: 480 818-0741

Pure Compounding Pharmacy label for Maintenance FM/placebo for each patient

Fecal Microbiota (FM) or Placebo **Qty: 21 caps**

Maintenance dose: Take 1 capsule by mouth on an empty stomach every four days for 12 weeks.

No caloric intake (solid or liquid) for 1-2 hours prior and 1 hour after dosing.

Take capsules with at least 1.5 ounces (3 tablespoons) of water.

For oral consumption only. Swallow capsules whole – do not open.

Keep refrigerated (36-46°F) – very important.
Prescribed by _____ and compounded by Pure Compounding Pharmacy (630-995-4300).

Caution: New Drug - Limited by Federal Law to Investigational Use

Produced for:
James B. Adams
Arizona State University
ECG 302, 501 E. Tyler Mall
Tempe, AZ 85287
Phone: 480 818-0741

Pure Compounding Pharmacy label for Vancomycin/placebo for each patient.

Vancomycin or Placebo **Qty: __ caps**

Take _____ capsules 3 times per day (after waking, mid-afternoon, bedtime), for 10 days.

For oral consumption only.

Prescribed by _____ and compounded by Pure Compounding Pharmacy (630-995-4300).

For investigational use only.

Produced for:
James B. Adams
Arizona State University
ECG 302, 501 E. Tyler Mall
Tempe, AZ 85287
Phone: 480 818-0741

Magnesium Citrate: No special label will be placed on the magnesium citrate since the real medication will always be used.

6.vi Outcome Measures

Below is a chart of the outcome measures, followed by a more detailed description of them.

The CGI will be conducted by the study physician. All other forms will be completed by the participant's parent/guardian, after receiving instructions from the study coordinator.

Primary Outcome Measures	Daily Stool Record A 14-day evaluation with the Bristol Stool Form Scale. Safety Measures Described in section 6.vii.a Methods to Assess Drug Safety
Secondary Outcome Measures	CGI for GI Disorder CGI for PTHS Symptoms PGI-PTHS GSRS FLACC pain
Tertiary/ Exploratory Outcome Measures	Microbiome Composition of Stool OT Evaluation
Possible Future Exploratory Outcome Measures	Metabolomics of Blood, Urine, and Stool Measurements of 200+ metabolites in blood, urine, and stool (we will be collecting samples for future testing, but do not yet have funding for those tests).

Detailed description of Outcome Assessments

Daily Stool Record (DSR)

For the Daily Stool Record, participants will record the type of stool(s) each day using the Bristol Stool Form Scale (see below). We will score it as the percentage of days (over 14 days) with a normal stool (defined as a stool with a score of 3-5 on the Bristol Stool Form Scale; abnormal scores are1-2 for very hard stools, and 6-7 for soft/liquid stools). Days without a bowel movement, or with 3 or more bowel movements, will be counted as an abnormal day. Days requiring a GI medication/treatment, such as a laxative or enema, will also be counted as an abnormal day. Days with significant abdominal pain would also be counted as an abnormal day.

The reason for this scale is that most PTHS patients have constipation, but some have diarrhea, and some alternate between the two. This simple form allows us to describe either type of problem as an "abnormal" day, and hence use a single number to describe constipation and/or diarrhea symptoms.

Subscales will include the percentage of days of types 1-2 (constipation), types 6-7 (diarrhea), days with no stool, days with 3 or more stools, days requiring GI medication/treatment, and days with significant abdominal pain.

In our Phase 1 study for autism (Kang 2017), we found that the DSR was easy for participants to understand and comply with, and it was able to detect significant changes due to MTT treatment.

THE BRISTOL STOOL FORM SCALE		
Type 1		Separate hard lumps, like nuts
Type 2		Sausage-like but lumpy
Type 3		Like a sausage but with cracks in the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces

Clinical Global Impression (CGI)

The CGI is a widely used tool for assessing severity of symptoms, and changes after treatment. We will use the CGI- Severity Scale (CGI-S) at baseline, and after treatment we will use the CGI-S, CGI-Improvement (CGI-I), and CGI-Efficacy. The study physician will conduct all the CGI evaluations.

