Abbreviated Title: BSS Gut Microbiome

National Institutes of Health (NIH) Institutional Review Board (IRB) #: 001631

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Title: An exploratory study of the effect of bismuth subsalicylate on the gut microbiome and host response in healthy adults

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice (GCP) and the United States Code of Federal Regulations (CFR) titles applicable to clinical studies (45 CFR §46, 21 CFR §50, §56, §312, and/or §812).

NIH-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

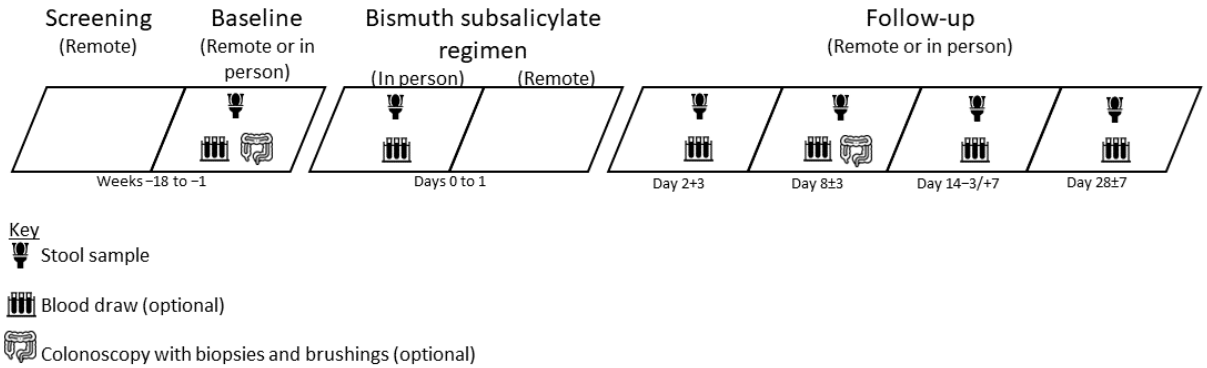
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	An exploratory study of the effect of bismuth subsalicylate on the gut microbiome and host response in healthy adults
Study Description:	This is a single-site, single-arm, open-label study to evaluate the effect of bismuth subsalicylate (BSS) on the human gut microbiome and host immune response. Upon confirmation of eligibility, healthy adult volunteers will provide stool and optional blood, saliva, urine, and intestinal biopsy samples for a baseline assessment of gut microbiome and host immune response. Up to 18 weeks later, participants will undergo a 2-day/8-dose regimen of oral BSS. Stool will be collected at baseline, days 2 (+3), 8 (±3), 14 (−3/+7) and 28 (±7). Blood, saliva, and urine are also optional at these time points. Participants may also undergo a second optional colonoscopy at day 8 (±3) to provide colon biopsies for research analysis.
Primary Objective:	To evaluate the effect of BSS on the human gut microbiome.
Secondary Objective:	To evaluate the effect of BSS on the human gut metabolome.
Tertiary/Exploratory Objective:	To evaluate the effect of BSS on the systemic and intestinal host response (immune and inflammatory responses).
Primary Endpoint:	Differences in the relative abundance of taxa in stool samples pre-BSS and approximately 1 month post-BSS. Differences in microbiome metrics of alpha diversity and beta diversity will also be assessed.
Secondary Endpoint:	Differences in the stool metabolome (including short chain fatty acids, bile acids, and untargeted metabolomics) pre-BSS and approximately 1 month post-BSS.
Tertiary/Exploratory Endpoint:	Differences in systemic host immune and inflammatory responses, such as cytokines and immune cells, and host intestinal immune responses, such as specific T-cell populations in intestinal biopsies pre-BSS and approximately 1 month post-BSS.
Study Population:	Healthy adult volunteers aged 18 to 50 years, n=20, accrual ceiling 40.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	NIH Clinical Center (CC), Bethesda, MD
Description of Study Intervention:	BSS oral suspension, self-administered at 4 doses of 1050 mg each per day (1 to 6 hours apart) for 2 days. This is within the recommended adult dose.
Study Duration:	3 years
Participant Duration:	Up to 22 weeks.

1.2 SCHEMA


Screening
(Remote)
Baseline
(Remote or in person)
Bismuth subsalicylate regimen
(In person)
(Remote)
Follow-up
(Remote or in person)
Weeks −18 to −1
Days 0 to 1
Day 2±3
Day 8±3
Day 14−3/+7
Day 28±7
Key
Stool sample
Blood draw (optional)
Colonoscopy with biopsies and brushings (optional)

1.3 SCHEDULE OF ACTIVITIES

	Screening	Baseline	Intervention	Follow-up			
	Week −18 to −1	Week −18 to Week −1[a]	Day 0	Day 2 (+3 days)	Day 8 (±3 days)	Day 14 (−3/+7 days)	Day 28 (±7 days)
Procedures	Remote	Remote or in-person	In-person	Remote or in-person	Remote or in-person	Remote or in-person	Remote or in-person
Informed consent	X[b]						
Physical exam			X				
Height			X				
Weight and vital signs			X				
Medical history and medication review	X[c]	X[d]	X	X	X	X	X
AE assessment			X	X	X	X	X
Provision of BSS			X				
Pregnancy test[e]		X[f]	X[g]		X[h]		
Stool collection		X[i]	X[i]	X	X[i]	X	X
Baseline questionnaire		X					
Survey (including diet)		X	X	X	X	X	X
Optional procedures							
Urine collection		X	X	X	X	X	X
Saliva collection		X	X	X	X	X	X
Colonoscopy with biopsies and brushings		X			X		
Chest X-ray		X[j]			X[j]		
GI consult		X[k]			X[k]		
Pre-anesthesia consult		X[k]			X[j]		
ECG		X[j]			X[j]		
Blood evaluations							
CBC/diff		X[k]			X[j]		
Acute care, hepatic, mineral panels		X[k]			X[j]		
PT/PTT/INR		X[k]			X[j]		
Platelet function assay		X[k]			X[j]		
Research blood draw[l]		X	X	X	X	X	X

	Screening	Baseline	Intervention	Follow-up			
	Week −18 to −1	Week −18 to Week −1[a]	Day 0	Day 2 (+3 days)	Day 8 (±3 days)	Day 14 (−3/+7 days)	Day 28 (±7 days)
Procedures	Remote	Remote or in-person	In-person	Remote or in-person	Remote or in-person	Remote or in-person	Remote or in-person

AE, adverse event; BSS, bismuth subsalicylate; CBC/diff, complete blood count with differential; ECG, electrocardiogram; GI, gastrointestinal; PT/PTT/INR, prothrombin time, partial thromboplastin time, and international normalized ratio; X, to be performed.

a Baseline will be done after confirmation of eligibility. The overlap between the screening and baseline windows accounts for the required 30-day interval between the optional colonoscopies at baseline and day 8. Participants who do not undergo colonoscopy may have a shorter interval between screening, baseline, and day 0.

b The informed consent form will be signed before any research procedures are conducted on this study.

c Limited review of existing medical records may be done before the participant signs the informed consent form.

d Continued eligibility will be confirmed at the baseline visit.

e For participants of childbearing potential only. If the visit will be done remotely, then the participant will be instructed to send a urine sample to the study team, which will be used for pregnancy testing.

f For participants who will have the baseline colonoscopy, the timing of baseline pregnancy test will be per Department of Perioperative Medicine guidelines.

g To avoid repetition of procedures, pregnancy test will only be done at day 0 if baseline was more than 14 days before.

h Pregnancy test will only be done at day 8 if the participant will undergo the colonoscopy.

i Day 0 stool, which will be collected before first dose of BSS, may be collected up to 7 days earlier. For participants undergoing colonoscopy, stool samples will be attempted to be collected prior to the start of the bowel preparation.

j These procedures will only be done if the participant is undergoing the colonoscopy and the procedures are determined to be needed per the Department of Perioperative Medicine guidelines at the NIH Clinical Center. These labs will be repeated for the second (day 8) colonoscopy per the Department of Perioperative Medicine and if there are more than 30 days between it and the first procedure. For the convenience of the participant and study team, blood for the second colonoscopy may be drawn at the day 0 or 2 visits (the latter only if in person at the NIH Clinical Center).

k These procedures will only be done if the participant is undergoing the colonoscopy.

l Research blood draw is optional and will only be offered at in-person visits.

