Official Title:	A Pilot Proof of Concept Study of the Effects of Administration of a Short Chain Fatty Acid (SCFA) Supplement in Rheumatoid Arthritis Inadequate Responders (EASi-RAIR)
NCT Number:	NCT05718583
Study Number:	22-01526
Document Type:	Study Protocol and Statistical Analysis Plan
Date of the Document:	• August 15, 2025

NYU Langone Health

A pilot proof of concept study of the Effects of Administration of SCFA in Rheumatoid Arthritis Inadequate Responders (EASi-RAIR)

Principal Investigator:	Jose U. Scher, MD Division of Rheumatology Department of Medicine New York University School of Medicine 301 East, 17th Street, Suite 1410 New York, NY 10003 Email: jose.scher@nyumc.org Tel: 212- 598- 6272
Additional Investigators:	Rebecca B. Blank, MD, PhD Email: Rebecca.blank@nyulangone.org
NYULMC Study Number:	s22-01526
Funding Sponsor:	Arthritis Foundation 1355 Peachtree Street, Suite 600, Atlanta, GA 30309 612-251-0952 NYU Clinical and Translational Science Institute (CTSI)
Study Product:	Oral Butyrate supplement (over the counter supplement)

Initial version: 01 December 2022
Amended: 13 July 2023
Amended: 08 December 2023
Amended: 20 June 2025
Amended: 08 August 2025

Statement of Compliance
This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation ("ICH") Guideline for Good Clinical Practice ("GCP") (sometimes referred to as "ICH-GCP" or "E6") will be applied only to the extent that it is compatible with FDA and DHHS regulations. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

Table of Contents

List of Abbreviations

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
CSOC	Clinical Study Oversight Committee
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
FFR	Federal Financial Report
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IR	Inadequate responder
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MOP	Manual of Procedures
MTX	Methotrexate
N	Number (typically refers to participants)
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RA	Rheumatoid arthritis
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
US	United States

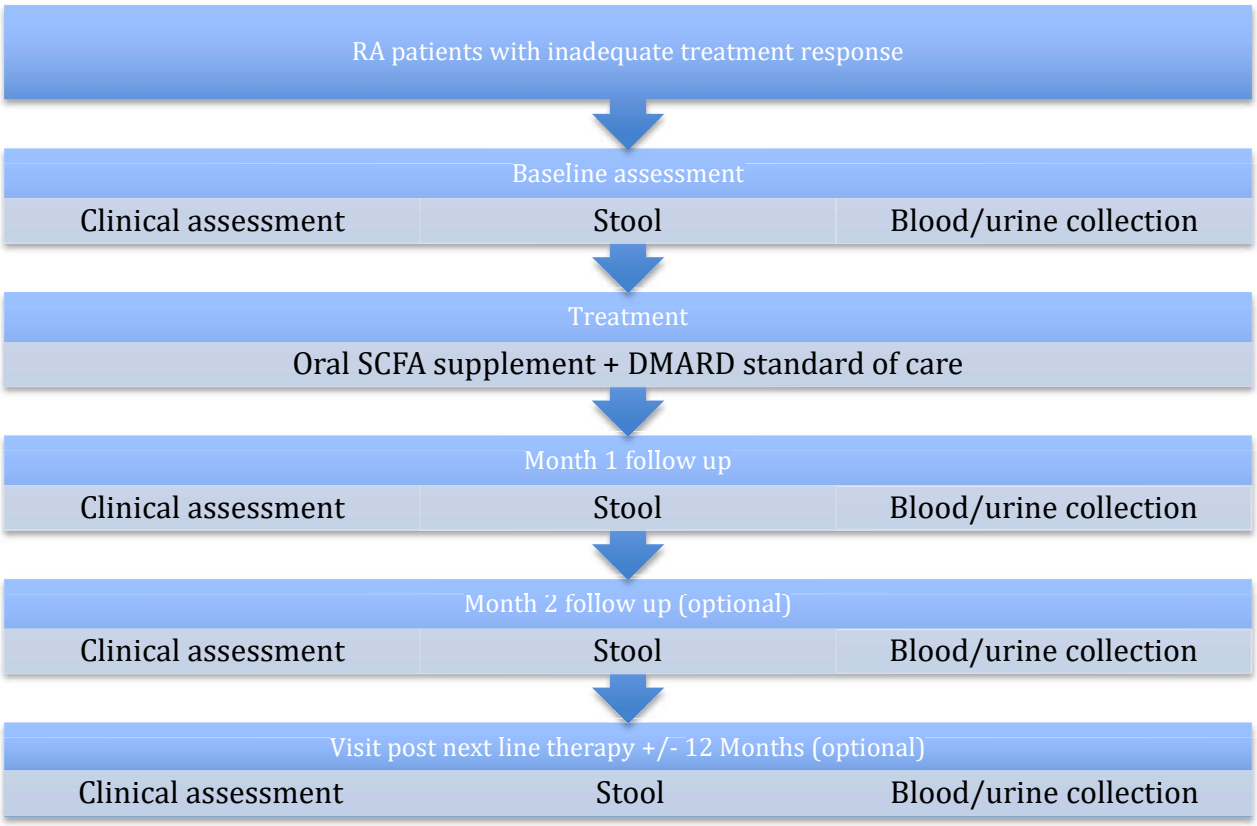
Protocol Summary

Title	A pilot proof of concept study of the Effects of Administration of SCFA in Rheumatoid Arthritis Inadequate Responders
Short Title	EASi-RAIR
Brief Summary	This study is a pilot, proof of concept study to determine the effects of administering an oral SCFA supplement to RA patients with inadequate response to methotrexate. We will include up to 65 total participants to obtain the following samples sizes below. We will include up to 65 participants to obtain a sample size of at least 25 participants taking the oral supplement. We hypothesize that oral SCFA will change the participants' gut microbiome and regulatory immune responses. Additionally, we will include participants with inadequate response to methotrexate who do not want to take oral SCFA supplements but rather proceed directly to the next medication their treating physician suggests. We will include up to 30 participants to obtain a sample size of at least 20 participants. Clinical data to assess for adverse events, stool, urine samples and peripheral blood will be collected at baseline, 1 month, with an optional 2-month time-point, and an optional visit up to 12-months following the last visit after participants have started treatments with the next line of therapy, Fecal microbiome will be analyzed. Adaptive immune responses will be analyzed from participant blood samples.
Phase	Pilot proof of concept study
Objectives	**Primary objective:** • To determine whether oral SCFA supplementation in RA inadequate responders induces changes in the gut microbiome composition **Key secondary objectives:** • To determine whether oral SCFA supplementation in RA inadequate responders induces changes in the peripheral Treg population • To measure changes in adaptive immune response over time with oral supplementation of SCFA • To measure changes in serum and fecal SCFA concentration with oral supplementation of SCFA • To measure MTX and its metabolites in fecal and urine samples after oral SCFA supplementation • To measure changes in the gut microbiome composition in methotrexate inadequate responders who do not want to take oral SCFA supplements. • To measure changes in the gut microbiome composition in methotrexate inadequate responders after they initiate next line therapy (as prescribed by their treating physician)
Methodology	Open label pilot proof of concept study
Endpoint	**Primary endpoint:** • The primary endpoint will be an increase in microbiome alpha diversity from baseline visit 1 to visit 2 after SCFA supplementation. **Important secondary endpoints:** • We will determine the changes to serum and fecal SCFA after SCFA supplementation and determine the changes in adaptive immune response including peripheral Treg cell populations.
Study Duration	Up to 4 years
Participant Duration	Up to 14 months

