

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

## Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised placebo-controlled trial

### The FLORA trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019231
Article Type:	Protocol
Date Submitted by the Author:	29-Aug-2017
Complete List of Authors:	Kragsnaes, Maja; Odense University Hospital, Department of Rheumatology; University of Southern Denmark, Odense Patient data Explorative Network (OPEN), Department of Clinical Institute Kjeldsen, Jens; Odense University Hospital, Department of Gastroenterology Horn, Hans; Odense University Hospital, Department of Rheumatology Munk, Heidi; Odense University Hospital, Department of Rheumatology Pedersen, Finn; Odense University Hospital, Department of Gastroenterology Holt, Hanne; Odense University Hospital, Department of Clinical Microbiology Pedersen, Jens Kristian; Odense University Hospital, Department of Rheumatology Holm, Dorte; Odense University Hospital, Department of Clinical Immunology Glerup, Henning; Silkeborg Regional Hospital, Diagnostic Centre Andersen, Vibeke; Hospital of Southern Jutland, IRS-Centre Sonderjylland; University of Southern Denmark, Institute of Molecular Medicine Fredberg, Ulrich; Silkeborg Regional Hospital, Diagnostic Centre Kristiansen, Karsten; University of Copenhagen, Laboratory of Genomics and Molecular Biomedicine, Department of Biology; BGI Christensen, Robin; Frederiksberg and Bispebjerg Hospital, Musculoskeletal Statistics Unit, Parker Institute Ellingsen, Torkell; Odense University Hospital, Department of Rheumatology
<b>Primary Subject Heading</b>:	Rheumatology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Psoriasis < DERMATOLOGY, Clinical trials < THERAPEUTICS, Faecal microbiota transplantation, Intestinal microbiota, Psoriatic arthritis

SCHOLARONE™  
Manuscripts

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1  
4  
5 2  
6  
7 3 Efficacy and safety of faecal microbiota transplantation in  
8 patients with psoriatic arthritis:  
9 4  
10 protocol for a 6-month, double-blind, randomised placebo-  
11 5  
12 controlled trial  
13 6  
14 7

15 7  
16 8  
17 8 The FLORA trial  
18 9  
19

20 10 *Kragsnaes MS<sup>1,2\*</sup>, Kjeldsen J<sup>3</sup>, Horn HC<sup>1</sup>, Munk HL<sup>1</sup>, Pedersen FM<sup>3</sup>, Holt HM<sup>4</sup>, Pedersen JK<sup>1</sup>, Holm*  
21 11 *DK<sup>5</sup>, Glerup H<sup>6</sup>, Andersen V<sup>7,8</sup>, Fredberg U<sup>6</sup>, Kristiansen K<sup>9,10</sup>, Christensen R<sup>11</sup>, Ellingsen T<sup>1</sup>.*  
22  
23 12

24 13 <sup>1</sup> Department of Rheumatology, Odense University Hospital, Denmark.

25 14 <sup>2</sup> Odense Patient data Explorative Network (OPEN), Department of Clinical Institute, University of Southern Denmark.

26 15 <sup>3</sup> Department of Gastroenterology, Odense University Hospital, Denmark.

27 16 <sup>4</sup> Department of Clinical Microbiology, Odense University Hospital, Denmark.

28 17 <sup>5</sup> Department of Clinical Immunology, Odense University Hospital, Denmark.

29 18 <sup>6</sup> Diagnostic Centre, Silkeborg Regional Hospital, Denmark.

30 19 <sup>7</sup> IRS-Centre Sonderjylland, Hospital of Southern Jutland, Denmark.

31 20 <sup>8</sup> Institute of Molecular Medicine, University of Southern Denmark.

32 21 <sup>9</sup> Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Denmark.

33 22 <sup>10</sup> Institute of Metagenomics, BGI-Shenzhen, Shenzhen, China.

34 23 <sup>11</sup> Musculoskeletal Statistics Unit, The Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

35 24  
36 24  
37 25 \* Corresponding author email address: [maja.kragsnaes@dadlnet.dk](mailto:maja.kragsnaes@dadlnet.dk).

## 1 ABSTRACT

2 **Introduction:** An unbalanced intestinal microbiota may mediate activation of the inflammatory  
3 pathways seen in psoriatic arthritis (PsA). A randomised, placebo-controlled trial of faecal  
4 microbiota transplantation (FMT) infused into the small intestine of PsA patients with active  
5 peripheral disease who are non-responsive to methotrexate (MTX) treatment will be conducted.  
6 The objective is to explore clinical aspects associated with FMT performed in PsA patients.

7  
8 **Methods and analysis:** The FLORA trial is a randomised, two-centre stratified, double-blind  
9 (patient, care provider and outcome assessor), placebo-controlled, parallel-group study. Eighty  
10 patients will be included and randomised (1:1) to either placebo (saline) or FMT provided from an  
11 anonymous healthy donor. Throughout the study, both groups will continue the weekly self-  
12 administered subcutaneous (s.c.) MTX treatment, remaining on the pre-inclusion dosage (15-25  
13 mg/week). The clinical measures of psoriasis and PsA disease activity used include the Health  
14 Assessment Questionnaire (2-page DI-HAQ), the Dermatology Quality of Life Index (DLQI), the  
15 Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index, the Psoriasis Area  
16 Severity Index (PASI), a dactylitis digit count, a swollen/tender joint count (66/68), plasma C-  
17 reactive protein as well as visual analogue scales for pain, fatigue, and patient and physician global  
18 assessments. The primary endpoint is the proportion of patients who experience a treatment  
19 failure during the 6-month trial period. The number of adverse events will be registered  
20 throughout the study.

21  
22 **Ethics and dissemination:** This is a proof-of-concept clinical trial and will be performed in  
23 agreement with Good Clinical Practice standards. Approvals have been obtained from the local  
24 Ethics Committee (DK-S-20150080) and the Danish Data Protection Agency (15/41684). The study  
25 has commenced in May 2017. Dissemination will be through presentations at national and  
26 international conferences and through publications in international peer-reviewed journals.

27  
28 **Trial registration number:** NCT03058900

### 29 30 **Strengths and limitations of this study**

- 31 • This is a double-blind, randomised, placebo-controlled trial of faecal microbiota  
32 transplantation in psoriatic arthritis (PsA).
- 33 • Subcutaneously administered MTX treatment.
- 34 • The primary endpoint is based on shared decision-making between patient and physician.
- 35 • Associated microbiome analyses can reveal novel insight into the PsA pathogenesis.
- 36 • A limitation of the study is that the content of the faecal transplant suspension cannot be  
37 fully standardized.

## 1 INTRODUCTION

2 Emerging data suggest a causal relationship between the intestinal microbiota and  
3 spondyloarthritis (SpA), thus, linking dysbiosis of the complex microbial communities with SpA  
4 pathogenesis.<sup>1-5</sup> Psoriatic arthritis (PsA) is one of five SpA categories in adults which also include  
5 ankylosing spondylitis, undifferentiated arthritis, reactive arthritis and arthritis associated with  
6 inflammatory bowel disease. While the association between the gut and the latter two disorders is  
7 well established,<sup>6</sup> only very recently, studies evaluating the faecal microbiota and the presence of  
8 subclinical gut inflammation in PsA patients have coupled this disease to a perturbation of the  
9 intestinal microbiota composition.<sup>7-12</sup>

10 PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum  
11 and a varied disease course.<sup>13</sup> The clinical manifestations include peripheral arthritis, enthesitis  
12 and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis,  
13 and dactylitis.<sup>14</sup> Nearly half of the patients with both early and established PsA also present with  
14 extra-musculoskeletal manifestations which can include bowel (16%), ocular, cardiovascular or  
15 urogenital involvement.<sup>15</sup> Without disease modifying intervention, 40-60% of PsA patients will  
16 develop erosive and deforming joint damage within a few years of disease onset.<sup>16</sup> Methotrexate  
17 (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug  
18 (csDMARD) for initial therapy.<sup>17</sup> However, a substantial number of patients does not benefit from  
19 such treatment.<sup>18</sup> Currently, other treatment options may include biological agents such as  
20 tumour necrosis factor (TNF- $\alpha$ ) inhibitors aiming to block some of the downstream molecular  
21 pathways driving the disease.<sup>19</sup> However, these drugs do not target the cause of PsA, which is  
22 believed to be multifactorial comprising genetic, immunological and environmental factors.<sup>20</sup> The  
23 interplay between these complex aetiological factors has yet to be fully understood.<sup>21,22</sup>

24 The classic pathophysiological concept of PsA is that it is an autoimmune disease of  
25 the skin and joints and that the pathological processes at both sites are driven by inflammatory  
26 responses involving the innate immune system, natural killer cells, T cells, and the expression of  
27 pro-inflammatory cytokines, including TNF- $\alpha$ , interleukin (IL)-1, interferon- $\gamma$ , IL-6, IL-12, IL-15, IL-18  
28 and the IL-17/IL-23 axis.<sup>23-27</sup> However, although microbial agents including dormant bacteria,  
29 bacterial products, mycobacteria and viral antigens have been implicated as potential  
30 initiators,<sup>28,29</sup> the true pathophysiological factors triggering the dysregulated immunological  
31 cascade underlying the disease remain to be identified.