2 INTRODUCTION

2.1 STUDY RATIONALE

BSS is a commonly used, widely available, over-the-counter (OTC) medication for a variety of gastrointestinal (GI) symptoms. Side effects are generally rare and short-lived. Our laboratory has recently shown in murine models that BSS, in doses scaled down to mice from recommended adult dosing, has a profound effect on the gut microbiome, with a depletion of *Lactobacillus* and increase in *Enterococcus.* This treatment also impacted the host immune response, including a decrease in CD4+ T cells in the small intestine. These mice were more susceptible to the pathogenic effects of a subsequent *Salmonella* infection (unpublished data, manuscript in preparation). The effect of BSS alone on the human gut microbiome has not previously been investigated. Given our recent findings in murine models and the known critical role of the host-microbiome response in human health and disease, this is an important area to study in humans as BSS is commonly used. Therefore, we propose to assess whether the effect of BSS seen on the gut microbiome and host response in murine models is also seen in healthy human volunteers. If an effect is seen, this lays the foundation for further studies to assess the clinical impact and potential health outcomes of these changes in humans.

2.2 BACKGROUND

2.2.1 Bismuth Subsalicylate: Clinical Use

BSS is a commonly used, widely available, OTC medication for a variety of GI symptoms. It is available in the generic form, but also under the more commonly known brands: Bismatrol, Diotame, Geri-Pectate, Kao-Tin, Peptic Relief, Pepto-Bismol, Pink Bismuth, and Stomach Relief. It received approval by the US Food and Drug Administration (FDA) in 1939. It is approved for the following indications in adults and children 12 years and older: 1) relief of diarrhea; 2) relief of upset stomach, including belching, fullness, gas, indigestion, heartburn, and nausea, and 3) relief of travelers' diarrhea. It is also used "off-label" for *Helicobacter pylori* eradication and prophylaxis against travelers' diarrhea. There are typically few adverse reactions when taken in the recommended dose (section 2.3.1).

Clinical trials have shown that BSS is effective in situations where patients are experiencing mild GI discomfort, as it reduces the severity and incidence of flatulence and diarrhea.(1) In comparison to a placebo, BSS was able to provide greater and faster relief in patients with indigestion.(2) BSS also demonstrated effectiveness in the acute treatment of traveler's diarrhea in patients with mild symptoms and was found to be superior to a placebo.(3, 4) This drug can be found OTC; as such, it has become a preferred self-treatment option for mild diarrhea, often replacing the need for an antimicrobial in traveler's diarrhea.(5, 6) As this medication is sold without the need for a prescription, the true usage in the USA is difficult to accurately estimate.

2.2.2 Bismuth Subsalicylate: Mechanism of Action

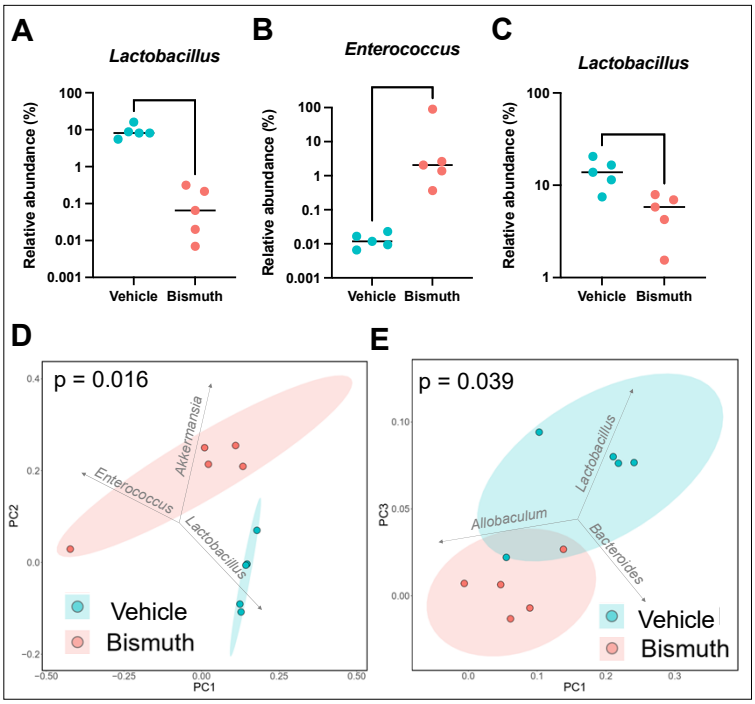
The mechanism of action of BSS is not fully understood. BSS exhibits both antisecretory and antimicrobial actions. In the stomach, it hydrolyzes into two compounds: bismuth and salicylic acid. The salicylate compound is almost completely absorbed into the bloodstream, while bismuth salt is minimally absorbed.(7) The bismuth salts contain bactericidal and antimicrobial activity, and prevent bacteria from binding and growing on the mucosal cells. Additionally, antisecretory effects are most likely due to 1) the reduction in prostaglandin formation, as BSS

inhibits cyclooxygenase (prostaglandin induces inflammation and hypermotility) and 2) the stimulation of reabsorption of fluids, sodium, and chloride (this action helps decrease fluid loss).(8) Importantly for this current study, BSS also sequesters local gut sulfides, with sulfides being produced by host cells and microbes.(9) Sulfide, an inhibitor of cellular respiration, is important for resistance to host colonization by pathogens.(9)

2.2.3 Bismuth Subsalicylate: Effect on the Gut Microbiome and Host Response in Murine Models

LHIM recently described for the first time that BSS affected the gut microbiome of mice through the mechanism of sequestering local gut sulfides, with a large increase in *Enterococcus*, after treatment with a high-dose regimen.(9) Building on this work, we then designed another mouse model of BSS treatment with doses scaled down to a mouse volume from those recommended in humans (rather than higher dose). Mice were gavaged with 5 mg of BSS in a 200-µL solution every 12 hours for 72 hours. This treatment substantially altered the microbiome composition. One day after the end of treatment, we noted a 2-log decrease in *Lactobacillus* genus abundance by 16S reads, a 3-log increase in *Enterococcus* abundance (Figure 1A-B), and a 1-log increase in *Akkermansia* abundance. Longitudinally, we observed alterations in the microbiome out to 30 days post treatment, with significant differences persisting for *Lactobacillus* (Figure 1C) (unpublished data, manuscript in preparation). Beta diversity (weighted unifrac distance) of control and treated animals are shown in Figure 1D-E for 1 and 30 days after the end of treatment, respectively.

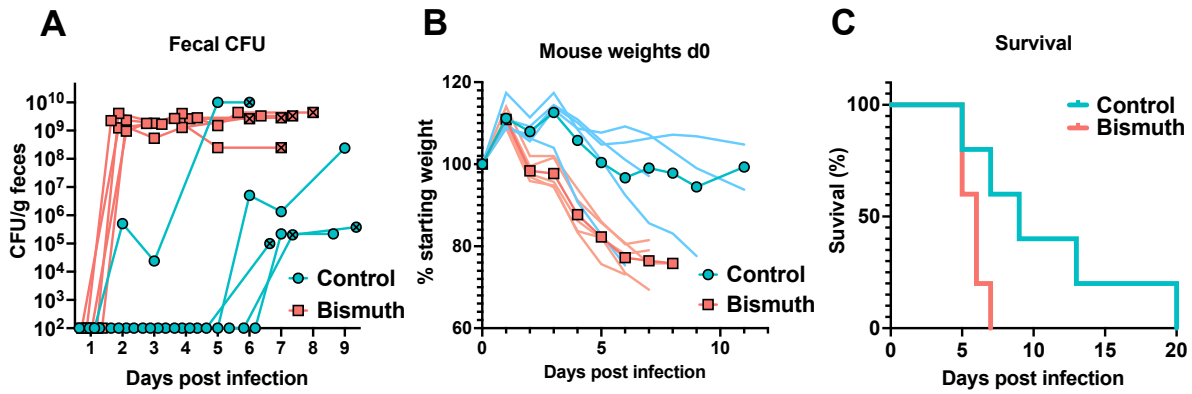
Figure 1: Changes in mouse fecal microbiome after bismuth subsalicylate treatment



We also analyzed local immunity in the gut and observed significant changes in the distal small intestine. There was a significant 3-fold decrease in CD4+ T cells in the small intestine lamina propria at 1 week after treatment. This was most acute in the ileum, where we noted 10-fold

fewer CD4+ T cells in this section of the gut. However, we observed no significant differences in the colon of these mice. These alterations were not detectable at 2 weeks post-treatment. Importantly, mice who were treated with BSS compared to controls were more susceptible to the pathogenic effects of *Salmonella* infection with an increased *Salmonella* load found in the feces, increased weight loss, and decreased survival (Figure 2).