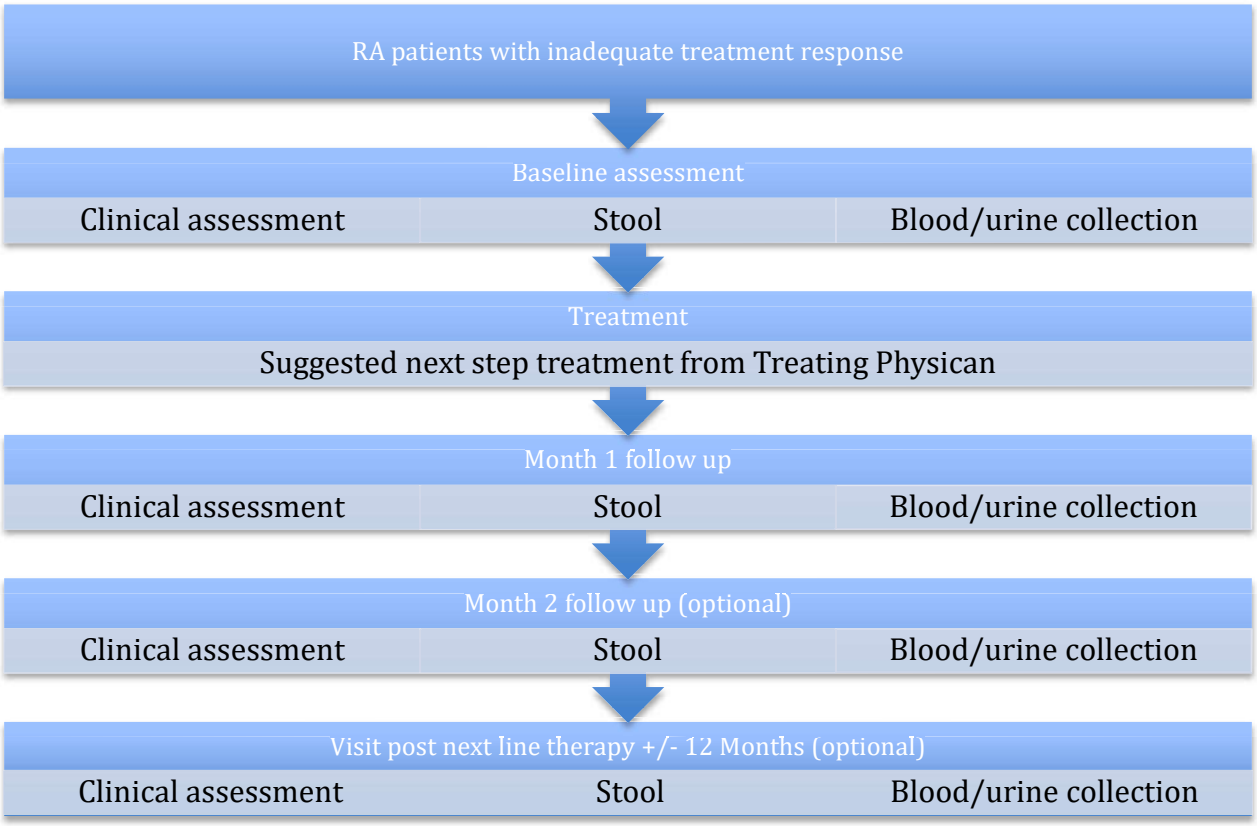
Duration of IP administration	Up to 2 months
Population	Any Rheumatoid Arthritis patient who is an inadequate responder to current RA treatment: • Age 18 years and above • Subjects not excluded based on gender, race or ethnicity, general health status, or geographic location
Study Sites	NYU Langone Health inpatient and outpatient sites, Bellevue Hospital
Number of participants	65 participants
Description of Study Agent/Procedure	Study agent is an oral SCFA supplement containing butyrate. It will be taken orally 1000mg three times a day with meals.
Key Procedures	Blood draws Stool collection Urine collection
Statistical Analysis	The primary endpoint of the trial is an increase in microbiome alpha diversity within subject pre- and post-SCFA supplementation at 1 month after initiation of treatment. According to our preliminary data in new-onset RA participants, SCFA supplementation leads to increase in alpha diversity with an effect size of 0.63. To be conservative, we will estimate an effect size of 0.60. A total of 25 participants will be needed to achieve an effect size of at least 0.60 while maintaining 80% power with a 0.05 type I error

Schematic of Study Design

Participants taking Oral SCFA Supplements



Participants Not Taking Oral SCFA Supplements



1 Key Roles

Principal Investigator

Jose U. Scher, MD
Associate Professor of Medicine
Director of the NYU – MiCRA
Director of the NYU Psoriatic Arthritis Center (PAC)
New York University Grossman School of Medicine
301 East 17th Street, Suite 1410
New York, NY 10003
Email: jose.scher@nyumc.org
Tel: 212-598-6272

Co-Investigators

Rebecca B. Blank, MD, PhD

2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

Methotrexate (MTX) is highly effective in about half of all rheumatoid arthritis (RA) patients[1-3] and remains the anchor drug for most of those who are recently diagnosed. It would therefore be beneficial to find a safe, effective, and inexpensive way to increase MTX response. A major mechanism by which MTX efficacy can be enhanced is through T regulatory cell (Treg) function[4]. The short chain fatty acid (SCFA) butyrate, a microbial fermentation product of certain gut microbes, has been shown to: i) induce a Treg immune response; and ii) promote changes in the gut microbiome in both murine models of inflammatory disease and in humans[5-11]. Importantly, butyrate supplementation in patients with inflammatory disease has been shown to modify the gut microbial community towards an abundance of potentially beneficial butyrate producers[11], supporting the notion that oral butyrate may promote a more tolerogenic gut microbial niche in autoimmune conditions. Based on this

body of evidence, along with both our preliminary data and our published work showing that the baseline gut microbiome can be predictive of MTX responsiveness in new-onset RA (NORA) patients[12], we **hypothesize** that in RA patients with initial inadequate MTX response, the co-adjuvant administration of butyrate (along with MTX), will induce modifications in gut microbial communities that favor a regulatory adaptive immune response which can ultimately increase drug efficacy.

To our knowledge, the proposed study will constitute the first of its kind. There have been mixed results in small trials of probiotics and/or prebiotics in inducing immunoregulatory activity in healthy and diseased participants[13-16] and in RA in particular[17-19]. We propose to circumvent the upstream mechanisms induced by the supplementation of probiotics and/or prebiotics and directly administer the gut microbial fermentation byproduct itself along with MTX to induce a more effective regulatory response in RA patients deemed refractory to the drug. If oral butyrate can induce even a modest increase in MTX efficacy, this strategy has the potential to allow patients to continue MTX monotherapy instead of unnecessarily switching to another DMARD. This would help reduce the dependence on alternative DMARDs, including targeted synthetic oral DMARDs and biologic DMARDs which are generally more prone to adverse events.

This is being submitted as a separate study from EASi-RA (i20-02068) because of the requirement for participants to have an inadequate response to MTX, while EASi-RA patients are new onset rheumatoid arthritis patients who are rheumatoid arthritis treatment naïve. The patients in this EASi-RAIR study are no longer "new-onset" rheumatoid arthritis compared to the patients in EASi-RA. Additionally, the primary outcomes in both studies are different, therefore necessitating implementation of a new IRB protocol.

Hypothesis
We **hypothesize** that in RA patients with inadequate MTX responses, the oral co-administration of butyrate along with MTX will induce modifications in gut microbial communities that favor a regulatory adaptive immune response that may ultimately increase MTX efficacy.