32 Intriguingly, it has recently been suggested that mucosal sites exposed to a high load  
33 of bacterial antigens, in particular the gut, may represent the initial site of immunological  
34 tolerance break in PsA.<sup>30</sup> Indeed, under normal conditions the host and the microbiota live in  
35 harmony and benefit from their mutualistic relationship. However, alterations of the normal  
36 intestinal microbiota can affect mucosal immunity which, in turn, can induce local inflammation  
37 and elicit systemic effects at distant sites.<sup>31</sup> Mechanisms through which the intestinal microbiota  
38 may be involved in the pathogenesis of PsA include an abnormal activation of the gut-associated  
39 lymphoid tissue,<sup>32</sup> a decrease in regulatory T cell activity,<sup>33</sup> and/or an altered mucosal permeability  
40 thus compromising the capacity of the intestine to provide adequate containment of luminal  
41 microorganisms and molecules.<sup>34,35</sup> In support of these theories, several studies have documented  
42 subclinical gut inflammation in PsA patients.<sup>36-41</sup> Moreover, a recent study reported that several  
43 intestinal bacteria including *Akkermansia* and *Ruminococcus* were practically absent in PsA

1 patients. These commensal bacteria are, in fact, known to play an important role in maintaining  
2 gut homeostasis.<sup>42</sup>

### 3 4 **Rationale**

5 If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen  
6 in PsA,<sup>8</sup> modifying the intestinal microbiota could be a novel treatment strategy for this disease.<sup>1-  
7 3,43</sup> Faecal microbiota transplantation (FMT) is currently being used to restore the balance of the  
8 intestinal flora.<sup>44,45</sup> Particularly, this procedure has demonstrated more than 90% clinical  
9 resolution of recurrent or refractory *Clostridium difficile* infections.<sup>46-50</sup> Also, multiple FMTs seem  
10 to be able to induce remission in patients with inflammatory bowel disease (IBD).<sup>51</sup> Due to these  
11 results, FMT is now being tested as a potential novel treatment for other gastrointestinal and  
12 extra-intestinal diseases.<sup>52</sup> To the best of our knowledge, no study has yet ascertained the efficacy  
13 and safety of FMT in patients with inflammatory rheumatic diseases.

### 14 15 **Evidence-based research**

16 To avoid waste of research no new studies should be initiated without a systematic review of the  
17 existing evidence.<sup>53</sup> We performed a pragmatic search in the biomedical literature via Pubmed  
18 combining different related MeSH terms: ("Microbiota"[Mesh] OR "Fecal Microbiota  
19 Transplantation"[Mesh] OR "Faecal Microbiota Transplantation"[Mesh] OR "Gastrointestinal  
20 Microbiome"[Mesh]) AND (arthritis[tiab] OR "Arthritis"[Mesh] OR "Arthritis, Psoriatic"[Mesh] OR  
21 "Arthritis, Reactive"[Mesh] OR "Spondylarthritis"[Mesh] OR "Arthritis, Gouty"[Mesh] OR "Arthritis,  
22 Rheumatoid"[Mesh] OR "Psoriasis"[Mesh]). From the search revealing 122 citations it became  
23 clear that the majority of papers were reviews/editorials (74 [61%]), where the overall conclusion  
24 was that the main challenges are to uncover the cause-effect relationship between the intestinal  
25 microbiota and rheumatic diseases, and to investigate the potential of microbiome-targeting  
26 strategies.<sup>1,3,5,6,20,32,43,54-60</sup> Also from the published literature it became evident that to date only  
27 three clinical interventional studies trying to modify the intestinal microbiota in arthritis patients  
28 have been performed; one in entheses-related arthritis using probiotics (n = 8),<sup>61</sup> one in juvenile  
29 idiopathic arthritis using exclusive enteral nutrition (n = 7),<sup>62</sup> and one in rheumatoid arthritis  
30 patients using probiotics in a placebo-controlled setting (n = 60).<sup>63</sup> Following the intervention, the  
31 latter two studies showed a moderate anti-inflammatory effect on the number of active joints, on  
32 the Disease Activity Score of 28 joints (DAS-28), and on the C-reactive protein concentrations. In  
33 the first study reporting no beneficial effects, the probiotics did not change the microbiota. No  
34 clinical trials performing FMT on arthritic patients were identified.

### 35 36 **Objective**

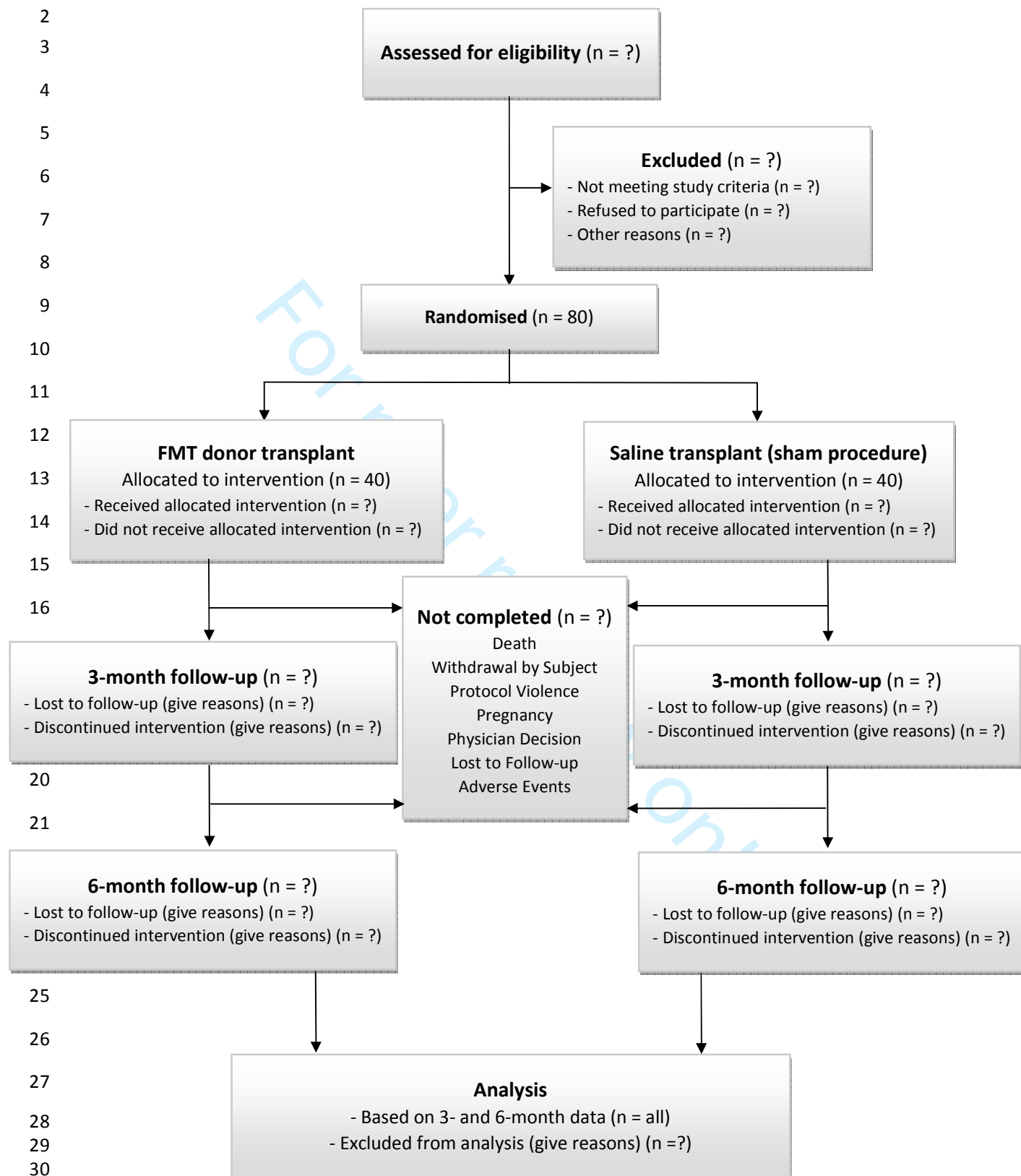
37 By conducting a double-blind, randomised, placebo-controlled trial, the objective of this study is to  
38 explore whether FMT is more effective than placebo in reducing disease activity in PsA patients  
39 with active peripheral arthritis concomitantly treated with weekly subcutaneously administered  
40 MTX. In addition, extensive bacteria taxonomic and metagenomic analyses will be performed on  
41 faecal samples before and after the FMT to get an indication of the functional capacity of the  
42 intestinal microbiota.