We will conduct two types of CGI evaluations. One evaluation will focus on GI symptoms (CGI-GI), and one will focus on PTHS-related symptoms (CGI-PH) not including GI symptoms. The rationale is that we hope that MTT will result in substantial improvements in GI symptoms, but it is unclear if it will have any effect on PTHS symptoms. Since GI symptoms are distinct from PTHS symptoms, we think it is best to separate the two by using two different assessments by the study physician.

Parent Global Impressions – Pitt Hopkins (PGI-PTHS)

There is no validated or unvalidated instrument for assessing symptoms in PTHS patients, as this is the first treatment study for PTHS. Therefore, we adapted the Parent Global Impressions of Autism – Revised (PGI-R3) for patients with PTHS. The PGI-R3 includes18 different

symptoms, and changes in symptoms are rated on a 7-point scale from much worse (-3) to zero (no change) to much better (+3). We used this scale for our Phase 1 study of MTT for ASD, and found it was very sensitivity to change. Therefore, we decided to use the PGI-R3 as an initial basis for the PGI-PTHS, since all of the original 18 items on the PGI-R3 have also been observed in patients with PTHS. Next, we added the following 9 items that are common in PTHS patients, based on a discussion with the Pitt Hopkins Foundation:

1. Hyperventilation
2. Daytime Apnea
3. Gross Motor Skills
4. Fine Motor Skills
5. Ataxia (lack of communication between brain and body)
6. Food allergies
7. Environmental allergies
8. Flushing
9. Rashes

An advantage of this scale is that it is quick to assess (10 minutes), so parents/guardians will use it frequently during the study to gather a quick assessment on possible changes in any PTHS-related symptoms.

Gastrointestinal Symptom Rating Scale (GSRS)

The GSRS is an assessment of GI symptoms based on 15 questions, which are then scored in five domains: abdominal pain, reflux, indigestion, diarrhea, and constipation. We use the GSRS-Likert, which rates each symptom on a 7-point scale. The GSRS is somewhat limited in that the scale is qualitative, but we think it provides good coverage of GI symptoms common in PTHS patients. Since it is not validated for PTHS patients, we are using it as a secondary outcome. In our Phase 1 study for children with ASD we found it to be very sensitive to change.

Revised Face Legs Activity Crying Consolability Pain Questionnaire for Children with Cognitive Impairment (FLACC)

The original FLACC was slightly revised for children with cognitive impairment (Malviya et al., 2006) by modifying the descriptions of some items. It assesses pain in five areas (Face Legs Activity Crying Consolability) using a three-point scale for each area, resulting in total FLACC pain scale ranging from zero to 10. The Interrater reliability has high intraclass correlation coefficients (ICC, ranging from 0.76 to 0.90) and adequate kappa statistics (0.44-0.57). The criterion validity is supported by good correlations between FLACC, parent, and child scores (rho = 0.65-0.87; $P < 0.001$). The construct validity was demonstrated by a significant decrease in FLACC scores following analgesic administration (6.1 +/- 2.6 vs 1.9 +/- 2.7; $P < 0.001$) in a small study. So, we believe it will be useful for assessing pain in children with PTHS.

Diet

The diet evaluation involves an estimate of the frequency of foods consumed during the last week, using the Block Brief 2000 Food Frequency Questionnaire from Nutrition Quest (child). Analysis of the form provides an estimate of both macro and micro nutrient intake. This

information is helpful in interpreting results of microbiome measurements. The diet form will be repeated at the end of the study, to determine if the treatment also results in changes in diet.

PTHS Registry

The Pitt Hopkins Research Foundation established a registry which collects a detailed 14-page medical history on people with PTHS. Participants will fill out that form prior to starting the study, and send us a copy to provide our study physician with a detailed understanding of their medical history.