2.2 Name and Description of the Investigational Agent: Butyrate – a short chain fatty acid

This study is looking at the investigational agent short chain fatty acids (SCFA), and in particular, a four-carbon SCFA called butyrate. Oral butyrate and other oral SCFA mixtures can be found as dietary supplements in local health food shops and pharmacies and are not regulated by the FDA.

2.2.1 Preclinical Data

There is a substantial amount of published work suggesting that oral butyrate supplementation ameliorates inflammatory arthritis in murine models[5,6]. Additionally, there have been a number of studies examining the effects of oral supplementation of SCFAs in humans in inflammatory diseases such as arthritis, MS and even the metabolic syndrome[8,]Cleophas, Ratter [10,13,16,17]. Butryate supplementation in patients with IBD alters the gut microbial community towards an abundance of butyrate producers[11]. In particular, SCFA such as butyrate promotes T regulatory cell differentiation, production of IL10, and a shift from Th17 dominant phenotype to Treg phenotype[5-10]. In our lab we can see systemic changes in Treg circulation within one week in healthy volunteers following butyrate supplementation (unpublished data). Further, our lab has characterized the gut microbial composition in RA patients that are either responders or non-responders to MTX[12]. One important mechanism of MTX is its role in promoting Treg function[4]. MTX responders have increased frequencies of regulatory T cells after MTX treatment whereas, nonresponders do not[20]. Therefore, we propose that addition of SCFA to MTX therapy will induce more circulatory Tregs.

2.2.2 Clinical Data to Date

There is no available clinical research data to date on this product (Oral butyrate supplement from Biotics Research or Bodybio, Inc.). Please reference above for data on SCFA, and butyrate, in related studies.

2.2.3 Dose Rationale

Butryate 1000 mg three times daily will be used in this study. This dose was based on doses found in the literature and over the counter general use guidelines that ranged from 1000mg – 6000 mg daily[8,10,11]. In our ongoing study in NORA patients, the proposed dosing regimen is feasible, tolerable and promotes changes in gut microbiome diversity (unpublished data).

2.3 Rationale

MTX is effective in up to only 40-50% of all RA patients yet MTX monotherapy is an anchor drug for most RA patients. It would be beneficial to find a safe, effective and inexpensive oral agent that could increase MTX effectiveness. A major mechanism of MTX is by enhancing Treg function[4]. SCFA have been shown to induce a Treg immune response and induce changes in the gut microbiome in both murine models of inflammatory disease and human patients. Taken together, we propose that an oral SCFA-based supplement given to RA patients on a daily basis along with physician prescribed MTX will induce changes in gut microbial communities that favor a regulatory adaptive immune response. We will analyze gut microbial communities from patients prior to SCFA initiation and evaluate whether SCFA can shift a non-responder gut microbiome into a responder gut microbiome phenoptype. We will also analyze blood samples from participants to characterize whether SCFA supplementation leads to increased production of T regulatory cells and regulatory cytokines. We will not have a placebo arm as this is a pilot proof of concept study.

2.4 Potential Risks & Benefits

2.4.1 Known Potential Risks

Risk of study supplement

Butyrate is a normal SCFA fermentation byproduct of our commensal gut microbiota. As such, no risks are expected other than those derived from ingestion of concentrated fatty acids, namely gastrointestinal intolerance (including pain and bloating) and (possibly) diarrhea. Subjects who experience any of these symptoms will be informed to contact their study doctor immediately at the telephone number provided by the PI.

The study team will work to minimize risks by monitoring subject experiences through the study and monitoring the withdrawal of subjects who experience certain risks or severe symptoms. This monitoring will be in the form of discussing with patients during their study visits and tracking of adverse events on case report forms and will be discussed with DSMB as required. There will not be no special monitoring in this case as this is a normal byproduct of gut microbiota and no serious risks are expected from the supplement.

Risks of all study related procedures

Blood draw: Possible side effects from drawing blood include: faintness, inflammation of the vein, pain, bruising, or bleeding at the site of puncture. There is also a slight possibility of infection.

Stool/Urine Sample Collection:
Potential contamination of surfaces or other people is the major risk of urine and stool collection procedure, which may transmit infectious diseases to animals and individuals.

Physical Examination
The physical examination will be performed by a qualified study physician as part of routine care. Minor discomfort may occur.

Loss of privacy and confidentiality: As with participation in any research study, there is always a risk that confidential or private information can be compromised. To minimize this risk, the study team will de-identify patient samples and replace identifying information, such as patient's name, with a bar code label that will not be associated with any date or identifiable information to the patient (for example birthday or street address). Samples will be stored in the PIs lab. Only Dr. Scher and his research team will have access to them.

2.4.2 Known Potential Benefits

The benefits of SCFA, particularly, butyrate, are currently unknown. Subjects will not benefit directly from the study. We hope the information gained will help us better understand the effects of SCFA in patients with RA.

3 Objectives and Purpose

- To determine whether SCFA alters the gut microbiome and to assess whether the changes in the gut microbiome alters systemic immune responses.
- Assess clinical responsiveness to oral supplementation of SCFA along with MTX in inadequate responders

3.1 Primary Objective

- To determine whether oral SCFA supplementation in RA inadequate responders induces changes in the gut microbiome composition

3.2 Secondary Objectives (if applicable)

- To determine whether oral SCFA supplementation in RA inadequate responders induces changes in the peripheral Treg population
- To measure changes in adaptive immune response over time with oral supplementation of SCFA
- To measure changes in serum and fecal SCFA concentration with oral supplementation of SCFA
- To measure MTX and its metabolites in fecal and urine samples after oral SCFA supplementation

4 Study Design and Endpoints

4.1 Description of Study Design

This study is a pilot proof of concept, open label study of the effects of oral SCFA supplementation in RA patients with an inadequate response to MTX. We will be enrolling all patients with RA who fulfill inclusion/exclusion criteria. This will be a single center study based in the NYU Langone hospitals and clinics as well as Bellevue Hospital which falls under its umbrella. Oral butyrate will be taken at 1000 mg three times daily with meals by RA patients who have active disease and are currently taking MTX at prescriber's recommended dose. There will be no dose escalation of the study supplement.

4.2 Study Endpoints

4.2.1 Primary Study Endpoints

The primary endpoint will be an increase in microbiome diversity (as measured by Shannon diversity index) from baseline visit 1 to visit 2 after SCFA supplementation with an effect size of at least 0.60.

4.2.2 Secondary Study Endpoints

We will determine changes in serum and fecal SCFA concentration over time to ensure that oral SCFA supplementation is localizing to the colon and/or peripheral circulation.

We will measure changes in adaptive immune response by flow cytometry, ELISA, T reg suppression assays and RNA-seq, over time with oral supplementation of SCFA with the hypothesis that oral SCFA supplementation will increase Treg and B regulatory cell responses over time.

We will measure changes in cytokine production over time with oral supplementation of SCFA with the hypothesis that oral SCFA supplementation will increase regulatory cytokine responses and decrease inflammatory cytokine responses.

Contingent upon observed changes in microbiome and/or changes in adaptive T and B cell populations, measurements of inflammatory transcripts may be performed on stored frozen PBMCs by measuring cell messenger RNA levels.

4.2.3 Exploratory Endpoints

We will determine the percentage change of gut microbiome composition from MTX-nonresponder (MTX-NR) phenotype to an MTX-responder (MTX-R) phenotype after SCFA supplementation. Our hypothesis is that oral SCFA supplementation will shift the microbiome to a more regulatory state and in parallel will shift the microbiome to a similar composition that is observed in patients who are MTX-R.