1

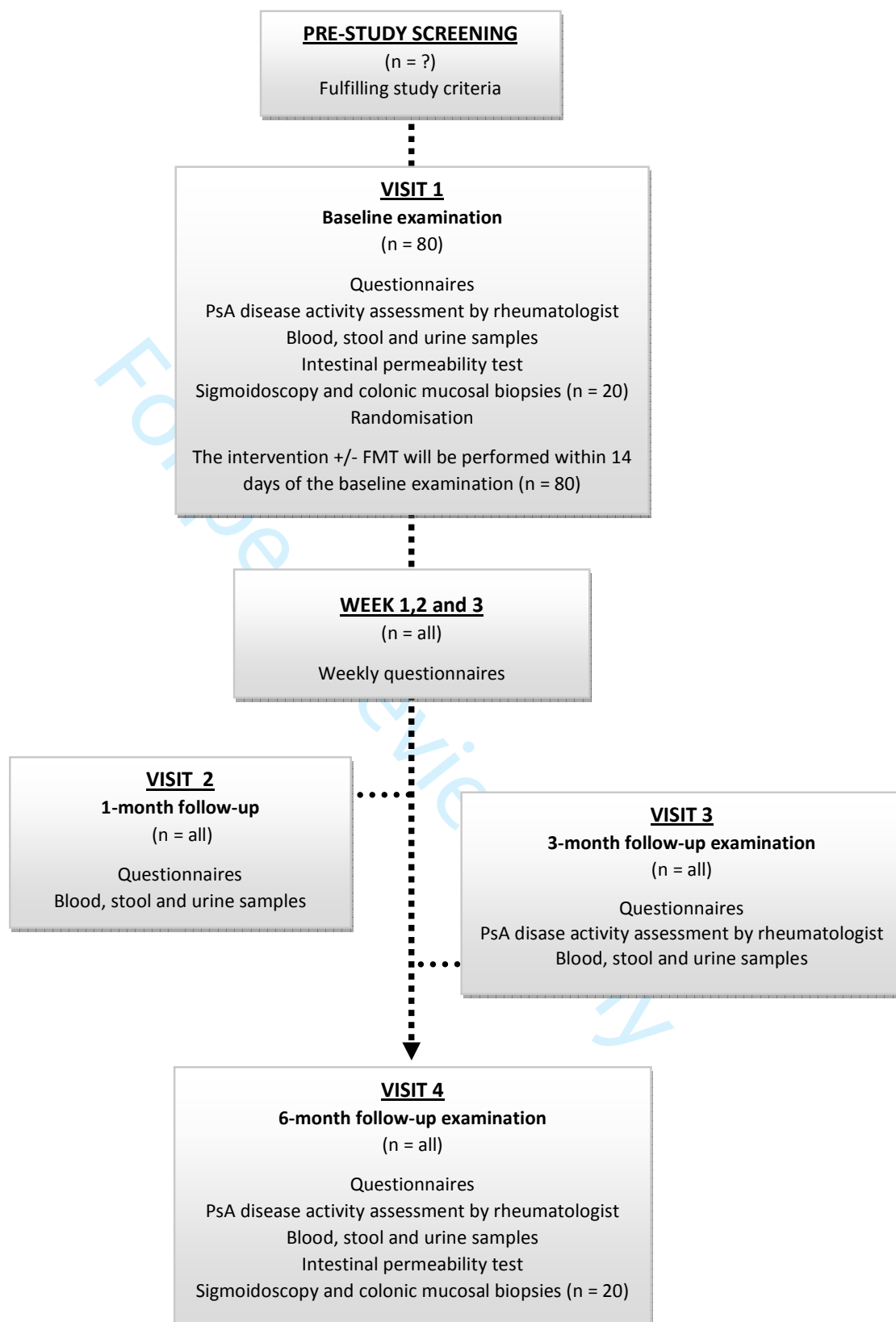
2  
3  
4 **METHODS AND ANALYSIS**5  
6 **Trial design**

7  
8 This is a randomised – patient, physician and outcome-assessor blinded, placebo-controlled, 6-  
9 month trial, which will be followed by an open-label extension trial for a minimum of 2 years.  
10 Patients will be randomly assigned in a 1:1 ratio to receive FMT or placebo (sham procedure).  
11 Outcome assessment will be based on follow-up by a rheumatologist and is scheduled to occur  
12 after 3 and 6 months (primary end-point evaluation), see Figure 1 and Figure 2.  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





**Figure 1.** Flow diagram of the randomised, placebo-controlled trial



**Figure 2.** Participation timeline and general characteristics of each visit.

## 1 Participants

2 Patients fulfilling the inclusion criteria will be offered participation. No treatment with biologics  
3 within 6 months, and no systemic and/or local intra-articular or peritendinous steroid injections,  
4 or non-MTX csDMARD treatment, or antibiotics are allowed within 3 months of inclusion. Non  
5 Steroidal Anti-Inflammatory Drugs (NSAIDs) must be paused within 14 days of study inclusion, and  
6 throughout the 6-month follow-up period. Patients, who do not wish to participate, will be  
7 characterised by sex and age. The recruitment has commenced in May 2017 and will continue until  
8 2019.

### 9 Psoriatic arthritis patients

10 A total of 80 PsA patients will be enrolled, and they will have to meet the following criteria:

#### 11 *Inclusion criteria:*

- 12 • Diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR).<sup>64</sup>
- 13 • Presence of active peripheral arthritis defined as  $\geq 3$  swollen joints.
- 14 • Subcutaneously administered MTX treatment ( $\geq 15$ mg/week (maximal tolerable dosage))  
15 for a minimum of 3 months prior to study inclusion.
- 16 • Age 18 to 70 years.

#### 17 *Exclusion criteria:*

- 18 • Other rheumatic inflammatory diseases than PsA.
- 19 • Clinical suspicion of current axial disease activity.
- 20 • History of severe MTX toxicity or allergic reactions.
- 21 • Biological treatment within the last 6 months.
- 22 • Non-MTX DMARD treatment within 3 months of inclusion.
- 23 • Systemic and/or local intra-articular or peritendinous steroid injections within 3 months of  
24 inclusion.
- 25 • NSAIDs within fourteen days.
- 26 • Antibiotics within 3 months of inclusion.
- 27 • Inflammatory bowel disease, celiac disease, food allergy, or other intestinal diseases.
- 28 • Pregnant or breastfeeding women.
- 29 • Not wishing to participate or unsuited for project evaluation.