Medical History

This form supplements the PTHS Registry form. It includes medical history focused on onset and history of PTHS and GI symptoms, other medical conditions, recent treatments, current medications/supplements, special diets, and level of exercise.

Clinical Diagnostic Criteria for PTHS

We will evaluate the clinical diagnostic criteria for PTHS for each participant using Table 2 of the Clinical Diagnostic Criteria from the International Consensus Report (Zollino et al 2019). This will only be used for informational purposes, as the verification of PTHS diagnosis will be done by review of previous genetic testing of the study participants.

Occupational Therapy (OT) Evaluation

At baseline (Day 0), an occupational therapist will conduct an occupational therapy evaluation including physical, sensory, and functional assessments such as posture, reaching, grasping, playing, sensory responses, and social/emotional behaviors. She will conduct an assessment of the autonomic nerve system (ANS) with a sensor that measures heart rate variability, skin conductance, and surface temperature. At the follow-ups, there will be a videocall discussion with the participant and their parents for approximately 15 minutes to determine if there have been any changes after treatment.

Microbiome Assessments

We will conduct microbiome assessments using the same approach as in our Phase 1 Study for ASD (Kang et al 2017). Specifically, we will first assess the overall diversity of gut bacteria at baseline (before MTT treatment) and investigate how it changes after the treatment, using a non-phylogenetic metric, Observed OTUs and a phylogenetic distance (PD) index. Next, we will collect individual bacterial profiles at baseline to identify specific bacteria significantly different between PTHS and controls (previously collected). We will also determine if there are significant changes in levels of either beneficial or harmful bacteria after MTT treatment. Finally, we will assess similarity of the gut microbiome of the recipients to that of their donor, to determine if there is successful engraftment.

Storage of biological samples

CBC & ChemPanel: these blood samples will be sent to LabCorp or similar lab immediately upon collection

Other blood samples, stool, urine, and buccal swab: these samples will be stored at -80^{o}C in a freezer in the laboratory of Prof. Krajmalnik-Brown, in the Biodesign Center at Arizona State University, until we are able to conduct the tests or send them to another lab for testing. Since we currently have funding for only some of the tests, it may be several years before funds are raised and samples can be tested. Each sample is stored with a subject code and date of collection – no other identifiers.

6.vii Safety Considerations and Monitoring

6.vii.a Methods to Assess Drug Safety

We will use several different approaches to monitor drug safety.

1) Adverse Effect Monitoring: Participants will be asked to report any adverse effects immediately if they occur. Any possible adverse effects will be followed up by research staff, and reported to the study physician if a possible adverse effect has been observed. Physicians will ask about possible adverse effects at each clinical visit. Research staff will check for possible adverse events in-between clinical visits (see section 6.iiii). Research staff will also review the PGI-PTHS and GSRS questionnaires to determine if there is any significant worsening of symptoms for any patients.

   For the primary outcome on safety, we will count the number of adverse effects (AE's) and determine if the treatment group has more AEs than the placebo group, both in terms of total AE's, and in terms of AE's for each severity category.

2) Vancomycin: Minor, temporary adverse behavior reactions (irritability and/or hyperactivity) were observed during the first few days of administration of vancomycin in both our Phase 1 study and the Sandler et al 2000 study (8 weeks of oral vancomycin for children with ASD) in 66% and 50% of participants with ASD, respectively. Therefore, participants will complete a brief Vancomycin Adverse Effect questionnaire daily during the vancomycin therapy, to determine if there were any adverse effects daily while taking the vancomycin. If adverse symptoms continue beyond that date, they will continue filling out the questionnaire until adverse symptoms stop. Similar to our Phase 1 study, this questionnaire will ask if there was any worsening of hyperactivity, irritability, or other adverse symptoms, and the severity of any symptoms, and the duration of any symptoms. If symptoms last longer than 2 weeks, we will continue to monitor them until they disappear, or until the end of Part 3 of the study.
3) For the primary outcome on safety, we will count the number of adverse effects (AE's) during vancomycin therapy, and determine if the treatment group has more AEs than the placebo group, both in terms of total AE's, and in terms of AE's for each severity category.