5 Study Enrollment and Withdrawal

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Diagnosis of RA meeting 2010 ACR/EULAR for RA and/or treating MD diagnosis
2. Inadequate response to MTX per treating MD at maximum tolerated dose.
3. Able and willing to provide written informed consent prior to any study specific procedures
4. Age 18 years and above at time of enrollment
5. Subjects not excluded based on race or ethnicity

5.2 Exclusion Criteria

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

1. Participants who are pregnant or are currently breastfeeding
2. History of sensitivity to study compound or any of their excipients
3. Previous intolerance to SCFA or related compounds
4. Current antibiotic treatment (within 3 months of screening) at discretion of PI
5. Current consumption of probiotics (within 3 months of screening) at discretion of PI
6. Severe hepatic impairment (eg, ascites and/or clinical signs of coagulopathy)
7. Renal failure (eGFR <30 or requiring dialysis) by history
8. History of other autoimmune disease at discretion of PI
9. Current immunodeficiency state (e.g., cancer, HIV, others)

5.3 Vulnerable Subjects

Vulnerable subjects will not be enrolled.

5.4 Strategies for Recruitment and Retention

Recruitment

Potential subjects will be informed of the study by their treating physicians. Treating physicians will be informed of the study verbally. Additionally, individuals with inadequate response to methotrexate who do not want to take oral SCFA supplements but rather proceed directly to the next medication their treating physician suggests; are also considered potential subjects, serving as the control group, and will be informed of the study by their treating physician. If patients agree to be contacted for participation, a member of the study team will approach them in person, by phone, via MyChart message. These recruitment materials are uploaded in Research Navigator. The study team also welcomes potential subjects from outside NYULH or Bellevue Hospital who may learn about the study from clinicaltrials.gov or by word-of-mouth.

Once contact is made, subjects will be told the reason they are being contacted and asked if they are interested in participating in this specific study. If the potential subjects agree, the study team will provide them with information regarding the next steps for participation.

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact study coordinator or have subjects contact research-contact-optout@nyumc.org or 1-855-777-7858.

Sex of Subjects

Both men and women will be equally recruited into the study. Since RA affects women over men by approximately 3-fold, we will likely recruit more women due to the epidemiology of this disease. There are no enrollment restrictions regarding gender of subject.

Age of Subjects.

Age of the enrolled subjects will be at least 18 years of age.

Racial and Ethnic Origin.

All racial and ethnic groups will be recruited into the study, without any restrictions.

Subject capacity:

Subjects with impaired cognitive capacity will not be consented or enrolled into the study.

Subject comprehension:

If any questions arise, the Study Staff will answer them until the subject has a clear understanding of what is expected if he/she agrees to participate in the study. Each subject will be asked to provide a statement of understanding of the study risks, procedures and what the study entails prior to signing the Study Informed Consent.

Documentation of consent:
Original documents of the Study Informed Consent will be maintained for study purposes and review, if needed.

5.4.1 Use of DataCore/Epic Information for Recruitment Purposes

This study will utilize EPIC to identify subjects.

Datacore may be used for subject recruitment. Subjects will be identified by clinician diagnosis of rheumatoid arthritis and by medications in order to screen for potentially eligible participants who have consented to be contacted for research purposes through their MyChart Study coordinator and Study PI will have access to this information in order to identify and recruit eligible participants.

Study personnel will submit a request to NYULH DataCore via iLab. Study team members who will have access to search results include Dr. Rebecca Blank (sub-I), Sydney Catron (research coordinator), Rhina Medina (research coordinator) and Sarah Moussavi (research coordinator). The PHI that will be accessed is the clinical diagnosis of the individual, medications, and age. The data will be discarded after use which will include deleting any identifiers that link the individual to the PHI.

The study team will search DataCore/EPIC weekly until recruitment ends.

Treating physicians will be notified of the study through word of mouth.

The patient's primary physician will recommend their patient for the study, after this, the study team will contact the patient by phone or MyChart to gauge their interest,

Any recruitment information sent by email will utilize Send Safe email.

Once potential subjects have been identified, the study team will notify the treating physician (TP) that they have patients eligible to participate as follows:

- Provide TP with an oral script to use when contacting potential subjects
- TP agrees to permit study team to directly contact potential subjects on behalf of TP.
- TP has been notified that the study team will contact potential subjects directly, by letter, phone, email, or the MyChart portal etc.

Once contact is made, approved recruitment language will be used to communicate the reason they are being contacted and subjects will be asked if they are interested in participating in this specific study. Should the potential subjects agree, the study team will provide the subjects with information regarding the next steps for participation.

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact research-contact-optout@nyumc.org or 1-855-777-7858.

5.5 Informed Consent Process

5.5.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting any research activities. The consent form is submitted with this protocol.

5.5.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their

surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. 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.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

When a participant consents to this study, his/her name, address, phone numbers, DOB, and copies of medical records will be collected and kept with the informed consent. At this time, he/she will be assigned a unique study identification number. All subsequent data analysis, specimen recording, and samples will be encoded with this identification number, only and the date of collection. No personal identifiers will be displayed. The principal investigator/project administrator will keep the original, signed informed consents in a locked cabinet in a locked office together with records of specimens donated and medical record copies secured for review. Information with participant names will not be used in any publications resulting from this investigation. The PI will retain the participant contact information until the conclusion of the study.

5.6 Duration of Study Participation

The duration of participation will be approximately 2 months with option to extend for additional visit up to 12 months after month 2 visit.

5.7 Total Number of Participants and Sites

Recruitment will end when approximately 65 participants are enrolled. It is expected that approximately 65 participants will be enrolled in order to produce 25 evaluable participants. Participants will be recruited from NYULH and Bellevue. The study team may also enroll any potential candidate from outside NYULH or Bellevue who contacts the study team. There will be no international site recruitment.

5.8 Participant Withdrawal or Termination

5.8.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

5.8.2 Handling of Participant Withdrawals or Termination

Any participant can withdraw his/her informed consent for the research use of their donated blood specimen and revoke his/her Authorization for the use and disclosure of his/her protected health information at any time and without any penalty. In this case, any unused specimens that have not been provided to the researchers will be destroyed. The NYU Langone Health research staff and System Administrator have received training in Good Clinical Practice and understand issues of patient/participant confidentiality. Given the fact that the research studies to be conducted are experimental and are not clinically validated, participants will not be notified of their tests results performed in the future.

5.9 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to Dr. Jose Scher, the funding agencies, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the NYU IRB and will provide the reason(s) for the termination or suspension.

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

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

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the sponsor and/or the NYU IRB.

Patients who are discontinued from study compound for any reason other than an SAE will be encouraged to remain on the study (not be officially withdrawn from the study) and complete all study visits. The data obtained will be used to understand variations in microbiome and Treg after discontinuation of SCFA.

6 Study Agent (Study drug, device, biologic, vaccine etc.) and/or Procedural Intervention

6.1 Study Agent(s) and Control Description

The SCFA oral supplement is an over the counter, non-FDA approved capsule containing butyrate. It is marketed as a dietary supplement. Butyrate and other SCFA are produced normally by gut commensal microbiota and are present at varying levels in the guts of all humans. Participants will self-administer the oral SCFA supplement three times daily, with meals for up to 2 months. This is an open label pilot proof of concept study therefore no placebo will be utilized.

6.1.1 Acquisition

The SCFA supplement will be acquired by purchase from the manufacturer and shipped to the investigator. All efforts will be made to acquire same lot number of the product.