Figure 2: The effect of *Salmonella* infection after bismuth subsalicylate treatment



Mice treated with 5-mg bismuth subsalicylate once every 12 hours for 3 days by gavage and were orally infected with 10^5 colony-forming units (CFU) *Salmonella* Typhimurium 1 day after end of treatment. Mice were assessed for fecal CFU (A) weight loss (B) and survival (C) each day post-infection.

2.2.4 Bismuth Subsalicylate: Effect on the Gut Microbiome and Host Response in Humans

To our knowledge, there are no studies that have investigated the impact of BSS on the human gut microbiome. There are a few studies using BSS in combination with an antibiotic regimen, but the impact of the antibiotics alone likely prevents interpretation of this data.(10, 11) Additionally, to our knowledge, no other studies have assessed immunity after BSS treatment, with the exception of one study investigating the response of bismuth on humoral immunity after vaccination.(12)

Given the findings in our murine models of the impact of BSS on the gut microbiome, host immunity, and susceptibility to subsequent infections, it is now important to study this in humans where BSS is widely used. It is known that in humans, host-microbiome responses are critical in health and disease.(13, 14) Moreover, dysbiosis or imbalance of the gut microbiome is implicated as a factor in the pathogenesis of numerous disease states, including but not limited to infection and responses to infection (such as *Clostridioides difficil*e and COVID-19), inflammatory diseases (such as autoimmunity, inflammatory bowel disease, and atopic and cardiovascular diseases), metabolic diseases (such as obesity, type 2 diabetes, and metabolic syndrome), neurologic diseases (including autism and depression), and cancers and responses to cancer treatments.(15-21) Therefore, we aim to assess whether the profound effect of BSS seen on the gut microbiome and host response in murine models is also seen in humans. If an effect is seen, this lays the foundation for further studies to assess the clinical impact and potential health outcomes of these changes in humans.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

Bismuth subsalicylate: There are no adverse reactions listed in the manufacturer's labeling. The following adverse reactions have been reported in post-marketing and clinical trials of BSS for different indications:

- Temporary darkening/discoloration of the tongue and stool that resolves after discontinuation of the medication.(22) The exact frequency of this is unknown, but it is common enough to be listed on the Pepto-Bismol website.(23)
- Anaphylaxis (extremely rare, one case report).(24)
- Acute esophageal necrosis (extremely rare, one case report with chronic overuse of BSS).(25)
- Staining of teeth (rare and with chronic overuse of BSS).(26)
- Neurotoxicity and encephalopathy (extremely rare with chronic overuse of BSS).(26)
- Tinnitus (extremely rare with chronic overuse of BSS).(26)
- Salicylate toxicity in those who have taken BSS inappropriately, whether through an overdose or for extended periods of time.(27) Symptoms may include impaired cognition, tremors, lethargy, somnolence, insomnia, delirium, myoclonus, seizures, depressed mood, anxiety, and death.
- Other warnings/precautions: bismuth absorbs X-rays and may interfere with diagnostic procedures of GI tract.

In addition, some study participants have reported nausea while taking BSS.

Given the study hypothesis and the observations in BSS-treated mice, there is the possibility that BSS will affect the gut microbiome and immune response of participants. The magnitude and duration of such effects and their clinical significance, if they occur, are unknown.

There are potential interactions between BSS and other drugs, so use of these drugs will be prohibited during the study (sections 5.2 and 6.4).

Overall, the risks of BSS in the dose taken in this study are mild and temporary (darkening/discoloration of the tongue and stool) or extremely rare. Risks will be minimized by using the recommended dosing for 2 days only (no excessive or chronic dosing), excluding anyone with an allergy to BSS or other salicylates, and excluding those on medications that may have drug interactions with BSS.

Venipuncture: The risks of drawing blood include pain, bruising, bleeding, fainting, and, rarely, infection. The amount of blood drawn for research purposes will be within the limits allowed for adult research participants by the NIH CC (Medical Administrative Policy 95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf).

Colonoscopy with biopsies and brushings: Serious risks of colonoscopy with biopsy and brushings include perforation, severe bleeding, and serious infection. These risks are rare. The risk of perforation is increased in people who have had perforation in the past, so people with a history of GI perforation will be excluded from colonoscopy. In addition, blood will be collected

before the procedure for a platelet function assay (PFA), so participants with prolonged bleed times will also not undergo colonoscopy.

More common risks of colonoscopy include abdominal discomfort, bloating, transient fever, diarrhea, and constipation. Preparation for colonoscopy may cause dehydration.

The procedure will be performed by an experienced physician gastroenterologist who specializes in GI diseases and is experienced with endoscopy. As a general practice, the risk of the procedure is minimized by monitoring pulse, blood pressure, and oxygen saturation throughout the procedure and by providing inpatient monitoring after the procedure. Any complication will be identified as soon as possible and treated appropriately.

Anesthesia: Participants will receive conscious sedation or general anesthesia for colonoscopy, depending on the preference of the endoscopist. Sedative drugs will be provided by the Critical Care Medicine Department and/or experienced anesthesiologists of the NIH CC.

Side effects from sedative medications may include cardiovascular and respiratory depression, bradycardia, hypotension, respiratory acidosis, apnea, and hypersensitivity reactions. Stinging or pain may occur at the injection site.

Side effects from conscious sedation include post-procedure drowsiness, cardiovascular and respiratory depression, hiccoughs, headache, and nausea.

Side effects from general anesthesia may include sore throat, hoarseness, dry mouth, muscle aches, itching, shivering, hiccoughs, nausea, vomiting, post-procedure drowsiness, and headaches. Rare complications include cardiovascular and respiratory depression, bradycardia, hypotension, and hypersensitivity reactions, including anaphylaxis. General anesthesia may also cause heart attack or stroke.

Participants will be closely monitored with vital signs and hemodynamic monitoring, and AEs will be treated as appropriate. To minimize risks, experienced anesthesiologists will be used.

Chest X-ray: Participants who undergo colonoscopy may have a chest X-ray before the procedure. The radiation exposure of one chest X-ray is 0.01 rem, so participants who have both colonoscopies would be exposed to a total of 0.02 rem for the study. The risk of this exposure is too low to be reliably measured.

Electrocardiogram (ECG): Participants who undergo colonoscopy may have an ECG before the procedure. The electrode pads may cause some discomfort or a skin rash, but there are otherwise no risks with the ECG.

Stool, urine, saliva collection: There are no foreseeable risks with these procedures.

Discovery of previously unknown conditions: Colonoscopy may identify unexpected abnormal but medically relevant findings. Return of medically actionable incidental findings is described in section 8.2.2.3. Participants may experience stress or anxiety from learning these results.

Breach of confidentiality: Because this study involves collecting personally identifiable information about participants, there is a risk of breach of confidentiality. To minimize this risk, samples will be labeled with a study number without identifiers. Data will be stored in a secure manner, such as in locked cabinets or password-protected computer files. Protection of confidentiality is described in more detail in section 10.3.

2.3.2 Known Potential Benefits

Participants will not benefit from this study.

The information learned in this study may improve the investigators' understanding of the effects of BSS on the human gut microbiome and host immune response. In the future, this may inform clinical care of patients with GI conditions.

2.3.3 Assessment of Potential Risks and Benefits

This is an exploratory study to investigate if and how the common OTC GI medication BSS affects the adult human gut microbiome and immune response. Participants will follow the approved dosing instructions for only 2 days, and given the well-understood safety profile, serious side effects are not expected. The only biological sample required from participants is stool. Saliva, urine, and blood are optional. Participants may also undergo two colonoscopies for collection of GI biopsies, once at baseline and a second time during follow-up. These procedures are also optional, and precautions to ensure participant safety are described in section 2.3.1.

For convenience of the study team and participants and to reduce the need to travel, most study visits can be done remotely over the phone or via telehealth. Stool, saliva, and urine samples can be collected at home and shipped to the NIH using collection kits with prepaid shipping.