### 30 Stool donors

31 The stool donor corps will consist of three to five anonymous (to the recipient) donors who must  
32 be healthy as assessed by a screening questionnaire, and be active members of the Danish blood  
33 donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m<sup>2</sup>, and an average alcohol  
34 intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of  
35 donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior  
36

1 to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie  
2 diets), and the donor must not be in a stressful life period. Before joining the stool donor corps,  
3 each potential donor will go through a screening process including stool analyses for faecal  
4 calprotectin and enteric pathogens (*Aeromonas*, *Campylobacter*, *C. difficile*, diarrhoeagenic  
5 *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, and multidrug-resistant  
6 bacteria, parasites including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA),  
7 *Cryptosporidium* (DNA) and *Giardia* (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus  
8 (RNA), human adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood  
9 tests for C-reactive protein (CRP), white blood cell count, haemoglobin, albumin, alanine  
10 aminotransferase (ALAT), glomerular filtration rate (eGFR) and coeliac disease, and blood test for  
11 infectious agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus  
12 (IgM), hepatitis A, B, C and E, tuberculosis (QuantiFERON® TB-Gold test), syphilis, human  
13 immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies),  
14 and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing  
15 the screening tests, the donor will donate stool for the next month after which, the donor will  
16 have to pass the screening programme once more before the stool can be released for  
17 transplantation.

## 18 19 **Interventions**

### 20 *Overall study interventions*

21 The FMT will be an add-on strategy for PsA patients with active joint disease despite ongoing  
22 treatment with weekly subcutaneously administered MTX. Therefore, all enrolled PsA patients will  
23 continue their MTX treatment throughout the study, and they will remain on the same individual  
24 dosage that they received at the time of study inclusion (a minimum of 15 mg/week cf. the patient  
25 inclusion criteria) in addition to folic acid supplement. Paracetamol and tramadol in recommended  
26 dosages are allowed during the trial but no NSAIDs can be taken.

### 27 28 *Active and sham comparator*

29 Patients will be randomised into two groups with an allocation ratio of active-to-placebo  
30 treatment of 1:1. The active comparator group (n = 40) will have an FMT with healthy donor  
31 faeces-suspension (250 mL) containing 50 g donor faeces, saline (NaCl 0.9%) and glycerol (10%),  
32 whereas, the sham comparator group (n = 40) will be treated with an identical appearing sham  
33 procedure where the transplant solution will consist of 250 mL brown coloured (brown food  
34 colourant) isotonic saline (NaCl 0.9%).

### 35 36 *Preparing the FMT suspension*

37 Donors will collect faeces at home and transport it in a cooling bag to the study site within 1 hour.  
38 Faeces will be sieved to remove particulate material, followed by dilution in sterile saline (0.9%  
39 NaCl) and 10% glycerol. The FMT suspension will be stored at - 80 °C until use. On the day of the  
40 FMT transplantation, the transplant suspension (250 mL) will be thawed to 37 °C and subsequently  
41 apportioned into five 50 mL syringes.

### *FMT procedure*

The FMT will take place within 14 days (preferably 7 days) of the baseline clinical examination. The evening prior to the FMT, patients will take one dose of oral proton-pump inhibitor. They will meet at the Department of Gastroenterology after a six-hour fast. A total of 250 mL transplant suspension (active or placebo) will be installed in the duodenum using an oral-duodenal tube. The correct placement of the tube will be confirmed using gastroscopic guidance.

### *Treatment strategy for FMT non-responders*

Patients who present with increased disease activity during follow-up will, depending on the clinical presentation, be offered another treatment strategy which may include local intra-articular steroid injections, change to another csDMARD or biological treatment. If the patient accept such treatment changes, this will be characterised as FMT treatment failure according to the primary outcome definition (one intra-articular steroid injection is allowed).

### *MTX toxicity and drop-outs*

Blood tests for MTX toxicity will be performed in accordance with our current clinical practice. In case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other diseases that contraindicate MTX treatment, MTX dosage will be decreased or the treatment will be paused. These patients will remain in the study (unless their condition contraindicates this), and they will be analysed as members of the treatment group to which they were randomised using intention-to-treat-type analyses.

## **Outcomes**

### *Primary Outcome Measure:*

Treatment failure [Time Frame: 6 months (+/- 14 days)]

Proportion of patients in each group who experience treatment failure according to shared decision making between patient and rheumatologist defined as at least one of the following:

- Need for more than one intra-articular glucocorticoid injection due to disease activity.
- Need for change to other csDMARDs (e.g., oral leflunomide or sulfasalazin) according to the updated Danish guideline treatment due to disease activity.
- Need for biologic treatment according to the updated Danish treatment guideline due to severe disease activity.

### *Secondary Outcome Measures:*

Change from baseline in the Short Health Assessment Questionnaire (2-page HAQ)<sup>65,66</sup>

[Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire<sup>67</sup> [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

1  
2  
3  
4 2 Changes from baseline in patient reported gastrointestinal side effects [Time Frame: 1 week, 2  
5 3 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
6  
7 4

8 5 Other non-gastrointestinal patient reported side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4  
9 6 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
10  
11 7

12  
13 8 Proportion of patients in each group achieving the American College of Rheumatology (ACR)<sup>68</sup>  
14 9 Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
15

16 10 I. ACR20 response criteria<sup>69</sup>

17 11 II. ACR50 response criteria<sup>70</sup>

18 12 III. ACR70 response criteria<sup>70</sup>  
19  
20  
21 13

22  
23 14 Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)<sup>68</sup>  
24 15 [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
25  
26 16

27  
28 17 Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis  
29 18 Index<sup>68</sup> in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7  
30 19 days), 6 months (+/- 14 days)]  
31  
32 20

33 21 Change from baseline in the Psoriasis Area Severity Index (PASI)<sup>71</sup> in the subset of patients who  
34 22 have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days) ]  
35  
36 23

37  
38 24 Change from baseline in the number of digits affected with dactylitis in the subset of patients who  
39 25 have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
40  
41 26

42 27 Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]  
43  
44 28

45 29 Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14  
46 30 days)]  
47  
48 31

49 32 *Tertiary (exploratory secondary) outcomes:* Proportion of patients in each group achieving changes  
50 33 in plasma CRP, changes in tender point count,<sup>72</sup> changes in faecal bacteria composition and  
51 34 metabolism, changes in intestinal permeability,<sup>73</sup> changes in plasma orosomucoid, changes in  
52 35 plasma and faecal calprotectin,<sup>74</sup> changes in serum 1,25-dihydroxyvitamin D, changes in  
53 36 cardiovascular risk factors including Body Mass Index (BMI), blood pressure, plasma triglyceride,  
54 37 plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, and HbA<sub>1c</sub> levels,  
55  
56  
57  
58  
59  
60

1  
2 1 changes in specific circulating inflammatory markers (i.e. cytokines, adipokines, and chemokines),  
3 2 and macroscopic and microscopic inflammatory changes of the colonic mucosa, see Table 1.  
4 3

#### 4 **Safety**

5 5 The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild  
6 6 diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the  
7 7 procedure.<sup>75</sup> A recent systematic review on the adverse events of FMT identified 50 relevant  
8 8 studies with a total of 1,089 patients. In this review, the incidences of serious adverse events  
9 9 (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The  
10 10 SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%),  
11 11 death (0.3%), auto-immune diseases and FMT procedure related injury.<sup>76</sup> Although most of the  
12 12 patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection  
13 13 and/or IBD), these findings warrant caution when performing FMT; especially when introducing  
14 14 the procedure in a new patient population. In addition, the potential long term side effects  
15 15 following FMT remains largely unknown.<sup>77</sup> Still, when strict donor screening is conducted and the  
16 16 procedure is performed by experienced practitioners, FMT is in general considered safe, and even  
17 17 elderly patients with a poor medical condition and multiple co morbidities as well as  
18 18 immunosuppressed patients have been proven to tolerate the FMT procedure well.<sup>78-82</sup>