6.1.2 Formulation, Appearance, Packaging, and Labeling

We will use a butyrate supplement from Biotics Research® or Bodybio, Inc. which is commercially marketed. The supplement comes in a capsule form that contains 1033 mg butyric acid (as calcium and magnesium butyrate), Vitamin A, Panthenic acid, ethyl vanillin (flavoring) and cellulose (capsule shell). It is gluten and dairy free. This product is commercially available for human use in the form, route and dose planned for this pilot study.

6.1.3 Product Storage and Stability

As per the manufacturer's recommendations, the product should be stored in a cool, dry area. According to manufacturer, the supplement expires approximately three years from manufacture date. We will plan to use supplement within at most one year from manufacture date.

6.1.4 Preparation

The SCFA oral supplement does not need any additional preparation prior to ingestion.

6.1.5 Dosing and Administration

The SCFA oral supplement should be stored in a dry place at room temperature. Participants will take two capsules by mouth three times daily with meals. If a participant misses a dose, the dose can be taken within 3 hours of the missed dose with or without food, otherwise participant can just wait until next dose. Do not double up on dose if missed previous dose.

6.1.6 Route of Administration

Oral

6.1.7 Starting Dose and Dose Escalation Schedule

Participants will take two capsules by mouth three times daily with meals. There will not be any dose escalation.

6.1.8 Dose Adjustments/Modifications/Delays

If a participant misses a dose, the dose can be taken within 3 hours of the missed dose with or without food, otherwise participant can just wait until next dose. Do not double up on dose if missed previous dose.

6.1.9 Duration of Therapy
Participants will self-administer the oral SCFA supplement three times daily, with meals for up to 2 months. The minimum duration necessary for an “evaluable” participant will be 2 weeks of SCFA supplementation.

6.1.10 Tracking of Dose
Participants will be given up to a 6-week supply at a time in an appropriate container. Patients will be asked to bring the container with them at follow up visits such that study coordinators can count remaining capsules to determine compliance. Participants will be called not more than on a weekly basis to discuss compliance to the study supplement for reinforcement.

6.1.11 Device Specific Considerations
Not applicable.

6.2 Study Agent Accountability Procedures
Biotics Research Butyric Cal-Mag supplements will be purchased from Agape Nutrition (agapenutrition.com; 640 Jadwin Ave, Suite L; Richland WA 99352). Alternatively, we can also procure Bodybio Calcium Magnesium Butyrate from Bodybio.com contingent on production lags by Biotics Research. The supplement bottles will be stored between 15° to 30° C at the NYU Langone Orthopedic Center, in a locked drawer in a locked room with a digital lock. Only those study team members delegated to dispense the supplements will have access.

The study compound will be labeled according to local regulatory requirements. Once dispensed to a subject, bottle labels will contain the following information:

- Bottle ID number
- Name of supplement
- Principal Investigator name
- Study team address and phone contact
- Date of dispensation
- Participant name/study ID
- Dosing instructions
- Date of expiration, if applicable

Records will be maintained by the study team indicating the receipt and dispensation of all supplies, including subject ID, date of dispensation, and bottle ID number.

At the conclusion of their participation, subjects will have the option to return any remaining product or dispose of the supplement on their own after end of the study.

7 Study Procedures and Schedule
7.1 Study Procedures/Evaluations
Once enrolled, participants will be asked to undergo physical examination (as standard of care), questionnaires facilitated by study staff, blood draws and collection of urine and stool samples.

7.1.1 Study Specific Procedures
- **Medical history** will be obtained by interview at screening and/or medical records
- **Medication history** will be obtained by interview and/or medical records. Assessment of eligibility will include a review of permitted and prohibited medications.
- **Physical examination** including assessments of tender and swollen joint counts will be performed by the physician and/or study staff at each clinic visit and recorded.
- **Biological specimen collection and laboratory evaluations** will be collected as above. Briefly,
    - Stool and urine will be collected by participant at home or in clinic per participant preference. Each participant will be given a stool/urine collecting kit along with detailed instructions on how to handle, store, and send specimen (should collection be at home) to the Scher research laboratory at the NYU Langone Orthopedic Hospital. Samples will be aliquoted into collection

tubes and stored at -80C until used for SCFA quantification, genomic, metabolomics and/or transcriptomic analyses.
    - Urine will be collected from participants in sterile containers. Urine will be stored and analyzed at a later time point for potential biomarkers, metabolic breakdown products of methotrexate and stored for analyses related to this study.
    - Blood samples will be collected as above for measurements of inflammation, immune activation, RNA transcripts and/or genomics.
- **Assessment of study agent adherence** will be performed at each clinic visit with quantification of remaining SCFA oral supplements given to the participant and with occasional phone calls reviewing compliance. Participants not taking oral SCFA supplements will not be assessed on study agent adherence.
- **Administration of questionnaires** DAS28 and MDHAQ will be performed at clinic visits by the study staff.

7.1.2 Standard of Care Study Procedures

Detailed physical exam including assessment of tender and swollen joint count will be completed at each clinic visit as part of regular standard of clinical care. Blood draws to look for markers of inflammation will also be completed as part of regular standard of clinical care. Treating physician will have discretion to make changes to methotrexate dosage at his/her discretion. If participant is switched to a new medication and methotrexate is discontinued, participant will no longer be eligible to continue in the study.

7.2 Laboratory Procedures/Evaluations

For each participant, approximately 5 tablespoons (75 cc) of blood will be collected in red (no anticoagulant) top, lavender (EDTA anticoagulant) top, green (heparin) and blue (sodium citrate anticoagulant) top tubes. Approximately 3 tablespoons (40 cc) of blood will be used for the different measurements of markers of inflammation and immune activation, and approximately 2 tablespoons (30 cc) of blood will be used to purify cells, RNA and DNA and then stored in -80C/liquid nitrogen for functional profiling. We will perform flow cytometry, ELISA, Treg suppression assays and RNA seq to determine changes in adaptive immune response. None of the above mentioned tests are standard of care. All of the above are necessary for the study to determine if oral butyrate induces a regulatory adaptive immune response in patients.

The drawing of a maximum of 5 tablespoons (75 cc) of blood is justified by the need for the above referenced tests, measurements, and purification and to ensure that enough PBMCs, RNA and serum/plasma are present. These tubes may be drawn at the time of the standard of care blood draws for routine patient care monitoring. This amount of blood is medically safe to draw. The physician will always ensure the safety of drawing this volume of blood.

7.2.1 Specimen Preparation, Handling, and Storage

Blood Collection

Samples will be collected by the NYU study staff or participant's physician or NYU laboratory. Samples will be processed for analysis as described in this protocol. Serum and plasma will be obtained from centrifuged samples of blood and stored for assays of protein levels and other mediators. In addition, peripheral blood cells may be collected, processed, and stored for analysis as well. Samples will be stored at the Scher research laboratory at the NYU Orthopedic Hospital (16th floor) in the dedicated - 80^0C freezer space in sterile plastic tubes and storage containers.

Stool and urine Collection

Stool and urine will be collected at the clinic visit or at participant's home as per participant preference. A stool and urine sample will be collected in the stool/urine sample collection kit and immediately placed on freezer packs and either shipped directly overnight to Scher lab, or if collected at clinic visit, will be directly transported to -80C freezer in Scher lab within 4 hours.

Back-Up Storage

All freezers are connected to a back-up generator in the chance that there is an adverse event. All freezers are also connected to the LPRO system, a 24-hour daily maintenance system that monitors temperature and overall status of freezer.

All samples will be taken directly from subjects and no samples will be collected from Pathology.