The alternative to participating in this clinical trial is not to participate.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the effect of BSS on the human gut microbiome.	Differences in the relative abundance of taxa in stool samples pre-BSS and approximately 1 month post-BSS. Differences in microbiome metrics of alpha diversity and beta diversity will also be assessed.	Hypothesis: there will be a decrease in beneficial bacterial taxa (eg, *Lactobacillus*) and increase in potential pathobionts (eg, *Enterococcus*). This will be most pronounced at day 2 (the day after therapy) and some changes may persist up to 1 month. This hypothesis is based on our findings in murine models (section 2.2.). These specific endpoints of relative abundance of taxa, alpha diversity, and beta diversity were chosen as they are standard metrics to assess changes in the microbiome.(28, 29)

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		
To evaluate the effect of BSS on the human gut metabolome.	Differences in the stool metabolome (including short chain fatty acids, bile acids, and untargeted metabolomics) pre-BSS and approximately 1 month post-BSS.	Hypothesis: there will be a change in specific stool metabolites (eg, a decrease in protective secondary bile acids and short chain fatty acids) with BSS therapy. This will correlate with gut microbiome changes. Stool metabolites are produced by gut microbiota and also contain host products. Importantly, they can assess the function of the microbiota, ie, "what are they doing" rather than just "what's there."(30) Moreover, bacterial metabolites have a key role in gut barrier function, host immunity, and inflammation, which is important in this study when assessing microbiome and host responses to BSS.(31) Targeted assays for metabolites of special interest, such as bile acids, short chain acids, and sulfides may be performed.(32, 33) Wide targeted or untargeted metabolite assays will also be performed to uncover other metabolites of relevance.
Tertiary/Exploratory		
To evaluate the effect of BSS on the systemic and intestinal host response (immune and inflammatory responses).	Differences in systemic host immune and inflammatory responses, such as cytokines and immune cells, and host intestinal immune responses, such as specific T-cell populations in intestinal biopsies pre-BSS and approximately 1 month post-BSS.	Hypothesis: there will be a change in the host immune/inflammatory response with BSS therapy, as seen in the murine models (section 2.2). For participants who have colonoscopies with biopsies before and after treatment, a decrease in CD4+ T cells in the small intestine lamina propria will be seen, as per murine models. These changes will correlate with microbiota changes found. Exploratory systemic immune and inflammatory profiling on blood, urine, and serum samples as applicable will also be performed via a multidisciplinary approach using techniques such as quantitative cytokine profiling and immune cell composition and function by flow cytometry.(34-36)

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is an exploratory, single-site, single-arm, open-label study of the effects of BSS on the human gut microbiome and host response. It is hypothesized that there will be a decrease in

beneficial bacterial taxa (eg, *Lactobacillus*) and increase in potential pathobionts (eg, *Enterococcus*) as seen in murine models (section 2.2).

Upon confirmation of eligibility, healthy adult volunteers (n=20) will undergo baseline assessment of gut microbiome and host response by providing a stool sample. Participants also have the option to provide blood, urine, and saliva, and to undergo colonoscopy for collection of biopsies and brushings. Approximately 1 to 18 weeks later, they will provide another stool sample (with optional blood, urine, and saliva), and then begin a brief regimen of BSS: 1050 mg 4 times daily (about 1 to 6 hours apart) for 2 days. Remote or in-person follow-up visits will be at days 2 (+3), 8 (±3), 14 (−3/+7), and 28 (±7). Participants will be assessed for AEs and provide stool samples, and have the option to provide blood, urine, and saliva. Participants may also have a second colonoscopy with collection of biopsies and brushings at the day 8 follow-up visit.

4.2 Scientific Rationale for Study Design

In our murine models, BSS caused a profound effect in the gut microbiome with subsequent changes in the host response. As there are no data on the effect of BSS on the human gut microbiome, an open-label pilot study is needed to gather preliminary data. The study design is strengthened by comparing the microbiome within a same individual before and after intervention as previously reported in other studies of the gut microbiome.(37) Moreover, in our murine models, longitudinal microbiome changes were seen within individual mice, in addition to between control and treatment groups. If an effect is seen, this lays the foundation for confirmation in a study with a randomized control design and further studies to assess the clinical impact and potential health outcomes of these changes in humans.

4.3 Justification for Dose

The BSS dose chosen for this study of 1050 mg 4 times daily (about 1 to 6 hours apart) for 2 days is the recommended maximum dosage of the medication. This dose was chosen as it was within the recommended dose range, to maximize the potential effect, and to avoid adverse effects of overdosing or chronic dosing.

5 STUDY POPULATION

5.1 Inclusion Criteria

An individual must meet all the following criteria to be eligible for this study:

1. Aged 18 to 50 years.
2. In generally good health.
3. Able to provide informed consent.
4. Willing to allow samples and data to be stored and shared for future research.
5. Participants who can become pregnant must agree to use one effective method of contraception when engaging in sexual activities that can result in pregnancy, beginning at the signing of the informed consent form (as early as week −18) until the final study visit. Acceptable methods of contraception include the following:
    a. External or internal condom with spermicide.
    b. Diaphragm or cervical cap with a spermicide.
    c. Hormonal contraception.
    d. Intrauterine device.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Use of systemic antibiotics in the last 3 months.
2. BSS use in the last 3 months.
3. Pregnant or breastfeeding.
4. Allergy to BSS.
5. Allergy to other salicylates (including aspirin).
6. Current use of other salicylates (including aspirin).
7. Current use of anticoagulant medications.
8. History of or active GI ulcers.
9. History of or active bleeding disorder.
10. Bloody stool within the last 3 months.
11. Diarrhea within the last 2 weeks (defined as three or more loose or liquid stools per day).
12. Current use of medications that may have a drug interaction with BSS (section 6.4).
13. Not proficient in written English.
14. Currently participating in another clinical trial that may affect current study procedures, per investigator's discretion.
15. Any condition that, in the opinion of the study team, contraindicates participation in this study.

Co-enrollment in other studies is restricted. Consideration for co-enrollment in clinical trials evaluating the use of a licensed medication will require the approval of the principal investigator. Study staff should be notified of co-enrollment on any other protocol as it may require the approval of the principal investigator.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Pregnant People, Fetuses or Neonates

BSS is not recommended for use during pregnancy, so pregnant people are excluded from this study. Pregnancy also may affect the gut microbiome, which would complicate comparisons with people who are not pregnant.

Neonates are excluded because of the risk posed by the study without corresponding benefit to the participant, and because of the differences between the gut microbiomes of neonates and adults.

5.3.2 Minors

Minors are excluded because of the risk posed by the study without corresponding benefit to the participant, and because of the differences between the gut microbiomes of minors and adults.

5.3.3 Adults Who Cannot Provide Informed Consent

Given the lack of benefit of study participation and the need to collect several biological samples, adults who are unable to consent are not eligible for enrollment in this protocol. Similarly, participants who lose the capacity to provide ongoing consent will be withdrawn from the study.

5.3.4 Adults Older than Age 50

The maximum age for participation is 50 years because older age is associated with perturbations of the microbiome. For this current study to assess the effect of BSS on the gut microbiome, a baseline microbiome with minimal factors that can cause perturbations is desired.(38)

5.3.5 Adults Who Are Not Proficient in Written English

The baseline questionnaire and follow-up surveys are only available in English and need to be completed for assessment of the study objectives. Thus, participants must be able to read and write in English to participate.

5.3.6 Participation of NIH Staff or Family of Study Team Members

NIH staff and family members of the study team may be enrolled in this study as these populations meet the study entry criteria. Neither participation nor refusal to participate in the research will have an effect, either beneficial or adverse, on the staff member's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the *Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* and the *Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3)*. Please see section 10.1.2 for consent of NIH staff and family members of the study team.

5.4 Lifestyle Considerations

Not applicable.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of any failed criterion may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.6 Strategies for Recruitment and Retention

This study is being conducted at a single site, the NIH CC. Participation of all racial and ethnic groups and genders will be actively encouraged. Recruitment will be supported by the Office of Patient Recruitment, which recruits participants for volunteer studies conducted at the NIH CC. IRB-approved flyers may also be posted for recruitment of participants.

Participants may be sent IRB-approved reminder emails, phone calls, and texts for administration and BSS, samples, surveys, and appointments.

5.6.1 Costs

There will be no costs to participants for participation in this study. Participants will be provided with collection kits with prepaid shipping for at-home stool, saliva, and urine collection, so there are no costs to the participant for those.

5.6.2 Compensation

Participants will be compensated as follows:

- $20 for return of each stool sample with accompanying survey (maximum 6 times).
- $20 for return of each set of saliva and urine samples (both required, maximum 6 times).
- $40 for in-person visit for first dose of BSS and completion of all 8 doses (maximum once).
- $30 per blood draw (maximum 6 times).
- $300 for first colonoscopy (maximum once).
- $500 for second colonoscopy (maximum once).