Microbiota Transplant Adverse Effect form: For the first seven days of microbiota transplant, participants will complete a daily questionnaire which includes questions on body temperature (to check for a fever) and changes in severity of any symptoms including abdominal pain, vomiting, diarrhea, constipation, bloating, flatulence, hyperactivity, irritability, and any other symptom.

For the primary outcome on safety, we will count the number of adverse effects (AE's) during microbiota transplant, and determine if the treatment group has more AEs than the placebo group, both in terms of total AE's, and in terms of AE's for each severity category.

4) Blood Tests: Complete Blood Count with differential (CBC) and a comprehensive blood metabolic panel including kidney and liver function will be assessed at baseline, the end of treatment, and at 2 and 12 months post-treatment. Since oral vancomycin and FM are generally well-tolerated, we believe this frequency is sufficient. However, if there is a worsening of symptoms the study physician may request additional measurements of CBC, chemistry panel, and/or other tests.

   For the primary outcome on safety, we will determine if the treatment group has any significant changes in any of the blood tests compared to baseline. Also, for each test, we will count the number of participants who have a test result outside the reference range for that test, and determine if the treatment group has more abnormal results than the placebo group, both for each test individually and also for a total of all of the tests.

5) Microbiome assessments: We will assess changes in the microbiome, and for safety purposes we will focus on decreases in bacterial diversity (associated with a worsening of GI health) and increases in any known pathogens (such as *C. Difficile*). These analyses require much more time (many weeks) than the other three assessments, but at the end of the study they will provide insight into whether or not there were any concerning changes in the microbiome of some individuals.

   For the primary outcome on safety, we will determine if the treatment group has a decrease (worsening) in bacterial diversity compared to the placebo group. We will also determine if the treatment group has an increase in levels of any known pathogen compared to the placebo group.

6.vii.b Adverse event monitoring

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital

anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All adverse events, regardless of severity, will be monitored for and reported to the FDA.

1.1.1.1.1.1.1.1 Participants and their Evaluators will be trained to contact the Study Coordinator if they experience any adverse events.

The severity of an adverse event will be determined using the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (available at http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf). Special focus will be given to symptoms relating to gastrointestinal symptoms, infections, and neurological conditions, but any other symptoms will also be evaluated for severity.

Reporting Procedures for all Adverse Events

Unexpected and related fatal or life-threatening events must be reported to the FDA by telephone or FAX within seven (7) calendar days. The FDA Medwatch 3500A mandatory form will be completed and submitted (www.fda.gov/medwatch).

We will send a written report to FDA within 15 calendar days for any serious unexpected adverse event considered related to study drug including those which have already been reported under the 7-day rule).

All adverse events occurring during the study, regardless of severity, and whether or not attributed to study drug, will be included in the investigator's annual IND report to FDA.

We will use a standardized form for evaluating and reporting any withdrawal from the study (see Appendix).

Criteria for Determining Causal Relationship between Adverse Events and Study Treatment.

It will generally be assumed that changes in gastrointestinal symptoms, PTHS symptoms, or neurological symptoms are due to the study treatment, unless another explanation is clearly present.

Adverse Events involving other symptoms will be assumed to be possibly due to the study treatment unless another cause is known. Based on our clinical experience, the type of event, and the temporal occurrence of the event, we will attempt to rate all Adverse Events as to their likelihood of being related to the study treatment, using a scale of unrelated, possibly related, probably related, or definitely related.

The following criteria will be used to assess the relationship of the Adverse Event to the study treatment:

□ Unrelated: the adverse event is clearly not related to the administration of the study treatment. Another cause of the event is most plausible, and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study treatment; and/or a causal relationship is considered biologically implausible;

□ Possibly related: Another cause of the event is plausible; however, a causal relationship is considered clinically/biologically plausible and there is a plausible temporal sequence between onset of the adverse event and administration of the study treatment.