Vial Labeling
Blood samples will be first collected into tubes labeled with donor's unique Study identification number, date of collection, and sample type. All samples will have freezer safe labels.

7.2.2 Specimen Shipment
If stool sample is NOT collected on day of visit (either day 0, month 1, month 2), participant can opt to collect specimen at home in a collection kit that we provide, and ship, on provided ice packs to

Rhina Medina/Scher MiCRA Lab
NYU Hospital for Joint Diseases
301 E 17th St FLR 1612
NY NY 10003

Specimens can be shipped Monday, Tuesday, or Wednesday with a shipping label that we provide.

7.3 Study Schedule

	Screening/Baseline	**Month 1**	**Month 2***	**Month 12***
Visit #	**1**	**2**	**3***	**4***
Day#	**− 15 to 0**	**30±15**	**60±15**	**61 to 421**
Informed consent	X			
Medical history	X			
Medications	X	X	X	X
MDHAQ Questionnaire	X	X	X	X
TJ/SJ counts	X	X	X	X
DAS28	X	X	X	X
Blood collection	X	X	X	X
Stool/urine sample	X	X	X	X
Dispense SCFA	X	X*		
Adverse events	X	X	X	X

Optional
Note: SCFA will not be dispensed for participants choosing to not take the supplement.

7.3.1 Screening/Enrollment/Baseline

Screening/Enrollment/Baseline (Visit 1, Days -15 to 0)

- Obtain informed consent of potential participant verified by signature on study informed consent form.
- Verify inclusion/exclusion criteria.
- Obtain urine pregnancy test.

- Obtain demographic information, medical history, medication history, alcohol and tobacco use history.
- Medical history and review of medications will be performed.
- DAS28 and MDHAQ questionnaire will be performed and recorded to monitor for adverse events.
- TJ/SJ counts will be measured by the physician during physical exam.
- Oral supplement will be dispensed for participants taking the supplement.
    - Oral supplement will not be dispensed for participants choosing to not take the supplement*
- Blood will be collected.
    - For each subject, approximately 5 tablespoons (75 cc) of blood will be collected in red (no anticoagulant) top, Pax gene tube for genomics, lavender (EDTA anticoagulant) top, green (heparin) and blue (sodium citrate anticoagulant) top tubes. Approximately 3 tablespoons (40 cc) of blood will be used for the different measurements of markers of inflammation and immune activation, and approximately 2 tablespoons (30 cc) of blood will be used to purify cells, RNA and DNA and then stored in -80C/liquid nitrogen for functional profiling.
    - The drawing of a maximum of 5 tablespoons (75 cc) of blood is justified by the need for the above referenced tests, measurements, and purification and to ensure that sufficient number of PBMCs, RNA and serum/plasma are present. These tubes will be drawn at the time of the standard of care blood draws for routine patient care monitoring. This amount of blood is medically safe to draw. The physician will always ensure the safety of drawing this volume of blood.
- Stool sample will be collected.
- Urine sample will be collected.
- All adverse events will be documented.

7.3.2 Intermediate Visits

Visit 2 (30 days ± 15 days)

- Inclusion/exclusion criteria will be reviewed with participant
- DAS28 and MDHAQ questionnaire will be performed and recorded to monitor for adverse events;
- TJ/SJ counts (if arthritis is present) will be measured by the physician during physical exam
- Oral supplement will be dispensed as per above table
    - Oral supplement will not be dispensed for participants choosing to not take the supplement. *
- Review of medications will be performed;
- Blood will be collected;
- For each subject, no more than approximately 5 tablespoons (75 cc) of blood will be collected in red (no anticoagulant) top, Pax gene tube for genomics, lavender (EDTA anticoagulant) top, green (heparin) and blue (sodium citrate anticoagulant) top tubes. Approximately 3 tablespoons (40 cc) of blood will be used for the different measurements of inflammation, immune activation, and other markers, and approximately 2 tablespoons (30 cc) of blood will be used to purify cells and RNA and then stored in -80C/liquid nitrogen for functional profiling.
- Stool sample will be collected as per above table;
- Urine sample will be collected;
- Adverse events will be reviewed and recorded;
- Oral supplement adherence will be reviewed with participant and recorded.
    - Oral supplement adherence will not be reviewed with participants choosing to not take the supplement. *

7.3.3 Study Visit (optional)

Visit 3 (60 ± 15 days)

- Inclusion/exclusion criteria will be reviewed with participant
- DAS28 and MDHAQ questionnaire will be performed and recorded to monitor for adverse events;
- TJ/SJ counts (if arthritis is present) will be measured by the physician during physical exam
- Review of medications will be performed;
- Blood will be collected;

- For each subject, no more than approximately 5 tablespoons (75 cc) of blood will be collected in red (no anticoagulant) top, Pax gene tube for genomics, lavender (EDTA anticoagulant) top, green (heparin) and blue (sodium citrate anticoagulant) top tubes. Approximately 3 tablespoons (40 cc) of blood will be used for the different measurements of inflammation, immune activation, and other markers, and approximately 2 tablespoons (30 cc) of blood will be used to purify cells and RNA and then stored in -80C/liquid nitrogen for functional profiling.
- Stool sample will be collected as per above table;
- Urine sample will be collected;
- Adverse events will be reviewed and recorded;
- Oral supplement adherence will be reviewed with participant and recorded.
    - Oral supplement adherence will not be reviewed with participants choosing to not take the supplement. *

7.3.4 Additional Optional Study Visit

Visit 4 (Up to 12 Months After Final Visit)

- Inclusion/exclusion criteria will be reviewed with participant
- DAS28 and MDHAQ questionnaire will be performed and recorded to monitor for adverse events;
- TJ/SJ counts (if arthritis is present) will be measured by the physician during physical exam
- Review of medications will be performed;
- Blood will be collected;
- For each subject, no more than approximately 5 tablespoons (75 cc) of blood will be collected in red (no anticoagulant) top, Pax gene tube for genomics, lavender (EDTA anticoagulant) top, green (heparin) and blue (sodium citrate anticoagulant) top tubes. Approximately 3 tablespoons (40 cc) of blood will be used for the different measurements of inflammation, immune activation, and other markers, and approximately 2 tablespoons (30 cc) of blood will be used to purify cells and RNA and then stored in -80C/liquid nitrogen for functional profiling.
- Stool sample will be collected as per above table;
- Urine sample will be collected;
- Adverse events will be reviewed and recorded;

7.3.5 Withdrawal/Early Termination Visit

If participant withdraws or if early termination occurs a final blood draw and stool and urine collection, medication list, MDHAQ, TJ/SJ count should be obtained, provided that the participant is willing.

7.3.6 Unscheduled Visit

Treating physician will examine participant and make any necessary medication adjustments as per his/her discretion. Study team will note medication history, TJ/SJ count and discuss oral supplement adherence with participant as necessary. All data collected will be documented as a separate entry in REDCAP.

7.4 Concomitant Medications, Treatments, and Procedures

All concomitant prescription medications taken during study participation will be recorded on the case report forms (CRFs). 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 CRF are concomitant prescription medications, over-the-counter medications, and non-prescription medications.

7.5 Prohibited Medications, Treatments, and Procedures

None.

7.6 Prophylactic Medications, Treatments, and Procedures

None.

7.7 Rescue Medications, Treatments, and Procedures

None.

7.8 Participant Access to Study Agent at Study Closure

The oral SCFA supplement will not be provided after participants are no longer enrolled in the study. If participants are interested in continuing oral supplementation, we will provide information on brand and dosage so that participants can purchase on their own from commercial vendors.