The minimum compensation (only stool samples and BSS doses) is $160, and the maximum compensation (including all optional procedures) is $1260. Payment will be provided as a gift card, check, debit card, direct deposit, or automated clearing house, given at the end of each visit (or upon receipt of send-in samples, as appropriate).

The NIH will cover some or all travel expenses related to participation in this study for participants who come to the NIH. The amount and form of these payments are determined by the NIAID Central Travel Policy for Research Protocol Participants. A copy of this policy will be provided to participants.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION ADMINISTRATION

6.1.1 Study Intervention Description

Use of BSS, which is lawfully marketed in the United States, is exempt from an investigational new drug application to the FDA in this study because its use meets the following criteria, per 21 CFR 312.2(b):

1. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
2. The investigation is not intended to support a significant change in the advertising for the drug.
3. The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug products.
4. The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR 56) and with the requirements for informed consent (21 CFR 50).

5. The investigation is conducted in compliance with the requirements of 21 CFR 312.7 (ie, the investigation is not intended to promote or commercialize the drug products).

6.1.2 Dosing and Administration

The oral suspension formulation of BSS will be used in this study. It is self-administered at 1050 mg 4 times per day (1 to 6 hours apart) for 2 days. The maximum dose in a 24-hour period is 4200 mg, which is consistent with the label.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

The study drug will be distributed and accounted for by the NIH pharmacy according to standard pharmacy procedures.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The oral suspension is provided in bottles. The bottle label will include the following:

- Name and address of the dispensing pharmacy.
- Serial number of the prescription.
- Date of the prescription.
- Name of the patient.
- Name and strength of the drug.
- Directions for use.
- Appropriate cautionary statements.
- Name of the prescriber.

6.2.3 Product Storage and Stability

This information will be provided on the bottle's label.

6.2.4 Preparation

Participants will be provided with a bottle of BSS and instructions for measuring the correct dose.

6.3 STUDY INTERVENTION COMPLIANCE

Compliance will be self-reported via the Research Electronic Data Capture (REDCap) system, email, or telephone.

6.4 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Clinical Research Information System of the NIAID (CRIMSON) are concomitant prescription medications, over-the-counter medications, and supplements.

Participants will be advised to avoid taking BSS during study participation outside the defined study regimen so the effects of the study regimen can be accurately evaluated.

The following drugs have potential interactions with BSS, and thus will be prohibited from use during the study:

- Agents with antiplatelet properties (eg, P2Y12 inhibitors, nonsteroidal anti-inflammatory drugs [NSAIDs], selective serotonin reuptake inhibitors): May enhance the adverse/toxic effect of salicylates. Increased risk of bleeding may result.
- Agents with blood glucose–lowering effects: salicylates may enhance the hypoglycemic effect of agents with blood glucose–lowering effects.
- Ajmaline: salicylates may enhance the adverse/toxic effect of ajmaline. Specifically, the risk for cholestasis may be increased.
- Ammonium chloride: may increase the serum concentration of salicylates.
- Angiotensin-converting enzyme inhibitors: salicylates may enhance the nephrotoxic effect of angiotensin-converting enzyme inhibitors. Salicylates may diminish the therapeutic effect of angiotensin-converting enzyme inhibitors.
- Anticoagulants: salicylates may enhance the anticoagulant effect of anticoagulants.
- Benzbromarone: salicylates may diminish the therapeutic effect of benzbromarone.
- Bismuth subcitrate: bismuth-containing compounds may enhance the neurotoxic effect of bismuth subcitrate.
- Carbonic anhydrase inhibitors: salicylates may enhance the adverse/toxic effect of carbonic anhydrase inhibitors. Salicylate toxicity might be enhanced by this same combination.
- Corticosteroids (systemic): salicylates may enhance the adverse/toxic effect of corticosteroids (systemic). These specifically include GI ulceration and bleeding. Corticosteroids (systemic) may decrease the serum concentration of salicylates.
- Dexketoprofen: salicylates may enhance the adverse/toxic effect of dexketoprofen. Dexketoprofen may diminish the therapeutic effect of salicylates. Salicylates may decrease the serum concentration of dexketoprofen.
- Ginkgo biloba: may enhance the anticoagulant effect of salicylates.
- Herbal products with anticoagulant/antiplatelet effects (eg, alfalfa, anise, bilberry): may enhance the adverse/toxic effect of salicylates.
- Hyaluronidase: salicylates may diminish the therapeutic effect of hyaluronidase
- Influenza virus vaccine (live/attenuated) within 2 weeks: may enhance the adverse/toxic effect of salicylates. Specifically, Reye's syndrome may develop.
- Loop diuretics: salicylates may diminish the diuretic effect of loop diuretics. Loop diuretics may increase the serum concentration of salicylates.
- Methotrexate: salicylates may increase the serum concentration of methotrexate.
- NSAIDs (nonselective): may enhance the adverse/toxic effect of salicylates. An increased risk of bleeding may be associated with use of this combination. NSAIDs (nonselective) may diminish the cardioprotective effect of salicylates. Salicylates may decrease the serum concentration of NSAIDs (nonselective).
- NSAIDs (topical): may enhance the adverse/toxic effect of salicylates. Specifically, the risk of GI toxicity is increased
- Potassium phosphate: may increase the serum concentration of salicylates
- Pralatrexate: salicylates may increase the serum concentration of pralatrexate. Salicylate doses used for prophylaxis of cardiovascular events are unlikely to be of concern.
- Probenecid: salicylates may diminish the therapeutic effect of probenecid.
- Salicylates: may enhance the anticoagulant effect of other salicylates.

- Sulfinpyrazone: salicylates may decrease the serum concentration of sulfinpyrazone.
- Tetracyclines: BSS may decrease the serum concentration of tetracyclines.
- Thrombolytic agents: salicylates may enhance the adverse/toxic effect of thrombolytic agents. An increased risk of bleeding may occur.
- Valproate products: salicylates may increase the serum concentration of valproate products.
- Varicella virus–containing vaccines within 2 weeks: salicylates may enhance the adverse/toxic effect of varicella virus–containing vaccines. Specifically, the risk for Reye's syndrome may increase.
- Vitamin K antagonists (eg, warfarin): salicylates may enhance the anticoagulant effect of vitamin K antagonists.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Study intervention may be discontinued for an individual participant (ie, pausing), or it may be discontinued for all participants and enrollment suspended (ie, halting). Pausing and halting rules and procedures are described below.

7.1.1 Pausing Rules for an Individual Participant

Pausing is the suspension of administration of study agent to a single participant until a decision is made whether to resume administration of the study agent.

The pausing criteria for a single participant in this study include any of the following:

- A participant experiences a grade 3 or higher AE that is unexpected and is possibly, probably, or definitely related to the study agent or study procedures.
- A participant experiences an SAE that is unexpected and is possibly, probably, or definitely related to the study agent or study procedures.
- Any safety issue that the investigator determines should pause administration of the study agent to a single participant.

The principal investigator will determine if study agent administration should be paused.

7.1.1.1 Reporting a Pause

If a pausing criterion is met, then a description of the AE(s) or safety issue must be reported to the NIH IRB per NIH Human Research Protection Program (HRPP) Policy 801.

7.1.1.2 Resumption of Study Agent After a Pause

The principal investigator will determine whether it is safe to resume administration of the study agent to the participant, and will notify the IRB of the decision on resumption of the study agent.

7.1.1.3 Discontinuation of Study Agent

A participant who does not resume study agent will continue to be followed for safety.

7.1.2 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all participants and suspension of enrollment until a decision is made whether to continue enrollment and study agent administration.

The halting rules are:

- Two or more participants experience the same or similar grade 3 or higher AEs that are unexpected and possibly, probably, or definitely related to the study agent or study procedures.
- Two or more participants experience the same or similar SAEs that are unexpected and possibly, probably, or definitely related to the study agent or study procedures.
- Any safety issue the investigators determine should halt the study.

The principal investigator will determine if the study should be halted. The IRB may halt the study at any time.

7.1.2.1 Reporting a Study Halt

If a halting rule is met, then a description of the AE(s) or safety issue must be reported to the IRB according to their requirements.

7.1.2.2 Resumption of a Halted Study

The principal investigator will determine if it is safe to resume the study, and will notify the IRB of the decision on resumption of the study.