□ Probably related: Another cause of the event is less likely, and a causal relationship is clinically/biologically plausible and there is a plausible temporal sequence between onset of the adverse event and administration of the study treatment.

□ Definitely related: the adverse event is temporally related to the administration of the study treatment, a causal relationship is considered biologically plausible, and there is no alternative cause that provides a more likely explanation.

In addition, we will compare the reports of adverse events between the treatment and placebo groups in Part 1, to determine if there is a significant difference between the groups.

Long-Term Follow-Up of Adverse Events

For any Adverse Events and Serious Adverse Events that occur, they will be followed by the study physician on at least a bi-weekly basis for the first 2 months, and then at least monthly for up to 6 months, or until the symptoms disappear, whichever occurs first. Phone follow-up may be used for some contacts, and the study coordinator may help with communication between the family and the study physician. These events will be reported to the Safety Monitoring Board.

6.vii.c Withdrawal from the Study

Reasons for Withdrawal

A study subject will be discontinued from further study agent/interventions in the study for:

- Completion of the study;
- Request by subject's parent/legal guardian to terminate participation;
- Requirement for prohibited concomitant medication or treatment;
- Unable to comply with requirements of the protocol;
- Lost to follow-up;
- At the request of the IRB;
- The subject's well-being, based on the opinion of the study physician or the Safety Monitoring Board.

- We will discontinue administration of the study product in any subject that develops a Grade 3 or higher adverse event or serious adverse event that is assessed as possibly, probably or definitely related to the study product.

Subjects who are discontinued from further study agent/interventions will be followed for safety until completion of the normal visit schedule.

Handling of Withdrawal

Subjects will be encouraged to complete the study; however, they may voluntarily withdraw at any time. The sponsor-investigator will provide a written explanation of the reason for withdrawal in a source document and the reason will be recorded on a case report form. Subjects will be asked for permission to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any Adverse Event resolve or the subject's condition becomes stable. Subjects who drop out of the study will not be replaced.

6.vii.d Study-Wide Halting Criteria

If there is a major safety concern that is study-wide, enrollment and dosing for the entire study may be halted. Possible criteria for stopping all enrollment and dosing pending review of safety data include:

a. Any serious adverse event assessed as at least possibly related to study treatment; and;

b. Any infection with an organism acquired from the Fecal Microbiota product.

c. Two or more subjects develop the same or similar Grade 3 adverse event that is considered related to the study product

d. An anaphylactic reaction that is considered possibly, probably, or definitely related to the study product.

The decision to halt enrollment and dosing study-wide may be made by the PI, the study physicians, the Data Safety Monitoring Board, the Research Monitor, the IRB, or the FDA.

6.vii.e Data Safety Monitoring Board

The primary goal of the DMSB is to review the data from the study, with a focus on data related to participants' safety.

We will use an external Data Safety Monitoring Board (DSMB) consisting of two physicians and one biostatistician: Dr. Alexander Khoruts (adult gastroenterologist at University of Minnesota who is experienced with FMT and helped develop the FM product we are using), Dr. Jessica Mitchell (naturopathic physician who is leading our study of MTT for adults with autism), and Prof. Juergen Hahn (chair of bioengineering at Rensselaer Polytechnic Institute, and experienced with biostatistics and with FMT research).

The DSMB will meet at least 1x prior to enrollment of subjects to review safety criteria, and then at least once every three months while patients are enrolled and being treated, until all treatments are completed. They will also meet at least once every six months while participants are in the follow-up phase (Part 3). The DSMB, the study physicians or the PI may call additional meetings if there is a safety concern, or if a Serious Adverse Event is reported.

The DSMB will review the lab safety tests and a summary of evaluations on GI and PTHS symptoms at each meeting. If any participant has a significant worsening of their GI or PTHS symptoms, the study physicians will report that to the DSMB. All Adverse Events will be reported to the Safety Board. If there is a Serious Adverse Event then they will report it to the Safety Board and the IRB within 24 hours.

The DSMB will have the right to stop treatment of an individual, or to order a halt to the entire study, by majority vote.