19 19 In the present study, we will carefully monitor and evaluate safety by means of open  
20 20 assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be  
21 21 monitored until resolution, stabilisation or until it has been shown that the study intervention is  
22 22 not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events,  
23 23 version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events.  
24 24 Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool  
25 25 type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a  
26 26 week for the first month following the randomised intervention. Routine blood screening for MTX  
27 27 toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by  
28 28 the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject  
29 29 incidence rates of all treatment-emergent AE will be tabulated by system organ class and  
30 30 preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant  
31 31 treatment-emergent adverse events, will also be provided. For the long-term extension portion of  
32 32 this study, exposure adjusted event rates will be summarised.  
33 33

#### 34 **Sample size and power considerations**

35 35 For a comparison of two independent binomial proportions using the Pearson's chi-squared  
36 36 statistic with a Chi-square approximation (a two-sided significance level of 0.05), a sample size of  
37 37 40 PsA patients per group has a power of 90% (0.895) if we assume that the proportions of  
38 38 treatment failures are 35% (FMT-active group) and 70% (FMT-sham group), respectively.  
39 39 Consequently, the inclusion of 80 PsA patients allocated (1:1) to two treatment arms is believed to  
40 40 be sufficient to reveal any difference of clinical importance between treatment groups (i.e., an

1 NNT <3 patients). Data will be analysed with the STATA statistical package (version 15; StataCorp  
2 LP), and SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA).

3 Assuming that there will be some attrition during the 6-month trial period, we also  
4 estimated how much drop out would be possible while still having a reasonable statistical power  
5 (80%): a total sample size of 62 PsA patients assuming a comparable level of withdrawals (31  
6 patients completing in each group) achieves a power of at least 0.8 with the proportion of  
7 treatment failures indicated above; i.e., even if we experience a drop-out rate of 20%, our trial will  
8 have 80% chance of detecting the intentional difference between groups  
9

### 10 **Randomisation, allocation concealment and blinding**

11 The randomisation was conducted using central-computer randomisation. Patients will be  
12 randomly allocated (1:1) to receive either a FMT or a placebo saline transplant (sham procedure).  
13 The randomisation lists was generated by the trial statistician and uploaded to the REDCap  
14 database by an independent data manager who will not be involved in any other aspects of the  
15 trial. Eligible patients will - after signing informed consent - be assigned randomly in permuted  
16 blocks with varying sizes of 4 and 6, according to computer-generated random numbers (SAS  
17 programming via SAS PROC PLAN), to undergo either FMT or a saline (sham) procedure using  
18 stratification for centre. The randomisation of each patient will be implemented by the local trial  
19 coordinator and allocation will be concealed as this is done independent of the pre-determined  
20 sequence generation (i.e. randomisation). The patients, care providers and outcome assessors will  
21 remain unaware of the group assignments, and only de-identified codes will be used to link  
22 participants to their data during the study to maintain their confidentiality.  
23

### 24 **Data collection, management and confidentiality**

25 Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central  
26 REDCap<sup>83</sup> database hosted by Odense Patient data Explorative Network (OPEN) at Odense  
27 University Hospital. Data obtained during the clinical examination will be entered directly into the  
28 database. Also, patient questionnaires will be fulfilled directly into the database. Access to the  
29 study data will be restricted, and a password system will be utilized to control access. All  
30 information about the patients' health and other private matters is covered by confidentiality. The  
31 authorisation from the Danish Data Protection Agency has been secured.  
32

### 33 **Statistical methods**

34 The full analysis set will consist of all randomised participants (i.e., the Intention to treat  
35 population). Participants will be analysed according to their randomised treatment group.  
36 Descriptive statistics will be provided for demographics, and baseline characteristics. The summary  
37 statistics of continuous variables will include: N, mean, standard deviation, median, interquartiles,  
38 and range. All summaries presenting frequencies and incidences will include counts, % and N,  
39 where N is the total number of participants in the corresponding arm.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1           The pre-specified efficacy analyses will be based data from the full-analysis set,  
2           which include all patients who underwent randomisation, have had their baseline measurement  
3           performed, and who have received the initial transplant (independent of group). The safety  
4           analysis set will include all patients who were randomly assigned to a study group and had  
5           exposure to a transplant (independent of group). Missing values will be imputed with the of a non-  
6           responder imputation by use of the baseline-observation-carried-forward method for  
7           measurements made after baseline. Thus, missing data for dichotomous endpoints will also be  
8           imputed using a “null responder” imputation, assuming that the patient did not have any benefit  
9           from being enrolled in the trial (e.g., for the primary endpoint will assume that the patient had a  
10          treatment failure).

11           Categorical changes for dichotomous end points will be analysed with the use of  
12          logistic regression with the model including treatment and centre as class effects. For continuous  
13          outcome measures an analysis of covariance (ANCOVA) model will be used to analyse mean  
14          changes in continuous end points. The model will include treatment, centre, with the baseline  
15          value of the relevant variable as a covariate. Sensitivity analyses, will be performed to assess the  
16          robustness of the primary analyses, including “worst” and “best” case imputation, repeated-  
17          measures and multiple-imputation analyses, using model-based approaches; repeated measures  
18          linear mixed models will also be used to model the potential group-dependent trajectories over  
19          time.

20           Additionally, completer analyses will be performed on those who complete 6 months  
21          of treatment. During follow-up, any medical treatments which could potentially modify the  
22          intestinal microbiota including antibiotics will be reported, but will not affect the statistical  
23          analysis. Statistical estimates will be calculated as odds ratios (OR) for the dichotomous variables  
24          and difference between means for continuous outcomes reported with 95% confidence intervals  
25          (95% CI). Two-sided confidence intervals, and P-values for primary, secondary and exploratory  
26          outcomes will be computed and will not be adjusted for multiplicity.

27           Exploratory stratified analyses will investigate whether the treatment effect varies  
28          with I) the faecal microbiota analysis performed at follow-up compared with baseline (+/- long-  
29          term changes in the intestinal microbiota and intestinal inflammation); and II) the demographic  
30          match (sex, age) between the stool donor and the recipient. Non-responders will represent the  
31          outcome group not fulfilling the primary outcome measure. Differences in demographics and  
32          baseline disease activity between this treatment-failure subpopulation and the remaining group  
33          will be examined in order to identify potential predictors for poor responders. Patients not  
34          participating in the follow-up examination will be classified as "drop-outs", and if possible, the  
35          reason for not participating will be registered.

Activity/assessment	Pre-study screening	Visit 1 Baseline	Week 1, 2 and 3	Visit 2 1 month	Visit 3 3 months	Visit 4 6 months
Patients	n = ?	n = 80	n = all	n = all	n = all	n = all
Screening log	x					
Inclusion/exclusion form	x					
Consent form		x				
Randomisation		x				
Study-composed questionnaire		x	x	x	x	x
Patient global (VAS 0-100 mm)		x	x	x	x	x
Patient fatigue (VAS 0-100 mm)		x	x	x	x	x
Patient pain (VAS 0-100 mm)		x	x	x	x	x
HAQ		x	x	x	x	x
BASDAI		x			x	x
BASFAI		x			x	x
DLQI		x	x	x	x	x
Gastrointestinal symptom diary		x	x	x	x	x
Eating habits questionnaire		x				
Clinical examination:						
- Height (m)		x				
- Weight (kg)		x			x	x
- Blood pressure (mmHg)		x			x	x
- Psoriasis Area Severity Index		x			x	x
- SPARCC Enthesitis Score		x			x	x
- Swollen joint count (66)		x			x	x
- Tender joint count (68)		x			x	x
- Doctors global (VAS 0-100 mm)		x			x	x
- BASMI		x			x	x
- Tender point count		x			x	x
Interview (AEs)				x	x	x
Blood sample analysis:						
- C-reactive protein (mg/L)		x		x	x	x
- Orosomucoid (g/L)		x		x	x	x
- Calprotectin		x		x	x	x
- 1,25-dihydroxyvitamin D (nmol/L)		x		x	x	x
- TSH (miu/L)		x				x
- Hgb (mmol/L)		x				x
- Triglyceride (mmol/L)		x				x
- LDL-cholesterol (mmol/L)		x				x
- HDL-cholesterol (mmol/L)		x				x
- Total-cholesterol (mmol/L)		x				x
- HbA <sub>1c</sub> (mmol/mol)		x				x
- HLA-B27 status (+/-)		x				
- Serology tests for <i>Yersinia</i> , <i>Campylobacter</i> , <i>Salmonella</i> (+/-)		x				
Faecal calprotectin		x		x	x	x
Faecal microbiota analysis		x		x	x	x
Sigmoidoscopy and mucosa biopsy		x				x
Stool, blood, and urine samples (biobank)		x		x	x	x
Intestinal permeability test		x				x
Intervention (+/- FMT)		x				
Serious adverse event forms				x		