7.1.2.3 Discontinuation of Study Agent

Participants who do not resume study agent will continue to be followed for safety.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

An individual participant will be withdrawn from the study for any of the following:

- An individual participant's decision. (The investigator should attempt to determine the reason for the participant's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the participant or to the integrity of the study data. For example, the participant does not complete the full BSS regimen.
- Participant is unable to provide ongoing informed consent.
- The participant becomes pregnant before day 0.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.

Participants who withdraw or are withdrawn from the study prior to day 14 will be replaced. If a participant is replaced, then all the data collected from that participant will still be included for the safety assessment.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if they fail to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the timeframe allowable by the study windows and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

The following minimal risk activities may be performed before the participant has signed the informed consent form:

- Email, written, in-person or telephone communications with prospective participants.
- Review of existing medical records, including but not limited to history and physical examination, laboratory studies, imaging (eg, X-ray or computed tomography), and diagnostic pathology specimens and reports.

8.2 STUDY EVALUATIONS & PROCEDURES

Targeted physical exam: The components of the exam will be at the discretion of the investigator. Weight and vital signs will be measured.

Baseline questionnaire: This will include questions regarding demographics, medical history, and medications. Participants may complete this questionnaire on paper or electronically via REDCap system.

Survey: A brief survey containing questions regarding diet and other factors than can affect the gut microbiome will be administered at each visit. Participants may complete this questionnaire on paper or electronically via REDCap.

Colonoscopy: Colonoscopy is an optional procedure for collection of intestinal biopsies and brushings. This procedure will be limited to those 30 years of age or younger. This is because the chance of incidental findings such as intestinal polyps are lower at this age.(39) If an incidental polyp was found, a polypectomy may need to be carried out at the discretion of the gastroenterologist performing the procedure, and this increases the risk of a colonoscopy.

Up to 20 research biopsies may be obtained from the colon and ileum. If a participant agrees to this procedure, then they will undergo it at baseline and the day 8 visit. Baseline has a window between weeks −18 and −1, so it will be scheduled relative to day 0 so there are at least 30 days between colonoscopy procedures. Colonoscopy preparation will be per standard process, and participants will be instructed accordingly. Before the first procedure to ensure participant safety,

participants will have a GI consultation, a pre-anesthesia consultation, clinical blood evaluations (section 8.2.1), and a pregnancy test (if of childbearing potential); an ECG and chest X-ray may be conducted if they are determined to be needed per the Department of Perioperative Medicine guidelines at the NIH CC. These procedures, except the pregnancy test, will only be repeated up to 8 days before the second procedure if there are more than 30 days between colonoscopies and if they are determined to be needed per the Department of Perioperative Medicine guidelines at the NIH CC. A pregnancy test will always be done before both colonoscopies for participants who can get pregnant. Biopsies and brushings will be collected for research purposes, as described in section 8.2.1.

8.2.1 Biospecimen Evaluations

Stool: Stool is the only required sample and will be collected at all study visits. For remote visits, participants will be provided with an at-home collection kit, which they can ship to the NIH. Participants may not be able to provide a stool sample at all visits. The minimum number of samples required for this study (ie, that does not constitute a protocol deviation, section 10.9) is 1 sample before starting the BSS regimen and 1 sample after completion of the regimen. Research evaluations on stool samples will include the following:

- Microbiome sequencing (including 16S ribosomal [r]RNA gene sequencing, shotgun metagenomic sequencing and evaluation of the resistome, virome, and parasitome).
- Metabolomics to evaluate intestinal metabolomic profiles, and when combined with microbiome/metagenomic studies, may suggest some functional role for candidate microbes of interest.
- Fecal flow (sorting and 16S and/or shotgun sequencing of immunoglobulin [Ig]A-bound organisms, which is an indication of pathogenicity).
- Lipocalin, calprotectin, and α-1-anti-trypsin levels (to evaluate mucosal inflammation and malabsorption).
- Measurement of secreted IgA and antimicrobial peptides.
- Culturing/polymerase chain reaction for specific organisms.
- Stool proteomics.
- Use of stool samples in murine models.
- Metatranscriptomics (the gene expression of the microbiota and host).
- Storage for future research.

Blood: Blood is an optional sample that will only be collected at in-person visits. Between 30 to 150 mL of blood will be collected per visit. The amount of blood drawn for research purposes will be within the limits allowed for adult research participants by the NIH CC (Medical Administrative Policy 95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf). Research evaluations on blood include the following:

- Host immune and inflammatory responses, and specific responses to the microbiome.
- Metabolomics.
- Lipopolysaccharide and lipopolysaccharide-binding protein (for evaluation of bacterial translocation and intestinal permeability).

- Isolation and cryopreservation of peripheral blood mononuclear cells and serum (for the creation of induced pluripotent stem [iPS] cells and future evaluation of systemic immune responses).
- Blood for RNA sequencing (seq) for evaluation of bacterial translocation and intestinal permeability, and host gene expression.
- Storage for future research.

Blood will be used for the following clinical evaluations only in those undergoing colonoscopy:

- Complete blood count with differential.
- Acute care, hepatic, and mineral panels.
- Coagulation studies: prothrombin time, partial thromboplastin time, international normalized ratio, PFA.

Urine: Urine is an optional sample. For remote visits, participants will be provided with an at-home collection kit, which they can ship to the NIH. Research evaluations on urine include the following:

- Metabolomics and trimethylamine N-oxide measurements.
- Microbiome sequencing (including 16S rRNA gene sequencing, shotgun metagenomic sequencing and evaluation of the resistome, virome, and parasitome).
- Immune and inflammatory responses (eg, using SomaLogic's SOMAscan assay).
- Oxalate level.
- Urinalysis.
- Storage for future research.

Saliva: Saliva is an optional sample. For remote visits, participants will be provided with an at-home collection kit, which they can ship to the NIH. Research evaluations on saliva include the following:

- Microbiome sequencing (including 16S rRNA gene sequencing, shotgun metagenomic sequencing and evaluation of the resistome, virome, and parasitome).
- Host immune and inflammatory responses.
- Metabolomics.
- Storage for future research.

Intestinal biopsies and brushings: As described in section 8.2, participants have the option to provide intestinal biopsies and brushings for the following research evaluations:

- Microbiome sequencing (including 16S rRNA gene sequencing, shotgun metagenomic sequencing and evaluation of the resistome, virome, and parasitome).
- Immunophenotyping.
- Immunohistochemistry and fluorescence in situ hybridization
- Generation of cell suspensions and sorting of cells for functional studies.
- Cell type–specific transcriptome and metabolomics analysis.
- Gene expression analysis (RNA-seq).
- Generation of iPS cells and organoids.
- Tissue fixing and stains for microscopy (eg, hematoxylin and eosin stain).
- Storage for future research.

8.2.2 Samples for Genetic/Genomic Analysis

8.2.2.1 Description of the Scope of Genetic/Genomic Analysis

Blood, stool, urine, and intestinal biopsy and brushing samples will be used for gene expression analysis (eg, RNA-seq and transcriptomics) and microbiome sequencing (eg, 16S rRNA gene sequencing and shotgun metagenomic sequencing, and potential evaluation of the resistome, virome, and parasitome).

These genetic analyses are for research purposes only and will not be validated in a laboratory certified under the Clinical Laboratory Improvement Amendments.

8.2.2.2 Description of How Privacy and Confidentiality of Medical Information/Biological Specimens Will Be Maximized

Confidentiality and privacy protections on this study, which includes genetic data, are described in section 10.3.

8.2.2.3 Management of Primary Results

Human sequences present in microbiome datasets will not be evaluated and will be removed prior to sharing in a repository. Therefore, there will be no genetic results to return to the participant.

8.2.2.4 Return of Secondary Genomic Research Results

Not applicable.

8.3 Safety and Other Assessments

Information collected from clinical and biospecimen evaluations (section 8.2) will be reviewed for ongoing safety assessments.

Any clinically relevant test results will be shared with the participant throughout the study. Results of research procedures or evaluations (including incidental findings) will be shared with participants if they are medically actionable. Such results will be discussed with the participant along with guidance for appropriate follow-up with their healthcare provider. Any research findings discovered beyond the completion of the primary research will not be returned.