8 Assessment of Safety

8.1 Specification of Safety Parameters

8.1.1 Definition of Adverse Events (AE)

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.1.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

8.1.3 Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets <u>all</u> the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- <u>Related or possibly related to participation in the research</u> (i.e. 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)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

8.2 Classification of an Adverse Event

8.2.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 Relationship to Study Agent

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- ***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 drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (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 drug, 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.*
- ***Possibly Related*** *– There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., 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 drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).*
- ***Not Related*** *– The AE is completely independent of study drug 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.*

8.2.3 Expectedness

Jose Scher or Rebecca Blank 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 agent.

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

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit,

the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

8.4 Reporting Procedures – Notifying the IRB

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though it should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.4.1 Adverse Event, Serious Adverse Event, and Unanticipated Problem Reporting

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment. All AE, SAE and UP will be documented in a report form by study staff and reported to the IRB by the PI.

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the DCC/study sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the DCC/study sponsor within 5 business days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 5 business days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within 30 days of the IR's receipt of the report of the problem from the investigator.

8.4.2 Reporting of Pregnancy

In case of pregnancy the participant should discontinue the oral supplement although there is no evidence that SCFA pose any risk for pregnant subjects or the fetus. In addition, the participant will be withdrawn from further study.

The patient should be followed by the Investigator until completion of the pregnancy. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e, postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for reporting a SAE.

8.5 Reporting Procedures – Notifying the Study Sponsor

The study clinician will complete a SAE Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the SAE Form and submitted to the PI within 24 hours of site awareness. See Section 1, Key Roles for contact information.
- Other SAEs regardless of relationship will be submitted to the PI within 72 hours of site awareness.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the DCC/study sponsor and should be provided as soon as possible.

As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse effects shall be provided promptly to the study sponsor.

8.6 Study Halting Rules

There are no formal halting rules.

8.7 Safety Oversight

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

Data Safety Monitoring

The Drs Scher and Blank are responsible for data safety monitoring reviews. This includes conducting systematic and periodic reviews of aggregate data and adverse events. These specific data and events include risks related to specimen collection and any reports of gastrointestinal intolerance. These data and events will be reviewed yearly.

A summary of the outcomes of these safety reviews along with accumulated adverse events and deviations will be submitted to the IRB as part of an annual progress report at the time of the Continuing Review submission.

9 Statistical Considerations

9.1 Statistical and Analytical Plans (SAP)

The primary endpoint will be an increase in microbiome diversity (as measured by Shannon diversity index) from baseline visit 1 to visit 2 after SCFA supplementation with an effect size of at least 0.60. Our preliminary data of NORA patients reveals an increase in alpha diversity after butyrate supplementation with an effect size of 0.63 (Cohen's *d*) (unpublished data). Using this effect size for a two-tailed Wilcoxon signed-rank test, to be powered at 80% with a 0.05 type I error rate, we would need 23 participants to see a difference in pre-post treatment response in alpha diversity. However, to be more conservative, we will aim for 25 participants in order to achieve an effect size of at least 0.6 while maintaining 80% power with a 0.05 type I error. This number of participants is in line with previous studies[11,21] and therefore should be sufficient for ours. These estimated sample sizes are based on before and after differences of the study group and we will not include a placebo group for the current proof-of-principle study. There will not be a formal SAP because due to the pilot nature of the proof of principle study.

Addition of a control group that do not take the SCFA supplement will not change the statistical analysis of the primary endpoint above since in the primary endpoint, the participants taking the SCFA supplement act as their

own controls. The additional control group will be used to explore secondary endpoints such as if there are gut microbial shifts after starting next line therapy as proposed by their treating physician.

It is expected that approximately 65 participants will be enrolled to produce at least 25 evaluable participants.

9.1.1 General Approach
This is a pilot proof of concept study.

9.1.2 Safety Analyses
Since the study is relatively small with no more than 65 subjects, there are no statistical measures for suspending enrollment or stopping the entire study.

9.1.3 Planned Interim Analysis
Not applicable.

9.1.3.1 Safety Review
Since the study is relatively small with no more than 65 subjects, there are no statistical measures for suspending enrollment or stopping the entire study.

9.1.3.2 Efficacy Review
Not applicable.

9.1.4 Multiple Comparison/Multiplicity
Not applicable.

9.1.5 Tabulation of Individual Response Data
Individual participant data will not be listed by measure and time point.

9.2 Sample Size
It is expected that approximately 65 participants will be enrolled to produce at least 25 evaluable participants.

The primary endpoint will be an increase in microbiome diversity (as measured by Shannon diversity index) from baseline visit 1 to visit 2 after SCFA supplementation with an effect size of at least 0.60. Our preliminary data of NORA patients reveals an increase in alpha diversity after butyrate supplementation with an effect size of 0.63 (Cohen's *d*) (unpublished data). Using this effect size for a two-tailed Wilcoxon signed-rank test, to be powered at 80% with a 0.05 type I error rate, we would need 23 participants to see a difference in pre-post treatment response in alpha diversity. However, to be more conservative, we will aim for 25 participants in order to achieve an effect size of at least 0.6 while maintaining 80% power with a 0.05 type I error. This number of participants is in line with previous studies[11,21] and therefore should be sufficient for ours. These estimated sample sizes are based on before and after differences of the study group.

10 Source Documents and Access to Source Data/Documents
Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents. If this is the case, it should be stated in this section what data will be collected on CRFs and what data will be collected from other sources.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct

such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

All information will be stored in the secure REDCap database.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethics/Protection of Human Subjects

11.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

11.2 Institutional Review Board

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. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

11.3 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

All data will be collected and stored on REDCap.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study participant’s contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NYU Langone Medical Center. This will not include the participant’s contact or identifying information. Rather, individual participants and their research data will be identified by a unique study

identification number. The study data entry and study management systems used by clinical sites and by NYU Langone Medical Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Langone Medical Center.

11.3.1 Research Use of Stored Human Samples, Specimens, or Data

- Intended Use: Samples and data collected under this protocol may be used to study the effects of oral supplementation with a SCFA on the gut microbiome and adaptive immune response of RA patients. No genetic testing will be performed.
- Storage: Access to stored samples will be limited using locked doors to the lab spaces. Samples and data will be stored using codes assigned by the investigators. Only investigators will have access to the samples and data.
- Tracking: Data will be tracked using REDCap
    - Disposition at the completion of the study: All stored samples will be maintained in the laboratory of the PI until further determination of usefulness or need for analysis validation. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.
    - Sample Donor Privacy and Confidentiality: Only study personnel authorized by the PI (Dr Jose U. Scher) will have a direct access to the sample storage facilities and samples.

11.4 Stored Specimens

All coded samples collected for the study, along with de-identified medical information, may continue to be used and stored under the conditions described in this protocol indefinitely unless subjects request, in writing, for samples to be destroyed.

The reasons for this is that if immunological and/or microbiological markers of interest are identified during this exploratory study, stored samples may be used for further analysis. Any future analysis that is not related to the current protocol will be submitted as a modification to the IRB or under a new study.

All samples will be taken directly from subjects and stored as described in section 7.2.1. Samples will be coded and only the NYU PI, Co-PI, and study coordinators will have access to the linking key between subject ID and subject identity. The PI, Co-PI, and research coordinators will have access to the banked samples.

12 Data Handling and Record Keeping

12.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the Study Staff under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Demographic, clinical data and laboratory data will be entered into REDCap, a password-protected database (as described above). Clinical data will be entered from the source documents by the Study Staff or directly into REDCap by the Study Staff.