**Table 1.** Protocol schedule of forms and procedures

## 1 ETHICS AND DISSEMINATION

2 This study is designed as a proof-of-concept clinical trial and will be performed in agreement with  
3 GCP-standards, and in accordance with the ethical standards of the responsible committee on  
4 human experimentation (institutional and national) and with the Helsinki Declaration (64th, 2013).  
5 The relevance of the study, the design and the recruitment strategy were evaluated with three  
6 patient research partners (PRPs), and alterations especially in primary outcome and recruitment  
7 strategy were embedded. Furthermore, a minimum of two PRPs (participating in the study) will be  
8 involved in the discussion regarding the progress of the recruitment phase and results, and will be  
9 offered the opportunity to comment on the manuscript draft. The Regional Committees on Health  
10 Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency  
11 (15/41684) have approved the study protocol, and the trial has been registered with  
12 ClinicalTrials.gov (NCT03058900). The Danish Health and Medicines Authority does not classify the  
13 FMT procedure as a medical intervention, and has had no objection to the use of FMT for this  
14 study and patient category. Thus, no GCP auditing is legally required. A report describing any  
15 potential side effects and adverse events will be submitted to the Ethics Committee yearly.

16 Although the Danish Health Authorities, for the time being, do not classify donor  
17 faecal microbiota as tissue, all steps of the stool donor recruitment, stool donation and FMT  
18 preparation will be in accordance with the Danish Tissue Law to ensure that the quality and safety  
19 standards laid down in the Danish Legislation BEK nr 764 of May 26, 2015 (implementing Directive  
20 2004/23/EC) are met. Three to five stool donors will be recruited from the South Danish  
21 Transfusion Service & Tissue Center, Department of Clinical Immunology, Odense University  
22 Hospital, and they will be carefully screened for potentially transmissible infections and other  
23 conditions associated with gut microbiota function before their stool can be released for FMT.  
24 Being a stool donor is voluntary, and no compensation fee will be given. Furthermore, to ensure  
25 donor traceability, each patient in the active treatment arm will only receive microbiota from one  
26 donor. Also, frozen samples will be clearly labeled with a unique donation code based on the ISBT  
27 128 coding and labeling system, and the release of the final product will adhere to the standards  
28 for tissue and blood donation.

29 Due to the well-documented risk of permanent joint destruction and occurrence of  
30 extra-articular manifestations in the PsA disease course, identification of new treatment modalities  
31 and biomarkers is essential to help the physician to slow down the disease development or  
32 ultimately to prevent it. All PsA patients participating in this study have significant activity in their  
33 joint disease despite treatment with the current guideline treatment and first-line drug, MTX, for  
34 this condition. This patient population will therefore benefit greatly from new treatment options.  
35 Consequently, when weighing the pros and cons of this study, this trial should be performed from a  
36 scientific and ethical perspective.

37 Dissemination will occur through presentations at national and international  
38 conferences and publications in international peer-reviewed journal(s).

39  
40

## DISCUSSION

Recent years have seen growing recognition of the complexity of the role of the microbiota in shaping the immune system and its potential effects for health and disease.<sup>22,84,85</sup> In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.<sup>86-89</sup> Intriguingly, an abnormal intestinal bacteria composition has been observed in PsA patients, and this association has fostered theories linking intestinal dysbiosis and PsA joint inflammation.<sup>90</sup> Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic disease are causal related,<sup>55</sup> and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.<sup>54,91</sup> We expect that this double-blind, randomised, placebo-controlled trial will shed new light on this highly relevant topic.

This is the first time that the efficacy and safety of FMT is being investigated in MTX immunosuppressed patients with rheumatic diseases. Subsequently, no data on the feasibility of conducting FMTs in the rheumatological setting is, so far, available. Nor do we know whether one FMT will be sufficient, or whether it should be repeated shortly after the first intervention to normalise the alterations of the intestinal microbiota, which would expectedly enhance any potential anti-inflammatory effects. In the present proof-of concept clinical trial, the FMT procedure is considered an add-on to the current guideline intervention and first-line drug, MTX. Therefore, from a pragmatic and ethical perspective, we have decided to perform only one FMT (or sham procedure) in each patient even if we are well aware that this approach may not be adequate to achieve long-lasting effects. Indeed, in patients with inflammatory bowel diseases it appears that performing a frequent dosing-regime repeating the FMT procedure up to five times a week for eight weeks provided the best results.<sup>51,92,93</sup> Hence, in contrast to the treatment of *C. difficile* infections where the microbiota is pushed past the point of homeostasis and can be restored following only one FMT,<sup>47</sup> the chronic nature of PsA and other autoimmune and inflammatory diseases, and the somewhat lesser degree of intestinal dysbiosis, may make the host microbiota more resistant to long-lasting modifications. Nevertheless, we strongly believe that the FMT procedure in the present study will be sufficient to boost the effects of MTX so that the participants who are all MTX-non-responders prior to study enrolment will achieve disease control without needing to add or switch to other non-MTX medication.

In the present trial, the primary endpoint is defined as the occurrence of treatment failure according to shared decision making between patient and physician evaluated at 6 months following the randomised intervention (FMT versus sham procedure). Shared decision making is a process in which both patient and health professional make a decision, taking into account the best evidence of available treatment options and the patient's values and preferences. This approach is considered a key element in the management of rheumatic diseases.<sup>94</sup> In addition to the primary end point evaluation at 6 months, patients will be asked to fill out a weekly questionnaire regarding side effects as well as skin and arthritis symptoms during the first month following the randomised intervention to reveal any short-term effects on patient-reported outcomes.

Next, only patients with active peripheral PsA will be included. One reason for this is that this will be the first time that FMT is performed on rheumatic patients. Therefore, it seems

1 reasonable only to enrol patients who have not had adequate effect from the initial guideline  
2 treatment (MTX), and consequently, on an individual basis could benefit the most from  
3 participating in new experimental clinical trials. Also, since patients need to have at least three  
4 swollen joints, we expect that we will be able to detect treatment effects of clinical importance.  
5 The fact that we do not include recent onset treatment naive patients will, of course, limit our  
6 ability to generalise our findings unto the entire PsA patient population. Indeed, in a recent  
7 randomised controlled trial of FMT in ulcerative colitis patients, participants with a recent  
8 diagnosis (< 1 year) were statistically significantly more likely to respond to FMT compared with  
9 those with longer disease duration.<sup>92</sup> That patients will have to subcutaneously administer MTX  
10 for at least three months prior to study enrolment will ensure that low intestinal MTX absorption  
11 is excluded as a potential effect modifier for the poor MTX response. In addition, as many drugs,  
12 including MTX, seem to affect the intestinal microbiological milieu,<sup>95-98</sup> bypassing the intestine  
13 during MTX administration will ensure that no local non-disease related effects on the intestinal  
14 microbiota will occur.

15 A great challenge when conducting a trial of FMT is that for the present being there  
16 is a lack of both national and international recommendations guiding the regulation and the best  
17 clinical practices for donor screening, stool sample handling and preparation of the FMT  
18 suspension.<sup>99-101</sup> Indeed, the variability in faecal bacterial communities can complicate or  
19 undermine treatment efficacy. This variability stems from both biological variation and variation  
20 introduced by sample handling. A recent study reported that oxygen exposure degraded faecal  
21 bacterial communities, whereas freeze-thaw cycles and lag time between donor defecation and  
22 transplant preparation had much more limited effects.<sup>102</sup> Given that many intestinal bacteria are  
23 obligate anaerobe, including many beneficial bacteria potentially possessing anti-inflammatory  
24 effects, exposure to oxygen during the preparation of FMT may potentially compromise the  
25 therapeutic value of FMT in PsA and other inflammatory diseases. Therefore, although frozen  
26 faecal preparations of stool suspended into physiological saline and glycerol have proven just as  
27 effective as fresh stool in treating *C. difficile* infections,<sup>103</sup> the optimal transplant preparation  
28 method in treating inflammatory diseases remain to be established.