Colonoscopic examination may lead to incidental discovery of lesions that require further study to rule out malignancy. Because this study is not designed for purposes of diagnosis or screening, the decision to perform follow-up examination or obtain biopsies of such lesions will be under the discretion of the principal investigator or the endoscopist. Biopsy-proven malignant or potentially malignant lesions will be immediately reported and discussed with the participant, and assistance for appropriate referral will be provided in a timely fashion. In addition, pathologic slides will be made available to an outside pathologist for review if requested in the future. Any suspicious-looking lesion where no biopsy is done will likewise be discussed with the participant for appropriate follow-up with the participant's healthcare provider.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event

AEs are any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening AE, 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Definition of Adverse Event of Special Interest

Adverse events of special interest (AESIs) are AEs that will be handled in a protocol/study-specific manner that differs from statutory and general rules for reporting. For the purposes of this protocol, AESIs are limited to any grade 2+ AEs probably or definitely related to gastrointestinal research biopsy collection.

8.4.4 Classification of an Adverse Event

8.4.4.1 Severity of Event

The investigator will grade the severity of each AE according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.1, July 2017 which can be found at https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf. Instructions provided in this toxicity table for estimating the severity grade for parameters not specifically identified in the table will be used for grading those events.

8.4.4.2 Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Potentially Related – There is some evidence to suggest a causal relationship (eg, the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (eg, the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (eg, the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (eg, the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.4.3 Expectedness

The investigators will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study drug/procedure.

8.4.5 Time Period and Frequency for Event Assessment and Follow-Up

At each contact with the participant, information regarding AEs will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include progress notes, laboratory reports, consult notes, phone call summaries, survey tools, and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and the principal investigator's judgment of the AE's relationship and expectedness to the study agent/intervention will also be recorded in CRIMSON.

AEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an AE (eg, the participant is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator in CRIMSON.

8.4.6 Adverse Event Reporting

AEs will be reported to the NIH IRB per NIH HRPP Policy 801.

8.4.7 Serious Adverse Event Reporting

SAEs will be reported to the NIH IRB per NIH HRPP Policy 801.

8.4.8 Adverse Events of Special Interest (Expedited Reporting)

Unless otherwise specified above, all AESIs must be reported to the NIAID Clinical Safety Office (CSO) as specified by the CSO (eg, Research Electronic Data Capture [REDCap] system; use the Safety Expedited Report Form [SERF]/email if REDCap is not available). If the preferred/indicated mechanism for reporting is not available, the CSO/medical monitor (MM)

should be contacted by telephone, fax, or other reasonable mechanism to avoid delays in reporting.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
Email: rchspsafety@mail.nih.gov
https://crimsonredcap.cc.nih.gov/redcap/index.php

Unless otherwise specified above, deaths and immediately life-threatening SAEs must be reported to the CSO/MM promptly, and no later than the **first business day** following the day of study personnel awareness.

All other SAEs must be reported to the CSO/MM no later than the **third business day** following the day of study personnel awareness.

8.4.9 Reporting of Pregnancy

Although pregnancy itself is not an AE, events occurring during pregnancy, delivery, or in the neonate (eg, congenital anomaly/birth defect) may be AEs or SAEs. We will assess pregnancy status at the baseline visit and before colonoscopy by urine pregnancy test in those who can become pregnant.

If a participant becomes pregnant before the first dose of BSS on day 0, then they will be withdrawn from the study (section 7.2). In the event of pregnancy after they start the BSS regimen, the following steps will be taken:

- Discontinue the study agent and procedures but continue to follow-up as scheduled for safety until the end of study participation to ensure no adverse effects have occurred.
- Report to the IRB.
- Advise research participant to notify the obstetrician of study participation and study agent exposure.

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a

greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report UPs to the NIH IRB as per HRPP Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

BSS will cause a change in the relative abundance of certain bacterial taxa approximately 1 month post-BSS compared to baseline (pre-BSS). We expect there to be a decrease in beneficial bacterial taxa (eg, *Lactobacillus*) and increase in potential pathobionts (eg, *Enterococcus*). This will be most pronounced at day 2 (the day after therapy) and some changes may persist up to 1 month. This hypothesis is based on our findings in murine models (section 2.2.).

9.2 Sample Size Determination

In our laboratory studies (section 2.2), on the day after BSS treatment, the average fold reduction of *Lactobacillus* reads pre- and post-BSS use was 1.57 and the standard deviation (SD) was 0.94. Given the possible larger individual variability and potential overall lower abundance of *Lactobacillus* in humans, for the range of SD between 0.5 to 3, Table 1 below gives the sample size and power calculation to detect a 2-log10 decrease of *Lactobacillus* reads pre- and post-BSS use for type one error of 0.05.

Table 1: Sample size and power calculation

SD	0.5	1	1.5	2	2.5	3
N=20	100%	99.9%	98%	87%	69%	54%
N=15	100%	99.9%	94%	75%	56%	42%

With 20 healthy volunteers, this study has a power 87% to detect the 2-fold change if the standard deviation is 2.

At the end of the study, for the primary endpoint, a paired t-test will be used to calculate the mean $\log_{10}$ change at each post-treatment time point compared to baseline and the 95% confidence interval will be provided. More sophisticated statistics will also be used for the overall microbiome data (section 9.4.2).

9.3 Populations for Analyses

To address the primary endpoint of a change in a relative abundance of bacterial taxa approximately 1 month post-BSS compared to baseline (pre-BSS), all participants with a stool sample for microbiome analysis at baseline and at least 1 stool sample for microbiome analysis after BSS treatment will be included.

9.3.1 Evaluable for Toxicity

All participants will be evaluable for toxicity from the time of their first treatment with BSS.

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

The summarized statistics such as mean (for continuous variables) or proportions (for categorical variables) as well as 95% confidence intervals will be provided. More sophisticated statistics will be applied with the microbiome data as below.

9.4.2 Analysis of the Primary Endpoints

In order to quantify changes in the stool microbiome with BSS treatment over a 1-month period, we will examine the following, comparing the baseline microbiome (pre-BSS treatment) with post-treatment samples:

- We will perform Linear discriminant analysis effect size to determine the relative abundance of taxa pre-treatment through approximately 1 month post-treatment. We hypothesize there will be a significant decrease ($p<0.05$) in certain beneficial taxa (eg, *Lactobacillus*) and increase ($p<0.05$) in potential pathobionts (eg, *Enterococcus*) post-treatment. Each timepoint post-BSS treatment will be compared to the pre-treatment baseline. If significant differences in the relative abundance of taxa are found in the early time points post-treatment, as per murine models, then we can longitudinally examine post-treatment samples to determine the length of time these differences last.
- We will examine microbial alpha diversity in the stool samples pre-treatment compared to stool samples at each time point through approximately1 month post-treatment using four different methods (chao1, Shannon diversity index, observed species, PD whole tree).
- We will examine stool sample beta diversity (microbial community structure) pre-treatment compared to post-treatment at each time point over a month using four different methods (unweighted UniFrac, weighted UniFrac, Bray-Curtis dissimilarity, Jaccard distance), and principal coordinate analysis will be used to explore and visualize differences in microbial community structure.

9.4.3 Analysis of the Secondary Endpoint(s)

Hypothesis: Differences in the stool metabolome, including increased primary bile acids and decreased short chain fatty acids, will be found in post-treatment samples compared to pre-treatment samples.

In order to quantify changes in the stool metabolome with BSS treatment over approximately a 1-month period, we will examine the following comparing the baseline metabolome (pre-BSS treatment) with post-treatment samples at each time point:

- Untargeted metabolomic analysis will be performed on serial stool samples by liquid chromatography and high-resolution tandem mass spectrometry. Metabolites will be identified by comparison to library entries of purified standards or recurrent unknown entities. In addition, as short chain fatty acids and bile acids are of particular interest, a separate targeted analysis for these will be performed.
- Metabolites will be compared pre-treatment to approximately 1 month post-treatment. Identified metabolites will be quantified by peak integration. Data will be scaled to a median of 1 for each biochemical in that sample set. Probabilistic principal component

analysis will be used to select for discriminative metabolites among pre-treatment and post-treatment sample. If significant differences in metabolites are found in the early time points post-treatment, as per murine models, then we can longitudinally examine post-treatment samples to determine the length of time these differences last.

- In a muti-omic data analysis, metabolite changes will be correlated with microbiome changes to assess which bacterial taxa may be driving the metabolite changes found.

9.4.4 Safety Analyses

As this study uses BSS, an already FDA-approved OTC medication, there are no safety endpoints that will be analyzed. However, safety will be assessed throughout the study as per section 8.3.

For the possible true probabilities of AEs described in the pausing rules (section 7.1.1), Table 2 below lists the early stop probabilities for the first 10 participants.