12.2 Study Records Retention

The study records will remain stored for twenty years, or longer as New York State law permits, to allow the study to be completed, and to allow for retesting of samples if necessary.

12.3 Protocol Deviations
A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. It is the responsibility of the PI to identify and report protocol deviations within 10 working days of identification. All deviations must be addressed in study source documents and reported to the IRB.

12.4 Publication and Data Sharing Policy
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.

13 Study Finances
13.1 Funding Source
This study is financed through a grant from the Arthritis Foundation, the NYU Clinical and Translational Science Institute (CTSI) and internal Departmental funding. Results of data analysis may be shared with the Arthritis Foundation. No identifiers will be shared with the Arthritis Foundation.

13.2 Costs to the Participant
There is no cost to the participants in the study. The PI of this study will provide the supplement.

13.3 Participant Reimbursements or Payments
Participants will be given the equivalent of $40 in either prepaid gift card or prepaid debit card at screening/baseline, 1, and 2 month follow up visits for a total of $120 at completion of the study. Reimbursements may be made available for travel expenses, such as parking reimbursement, where appropriate. Participants may receive up to $40 for travel expenses per visit.

14 Study Administration
14.1 Study Leadership
The Executive Committee will govern the conduct of the study. It will be composed of the PI and the Co-Investigators. The Executive Committee will discuss the study progress and any issues that arise every 6 months throughout the duration of the study. The Study Team will be composed of the PI, Co-Investigator, Collaborators and Study Staff. The Study Team will be responsible for executing the study according to the protocol.

14.2 Use of Non-Traditional Volunteers
Volunteers will be trained by the study team and PI on the study protocol and study procedures. Volunteers are gaining insight into the clinical setting as well as observing topics of research and seeing patient interactions that will greatly benefit their journey in medicine. The volunteers will be trained on the protocol and receive training on shipping study material. Volunteers will not be a part of the consenting process.

15 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 study 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 study. The study leadership 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.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

16 References

1. Detert J, Bastian H, Listing J, et al. Induction therapy with adalimumab plus methotrexate for 24 weeks followed by methotrexate monotherapy up to week 48 versus methotrexate therapy alone for DMARD-naive patients with early rheumatoid arthritis: HIT HARD, an investigator-initiated study. Ann Rheum Dis 2013;72(6):844-50. DOI: 10.1136/annrheumdis-2012-201612.
2. Smolen JS, Emery P, Fleischmann R, et al. Adjustment of therapy in rheumatoid arthritis on the basis of achievement of stable low disease activity with adalimumab plus methotrexate or methotrexate alone: the randomised controlled OPTIMA trial. Lancet 2014;383(9914):321-32. DOI: 10.1016/S0140-6736(13)61751-1.
3. Emery P, Breedveld FC, Hall S, et al. Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis (COMET): a randomised, double-blind, parallel treatment trial. Lancet 2008;372(9636):375-82. DOI: 10.1016/S0140-6736(08)61000-4.
4. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nature Reviews Rheumatology 2020;16(3):145-154. DOI: 10.1038/s41584-020-0373-9.
5. Mizuno M, Noto D, Kaga N, Chiba A, Miyake S. The dual role of short fatty acid chains in the pathogenesis of autoimmune disease models. PLoS ONE 2017;12(2):1-15. DOI: 10.1371/journal.pone.0173032.
6. Kim DS, Kwon JE, Lee SH, et al. Attenuation of rheumatoid inflammation by sodium butyrate through reciprocal targeting of HDAC2 in osteoclasts and HDAC8 in T cells. Frontiers in Immunology 2018;9(JUL):1525-1525. DOI: 10.3389/fimmu.2018.01525.
7. Asarat M, Apostolopoulos V, Vasiljevic T, Donkor O. Short-chain fatty acids regulate cytokines and Th17/treg cells in human peripheral blood mononuclear cells in vitro. Immunological Investigations 2016;45(3):205-222. DOI: 10.3109/08820139.2015.1122613.
8. Roshanravan N, Mahdavi R, Alizadeh E, et al. Effect of Butyrate and Inulin Supplementation on Glycemic Status, Lipid Profile and Glucagon-Like Peptide 1 Level in Patients with Type 2 Diabetes: A Randomized Double-Blind, Placebo-Controlled Trial. Hormone and Metabolic Research 2017;49(11):886-891. DOI: 10.1055/s-0043-119089.
9. Säemann MD, Böhmig GA, Österreicher CH, et al. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. The FASEB Journal 2000;14(15):2380-2382. DOI: 10.1096/fj.00-0359fje.
10. Cleophas MCP, Ratter JM, Bekkering S, et al. Effects of oral butyrate supplementation on inflammatory potential of circulating peripheral blood mononuclear cells in healthy and obese males. Scientific Reports 2019;9(1):1-10. DOI: 10.1038/s41598-018-37246-7.
11. Facchin S, Vitulo N, Calgaro M, et al. Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease. Neurogastroenterology and Motility 2020. DOI: 10.1111/nmo.13914.
12. Artacho A, Isaac S, Nayak R, et al. The Pretreatment Gut Microbiome Is Associated With Lack of Response to Methotrexate in New-Onset Rheumatoid Arthritis. Arthritis and Rheumatology 2021;73(6):931-942. DOI: 10.1002/art.41622.
13. Healey G, Murphy R, Butts C, Brough L, Whelan K, Coad J. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: A randomised, double-blind, placebo-controlled, cross-over, human intervention study. British Journal of Nutrition 2018;119(2):176-189. DOI: 10.1017/S0007114517003440.
14. Alfa MJ, Strang D, Tappia PS, et al. A randomized trial to determine the impact of a digestion resistant starch composition on the gut microbiome in older and mid-age adults. Clinical Nutrition 2018;37(3):797-807. DOI: 10.1016/j.clnu.2017.03.025.
15. Serrano-Villar S, Vázquez-Castellanos JF, Vallejo A, et al. The effects of prebiotics on microbial dysbiosis, butyrate production and immunity in HIV-infected subjects. Mucosal Immunology 2017;10(5):1279-1293. DOI: 10.1038/mi.2016.122.
16. Clarke ST, Green-Johnson JM, Brooks SPJ, et al. β2-1 Fructan supplementation alters host immune responses in a manner consistent with increased exposure to microbial components: Results from a double-blinded, randomised, cross-over study in healthy adults. British Journal of Nutrition 2016;115(10):1748-1759. DOI: 10.1017/S0007114516000908.

17. Zamani B, Farshbaf S, Golkar HR, Bahmani F, Asemi Z. Synbiotic supplementation and the effects on clinical and metabolic responses in patients with rheumatoid arthritis : a randomised , double-blind , placebo-controlled trial. 2018(2017):1095-1102. DOI: 10.1017/S000711451700085X.
18. Zamani B, Golkar HR, Farshbaf S, et al. Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. International Journal of Rheumatic Diseases 2016;19(9):869-879. DOI: 10.1111/1756-185X.12888.
19. Häger J, Bang H, Hagen M, et al. The role of dietary fiber in rheumatoid arthritis patients: A feasibility study. Nutrients 2019;11(10). DOI: 10.3390/nu11102392.
20. Peres RS, Liew FY, Talbot J, et al. Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. Proceedings of the National Academy of Sciences of the United States of America 2015;112(8):2509-2514. DOI: 10.1073/pnas.1424792112.
21. West NP, Christophersen CT, Pyne DB, et al. Butyrylated starch increases colonic butyrate concentration but has limited effects on immunity in healthy physically active individuals. EIR. NPW, 2013.