29 Our stool handling setup is in line with the prevailing practice, which includes mixing  
30 and filtration of the stool suspension and adding saline and glycerol as a cryopreservative before  
31 storage at minus 80 °C.<sup>101</sup> In addition, we have sought to limit the oxygen exposure during  
32 transport by placing the donor stool within a plastic bag, which is subsequently put into a tightly  
33 closed small plastic container. Supplementary, during preparation the solution will not be  
34 homogenized for more than 10 seconds. Nevertheless, the lack of optimal anaerobe conditions  
35 during stool handling could possibly undermine the therapeutic potential of our FMT procedure.  
36 Furthermore, although we aim to use 50g of faeces for each transplant, we acknowledge that the  
37 exact weight between donations could vary with an estimated +/- 5 g. Also, due to the wide  
38 variability in microbial content in stool between donations, the content cannot be fully  
39 standardized, and may likely differ between each FMT procedure. However, to meet this challenge  
40 we will collect and store samples from each donation which will enable us to determine the  
41 microbiota composition of each donation in case some donations prove more effective than  
42 others.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 Stool donor selection is another important issue that needs to be addressed. The  
2 composition of the normal microbiota composition has only recently been mapped,<sup>104</sup> and the  
3 existence of a limited number of well-balanced host–microbial symbiotic states, where one or  
4 more bacteria species are considered the main functional driver(s), have been identified using  
5 clustering of metagenomic sequences.<sup>105</sup> Still, the most favourable donor microbiota composition  
6 for treating inflammatory diseases has yet to be determined. Therefore, it also remains to be  
7 established whether donors with a high stool bacteria diversity should be preferred over isolation  
8 of specific bacteria, or if pooled stool samples from several donors outperforms a single-donor  
9 transplant.<sup>51,106</sup> We have chosen to use only single donations from three to five different  
10 anonymous stool donors to ensure donor traceability and to enable us to identify any individual  
11 donor-specific microbial effects. Also, since host intrinsic-, environmental-, dietary- and  
12 medication factors have been associated with gut bacteria composition and  
13 functionality,<sup>95,96,107,108</sup> the donors must eat a balanced diet, not be overweight or take any  
14 medications or be physical or psychological stressed, smoke or consume alcohol during the  
15 donation period in order to limit the risk of transferring "abnormal" microbiota to the recipients.  
16 These donor criteria have been set for safety reasons, and we acknowledge, that this could  
17 potentially limit the inter-donor microbiota diversity due to shared lifestyle characteristics.

18 Another factor to keep in mind is the concept of matching donor and recipient, which  
19 may be of importance for enhancing the colonisation capabilities of the donor microbial  
20 communities. In fact, Rossen et al<sup>93</sup> did find that in patients with ulcerative colitis, the microbiota  
21 of FMT responders shifted to their respective donors, whereas non-responders did not. Li et al<sup>109</sup>  
22 reported that donor bacteria strains established extensively in the recipient and persisted for at  
23 least 3 months with a negligible decline of donor-strain populations detected between 45 days  
24 and 3 months following FMT in metabolic syndrome patients. However, they also found that  
25 recipients receiving the same donor transplant displayed varying degrees of microbiota transfer,  
26 indicating individual patterns of microbiome resistance and donor-recipient compatibilities. In  
27 addition, host genetics is known to effect the gut microbiota,<sup>110</sup> and animal models have shown  
28 that sex<sup>111</sup> and age<sup>112</sup> also can be potentially modifiers of the gut bacteria composition. These  
29 observations may prove to be of importance for the outcome of FMT in inflammatory diseases.<sup>113</sup>  
30 However, whether sex- and/or age-matching between donor and recipient is crucial for a  
31 successful FMT in humans remains to be enlighten. Therefore, in the present study, no donor-  
32 recipient matching will be conducted. However, a subgroup analysis will be performed to reveal  
33 any trend that could indicate better results in sex- or age-match cases.

34 Furthermore, as the interactions between the microbiota and the host are influenced  
35 by cooperation and competition between pathogenic and commensal microbes and multiple  
36 environmental variables, the lifestyle of the recipient following the FMT may be of importance.  
37 Nevertheless, due to the uncertainty of how to define the optimal lifestyle and the lack of  
38 knowledge on how different lifestyle factors may interfere with the microbiota, we have decided  
39 that the patients in the present study will not have to adhere to any predefined lifestyle "regime"  
40 or diet following the randomised intervention. However, every participant will fulfil an eating habit  
41 questionnaire at the beginning of the trial.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 Finally, non-bacteria microorganisms such as bacteriophages, viruses and fungi may  
2 also be of importance when targeting components of the microbiota or host cells for therapeutic  
3 purposes.<sup>114-116</sup> Other complicating factors may include the composition of other microbiological  
4 niches such as the oral, lung, genitourinary, and skin microbiota.<sup>117,118</sup> Indeed, the latter could  
5 likely prove to be of significance in patients with skin psoriasis. However, these factors will not be  
6 assessed in the present study.

## 8 CONCLUSION

9 Autoimmune and inflammatory rheumatic diseases are characterised by an abnormal gut bacteria  
10 composition. This trial has the potential to substantially expand the growing body of literature on  
11 the role of the intestinal microbiota in PsA, thereby enhancing our understanding of cause and  
12 effect. The results of this study, when completed, may be exploited for biomarker discovery, and  
13 for diagnostic and therapeutic purposes.

## 15 AUTHORS' CONTRIBUTION

16 T. Ellingsen, M.S. Kragtsnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for  
17 the study. T. Ellingsen and M.S. Kragtsnaes are the principal investigators and wrote the first study  
18 protocol draft. T. Ellingsen and M.S. Kragtsnaes were responsible for all communication with the  
19 scientific ethical committee, the Danish Data Protection Agency and the National Health Board. T  
20 Ellingsen is the responsible party and sponsor. M.S. Kragtsnaes, T. Ellingsen, H.C. Horn, J.K.  
21 Pedersen, H.L. Munk, and U. Fredberg sat up the inclusion and exclusion criteria for the psoriatic  
22 arthritis patients, and the latter five rheumatologists are conducting all the clinical examinations. J.  
23 Kjeldsen, F.M. Pedersen, and H. Glerup discussed and planned the FMT procedure, and they are  
24 conducting the FMTs and the sigmoidoscopies (obtaining mucosae tissue samples). D.K. Holm and  
25 H.M. Holt helped set up the donor screening programme, and they were responsible for  
26 conducting this programme and performing the microbiological and immunological tests. V.  
27 Andersen and K. Kristiansen are responsible for the microbiome and omics analyses, and  
28 have advised on how the tissue collection should be performed and what kind of tissue would be  
29 relevant to collect. R. Christensen has written the analysis plan and will be responsible for the final  
30 statistics analyses. In conclusion, all participants designated as authors have contributed to the  
31 conception and design of the study, and have critically either drafted or revised the first draft of  
32 the study protocol and the protocol paper. Also, all authors have approved the final version before  
33 submission.