Table 2: Probability of Early Stop for Possible True Probabilities of Any Adverse Event in the First 10 Participants

True prob. of any AE	0.01	0.05	0.10	0.2	0.3	0.4
Prob. of ≥1 with any AE in the first 10 participants	0.10	0.40	0.65	0.80	0.97	0.99

9.4.5 Sub-Group Analyses

Subgroup analysis is not warranted given the small sample size of this study.

9.4.6 Tabulation of individual Participant Data

Individual participant data will be listed by measure and time point.

9.4.7 Exploratory Analyses

Exploratory analyses of the tertiary/exploratory endpoints (section 3) will be performed if enough data are available for these endpoints.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 Informed Consent Process

10.1.1 Consent Procedures and Documentation

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research participant. It is an ongoing conversation between the human research participant and the researchers that begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include purpose, duration, experimental procedures, alternatives, risks, and benefits. Coercion and undue influence will be minimized by informing participants that their decision to join the study will not affect any medical care they are currently receiving, or their eligibility to participate in other research studies. Participants will be given as much time as they need to read the consent form and ask questions of the investigators. Participants will also be given time to discuss their participation with family members, friends, and other healthcare providers.

The initial consent process as well as re-consent, when required, may take place in person or remotely (eg, via telephone or other NIH-approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant. If conducted remotely, permission to mail, fax, or email the consent document in advance will be obtained from the participant. Once the consent is received, the participant is instructed to contact the investigator for discussion of the study and consent document at a time that is convenient.

Whether in person or remote, the privacy of the participant will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (eg, clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document as a hard copy or on screens at their respective locations; the same document or screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Informed consent will be obtained by the principal investigator or a designated associate investigator on this protocol. The study will be thoroughly explained with ample time for questions or concerns related to participation. When consent is conducted remotely, the participant will identify themselves by name and date of birth, and state that they give consent to participate in this study.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. When consent is conducted by telephone, the consent signed and dated by the participant (witnessed as applicable) must be sent to the investigator or designee via mail, secure email, or another secure platform for signature by the principal investigator or a designated associate investigator. Once received, the consent will be checked for accuracy and signed by the NIH investigator.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the iMedConsent platform (which is compliant with 21 CFR 11) to obtain the required signatures. Both the investigator and the participant will sign the electronic document using a finger, stylus, or mouse. Electronic signatures (ie, the “signature” is digitally generated) will not be used.

The investigator will confirm that written legally effective consent has been obtained prior to initiating any study interventions (except some screening procedures, as described in section 8.1). If a participant is shipping a signed physical consent document with samples after being consented remotely, no testing of the samples will occur until the investigator confirms that written legally effective consent has been obtained. The signed consent (ie, electronic or hard copy) will be filed in the medical record, and the manner of obtaining consent and the names of the person administering and providing consent will be documented in the medical record, signed, and dated. A hard copy or access to an electronic copy of the fully signed consent form will be provided to the participant.

The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The participants may withdraw consent at any time throughout the course of the study.

10.1.2 Considerations for Consent of NIH Staff or Family of Study Team Members

Consent for NIH staff and family members of the study team will be obtained as detailed above and will comply with the requirements of NIH HRPP Policy 404 *Research Involving NIH Staff as Subjects*.

Consent from NIH staff for whom this research is taking place within their own work unit or is conducted by any of their supervisors will, when possible, be obtained by an individual in a non-supervisory relationship with that staff member. When consent of that staff member is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on the staff member. Similarly, for family of the study team, a study team member unrelated to the participant will obtain their informed consent. When consent of that staff member's relative is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on them.

10.1.3 Participation of Individuals Who Are/Become Decisionally Impaired

Participants who become decisionally impaired during their participation in this study, and can therefore no longer provide their ongoing informed consent, will be withdrawn from the study (section 7.2).

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the principal investigator will promptly inform study participants and the IRB. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient compliance to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility.

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

10.3 Confidentiality and Privacy

All records will be kept confidential to the extent provided by federal, state, and local law. Authorized individuals may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the participant,

except as necessary for monitoring by the IRB, NIAID, and the Office for Human Research Protections.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

Coded specimens and data will be stored at the NIH indefinitely for future research after the study is complete. Human genetic testing may be performed. Plans for future use of specimens and data will be described in the informed consent document. Specimens will be stored at the NIH CC in a locked facility with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the code key.

Other investigators (both at NIH and outside) may wish to use these specimens and/or data. If the planned research falls within the category of "human subjects research" on the part of the investigators, NIH IRB review and approval will be obtained. This includes the investigators sending out coded and linked samples or data and getting results that they can link back to their participants.

10.5 SAFETY OVERSIGHT

All data will be collected in a timely manner and reviewed by the principal investigator and/or a designee on a regular basis. Events meeting requirements for expedited reporting as described in the NIH HRPP Policy 801 will be submitted to the NIH IRB within the required timelines.

The principal investigator will review all data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10.5.1 Safety Review and Communications Plan

A safety review and communications plan (SRCP) is required for this protocol. The SRCP is an internal communications document between the principal investigator and the CSO, which delineates key safety oversight responsibilities of the principal investigator, the CSO, and other stakeholders. The SRCP includes a plan for conducting periodic safety surveillance assessments by the CSO for reportable events and AESIs.

10.5.2 Medical Monitor

An MM, representing the CSO, has been appointed for oversight of safety related to AESIs in this protocol. The MM or designee will be responsible for performing safety assessments as outlined in the SRCP.

10.6 CLINICAL MONITORING

The data gathered during this study will be monitored by the principal investigator for safety and compliance with protocol-specified requirements.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

To help ensure that NIH Office of Research Support and Compliance procedures and GCP are being carried out, a Clinical Trials Management designee within the Office of Clinical Research Policy and Regulatory Operations, Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH Office of Human Subjects Research Protections reporting requirements for reportable events. In addition, the quality management and data management plan for the study will be reviewed.

During the study, the principal investigator and study team will be responsible for implementing a quality management plan. Additionally, the study team will be responsible for completing and submitting a summary report on the quality plan to the NIAID Clinical Director or designee at least annually as detailed in the quality management plan. A courtesy copy will also be sent to Clinical Trials Management.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Study data will be maintained in REDCap and CRIMSON and collected directly from participants during study visits and telephone calls or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into REDCap and CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data, including cumulative participant accrual numbers, should be generated via the chosen data capture method and submitted to study oversight bodies as needed.

10.8.2 Study Records Retention

Study documents will be retained in accordance with regulatory and institutional requirements, ICH GCP guidelines, and the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the principal investigator and sponsor, as applicable.

10.9 PROTOCOL DEVIATIONS AND NON-COMPLIANCE

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH IRB as per HRPP Policy 801. All deviations must be addressed in study source documents and reported as specified in the protocol quality management plan and/or monitoring plan. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the participant, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety, or welfare of participants or others, or the scientific integrity or validity of the study.

10.10 REPORTING TO THE NIAID CLINICAL DIRECTOR

UPs, major protocol deviations, and deaths will be reported to the NIAID clinical director according to institutional timelines.

10.11 HUMAN DATA SHARING, INCLUDING GENOMIC DATA SHARING, AND PUBLICATION

10.11.1 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy Compliance

This study will comply with the NIH Data Management and Sharing Policy, which applies to all new and ongoing NIH-funded research in the intramural research program (IRP) as of January 25, 2015 that is associated with a ZIA, with a clinical protocol that undergoes scientific review and/or will involve genomic data sharing.

This study will comply with the NIH Genomic Data Sharing Policy, which applies to all new and ongoing NIH IRP-funded research as of August 31, 2015 that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies, single nucleotide polymorphisms arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.11.2 NIH Public Access Policy Compliance

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIAID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
BSS	Bismuth subsalicylate
CC	Clinical Center
CFR	Code of Federal Regulations
COVID-19	Coronavirus disease of 2019
CRIMSON	Clinical Research Information System of the NIAID
CSO	Clinical Safety Office
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastrointestinal
HRPP	Human Research Protection Program
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
Ig	Immunoglobulin
iPS	Induced pluripotent stem (cell)
IRB	Institutional review board
IRP	Intramural Research Program
LHIM	Laboratory of Host Immunity and Microbiome
MM	Medical monitor
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NSAID	Nonsteroidal anti-inflammatory drug
OTC	Over the counter
PFA	Platelet function assay
REDCap	Research Electronic Data Capture
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
rRNA	Ribosomal RNA
SAE	Serious adverse event
SD	Standard deviation
UP	Unanticipated problem
US	United States

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