6.vii.f Site Monitor

The site monitor will perform oversight functions regarding recruitment, enrollment procedures, and the consent process for participants; oversee study interventions and interactions; review monitoring plans; and oversee data matching, data collection, and analysis; and report their observations and findings to the Study Sponsor (who will then notify the IRB and FDA if needed).

The Site Monitoring will be done by FDA Connections which is a 3rd party FDA consulting alliance specializing in analytical, clinical, regulatory, and compliance affairs specific to FDA (GCP/GMP/QSR/ISO) requirements in the Pharmaceutical, Medical Device, and Biologic industries. They are located 3104 E. Camelback Road, Suite 571, Phoenix, Arizona 85016 and their website is www.FDAConnection.com. Their work will be performed in accordance with all applicable United States Food and Drug Administration (FDA) regulations, Good Clinical Practice (GCP) regulations, and International Committee for Harmonization (ICH) guidelines. These duties will include, but may not be limited to:

1. Site Monitoring Visits - these activities will include:

a) Site Visit Scheduling

b) Mailing of Visit Confirmation Letters

c) Review completed Case Report forms and compare with source data

d) Track status of data queries generated by data management personnel

e) Track Serious Adverse Events occurring during the conduct of clinical trial(s) to ensure appropriate reporting to Sponsor and Institutional Review Board (IRB).

f) Function as a communication liaison between site(s) and sponsor to expedite conduct of the clinical trial(s).

g) Prepare and submit Site Monitoring Visit reports

h) Ensure that all outstanding data queries have been satisfactorily resolved and documented.

i) Ensure that all Clinical Trial Material and/or supplies have been accurately accounted for and either returned or properly destroyed.

j) Ensure that all regulatory documents are complete and appropriately maintained by site personnel

The site visits will include:

One Site Initiation Visit (SIV)

One monitoring visit, at the end of Part 2 of the study.

Activities at site:

a. Source data verification

b. Queries / query resolution

c. Unblinded Pharmacy review

d. Regulatory documents review

e. Report & Follow-up Letter

f. Conference calls – as needed

6.viii Mechanisms in place to protect subjects' confidentiality

1) All study staff interacting with participants or with their identifiable info are required to complete the CITI training course, and sign a Confidentiality Statement, which reads:

> "As a researcher working on the above research study at Arizona State University, I understand that I must maintain the confidentiality of all information concerning research participants. This information includes, but is not limited to, all identifying information and research data of participants and all information accruing from any direct or indirect contact I may have with said participants. In order to maintain confidentiality, I hereby agree to refrain from discussing or disclosing any information regarding research participants, including information described without identifying information, to any individual who is not part of the above research study or in need of the information for the expressed purposes on the research program."

2) In order to maintain participant confidentiality, each participant will be assigned a code number, and their information will be stored by code number in our electronic database which is HIPPA certified. All study forms will use only the code number for the form. All of our computers are pass-word protected. Hard copies of data will be kept in a locked file cabinet in a locked office, accessible only by research team staff.

6-ix. – Temporary changes to study protocol due to Coronavirus/COVID-19

Due the disruption of travel and other activities due to the COVID-19 virus, we propose the following temporary changes be made to the study protocol on an "as-needed" basis as determined by the PI's and the study physician. These changes are being made to increase the safety of the participants.

1) Physical Exam: The physical exams will be done with the participant's personal physician, and he/she will be asked to report the results to the study physician Dr. Hellmers,who will also have telehealth consults with each participant at those times.
2) Blood draw: The blood draw can be done by a local laboratory. The blood draw will include our standard safety tests (comprehensive metabolic panel and complete blood count with differential). In addition, if possible the lab will also draw the extra blood samples for additional tests, and those samples will be shipped frozen to ASU.
3) There may be some delays in scheduling different evaluations and sample collections, so the schedule of events may change somewhat, but the treatment duration for each medication will remain unchanged.
4) The occupational therapy exam will be done via telehealth.

End of Protocol 1