## 35 REGISTRATION

36 The trial has been registered with ClinicalTrials.gov (NCT03058900).

## 38 FUNDING STATEMENT

39 This work was supported by the Region of Southern Denmark, Odense University Hospital, the  
40 Danish Regions, University of Southern Denmark, the Danish Rheumatism Association, the

1  
2 1 Psoriasis Research Fund, and Odense Patient Explorative data Network (OPEN). Musculoskeletal  
3 2 Statistics Unit at the Parker Institute, Bispebjerg and Frederiksberg Hospital (R. Christensen), is  
4 3 supported by a core grant from the Oak Foundation (OCAY-13-309). K. Kristiansen is supported by  
5 4 BGI-Research, BGI-Shenzhen, China.  
6  
7  
8  
9

#### 10 6 COMPETETING INTEREST STATEMENT

11 7  
12 8 None of the team members of this research project has declared any potential conflict of interest.  
13  
14  
15

#### 16 9 ACKNOWLEDGEMENTS

17  
18 10 A great thanks to Lene Albjerg, biomedical laboratory technologist and quality control manager at  
19 11 the Dept. of Clinical Immunology, Odense University Hospital, Denmark, for her expertise and  
20 12 dedicated work regarding the practical handling of the FMT suspension.  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



## References

1. Scher JU, Littman DR, Abramson SB. Microbiome in Inflammatory Arthritis and Human Rheumatic Diseases. *Arthritis Rheumatol*. 2016;68:35-45.
2. Asquith M, Elewaut D, Lin P et al. The role of the gut and microbes in the pathogenesis of spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2014;28:687-702.
3. Stoll ML. Gut microbes, immunity, and spondyloarthritis. *Clin Immunol*. 2015;159:134-142.
4. Costello ME, Ciccio F, Willner D et al. Intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol*. 2014.
5. Yang L, Wang L, Wang X et al. A Possible Role of Intestinal Microbiota in the Pathogenesis of Ankylosing Spondylitis. *Int J Mol Sci*. 2016;17.
6. Manasson J, Scher JU. Spondyloarthritis and the microbiome: new insights from an ancient hypothesis. *Curr Rheumatol Rep*. 2015;17:10.
7. De WK, Debusschere K, Beeckman S et al. Integrating the pathogenesis of spondyloarthritis: gut and joint united? *Curr Opin Rheumatol*. 2015;27:189-196.
8. Eppinga H, Konstantinov SR, Peppelenbosch MP et al. The microbiome and psoriatic arthritis. *Curr Rheumatol Rep*. 2014;16:407.
9. Coit P, Sawalha AH. The human microbiome in rheumatic autoimmune diseases: A comprehensive review. *Clin Immunol*. 2016;170:70-79.
10. Ciccio F, Ferrante A, Guggino G et al. The role of the gastrointestinal tract in the pathogenesis of rheumatic diseases. *Best Pract Res Clin Rheumatol*. 2016;30:889-900.
11. Tito RY, Cypers H, Joossens M et al. Brief Report: Dialister as a Microbial Marker of Disease Activity in Spondyloarthritis. *Arthritis Rheumatol*. 2017;69:114-121.
12. Eppinga H, Sperna Weiland CJ, Thio HB et al. Similar Depletion of Protective Faecalibacterium prausnitzii in Psoriasis and Inflammatory Bowel Disease, but not in Hidradenitis Suppurativa. *J Crohns Colitis*. 2016.
13. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. *N Engl J Med*. 2017;376:2095-2096.
14. Terslev L, Naredo E, Iagnocco A et al. Defining enthesitis in spondyloarthritis by ultrasound: results of a Delphi process and of a reliability reading exercise. *Arthritis Care Res (Hoboken)*. 2014;66:741-748.
15. Peluso R, Iervolino S, Vitiello M et al. Extra-articular manifestations in psoriatic arthritis patients. *Clin Rheumatol*. 2014.
16. Gladman DD. Psoriatic arthritis. *Dermatol Ther*. 2009;22:40-55.
17. Gossec L, Coates LC, De WM et al. Management of psoriatic arthritis in 2016: a comparison of EULAR and GRAPPA recommendations. *Nat Rev Rheumatol*. 2016;12:743-750.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
18. Kingsley GH, Kowalczyk A, Taylor H et al. A randomized placebo-controlled trial of methotrexate in psoriatic arthritis. *Rheumatology (Oxford)*. 2012;51:1368-1377.
  19. Gossec L, Smolen JS, Ramiro S et al. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis*. 2016;75:499-510.
  20. Asquith M, Rosenbaum JT. The interaction between host genetics and the microbiome in the pathogenesis of spondyloarthropathies. *Curr Opin Rheumatol*. 2016;28:405-412.
  21. Benham H, Robinson PC, Baillet AC et al. Role of genetics in infection-associated arthritis. *Best Pract Res Clin Rheumatol*. 2015;29:213-225.
  22. Shamriz O, Mizrahi H, Werbner M et al. Microbiota at the crossroads of autoimmunity. *Autoimmun Rev*. 2016;15:859-869.
  23. Lories RJ, de VK. Is psoriatic arthritis a result of abnormalities in acquired or innate immunity? *Curr Rheumatol Rep*. 2012;14:375-382.
  24. Mortezaei M, Thiele R, Ritchlin C. The joint in psoriatic arthritis. *Clin Exp Rheumatol*. 2015;33:20-25.
  25. Acosta Felquer ML, Fitzgerald O. Peripheral joint involvement in psoriatic arthritis patients. *Clin Exp Rheumatol*. 2015;33:26-30.
  26. Matt P, Lindqvist U, Kleinau S. Up-regulation of CD64-expressing monocytes with impaired FcγR function reflects disease activity in polyarticular psoriatic arthritis. *Scand J Rheumatol*. 2015;44:464-473.
  27. Al-Mossawi MH, Ridley A, Kiedel S et al. The role of natural killer cells, gamma delta T-cells and other innate immune cells in spondyloarthritis. *Curr Opin Rheumatol*. 2013;25:434-439.
  28. Ryan C, Korman NJ, Gelfand JM et al. Research gaps in psoriasis: opportunities for future studies. *J Am Acad Dermatol*. 2014;70:146-167.
  29. Berthelot JM, de la Cochetiere MF, Potel G et al. Evidence supporting a role for dormant bacteria in the pathogenesis of spondylarthritis. *Joint Bone Spine*. 2013;80:135-140.
  30. Abdollahi-Roodsaz S, Abramson SB, Scher JU. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nat Rev Rheumatol*. 2016;12:446-455.
  31. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature*. 2016;535:75-84.
  32. Ciccía F, Rizzo A, Triolo G. Subclinical gut inflammation in ankylosing spondylitis. *Curr Opin Rheumatol*. 2016;28:89-96.
  33. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med*. 2009;361:888-898.
  34. Ciccía F, Guggino G, Rizzo A et al. Dysbiosis and zonulin upregulation alter gut epithelial and vascular barriers in patients with ankylosing spondylitis. *Ann Rheum Dis*. 2017.
  35. Pianta A, Arvikar SL, Strle K et al. Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. *J Clin Invest*. 2017.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 36. Scher JU, Ubeda C, Artacho A et al. Decreased bacterial diversity characterizes an altered gut microbiota in psoriatic arthritis and resembles dysbiosis of inflammatory bowel disease. *Arthritis Rheumatol.* 2014.
  - 4 37. Lindqvist U, Kristjansson G, Pihl-Lundin I et al. Patients with psoriatic arthritis have an increased number of lymphocytes in the duodenal mucosa in comparison with patients with psoriasis vulgaris. *J Rheumatol.* 2006;33:924-927.
  - 7 38. Scarpa R, Manguso F, D'Arienzo A et al. Microscopic inflammatory changes in colon of patients with both active psoriasis and psoriatic arthritis without bowel symptoms. *J Rheumatol.* 2000;27:1241-1246.
  - 10 39. Van PL, Van den Bosch F, Mielants H et al. Mucosal inflammation in spondylarthritides: past, present, and future. *Curr Rheumatol Rep.* 2011;13:409-415.
  - 12 40. Schatteman L, Mielants H, Veys EM et al. Gut inflammation in psoriatic arthritis: a prospective ileocolonoscopy study. *J Rheumatol.* 1995;22:680-683.
  - 14 41. Ciccia F, Guggino G, Ferrante A et al. Interleukin-9 Overexpression and Th9 Polarization Characterize the Inflamed Gut, the Synovial Tissue, and the Peripheral Blood of Patients With Psoriatic Arthritis. *Arthritis Rheumatol.* 2016;68:1922-1931.
  - 17 42. Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunol.* 2017;18:2.
  - 19 43. Gill T, Asquith M, Rosenbaum JT et al. The intestinal microbiome in spondyloarthritis. *Curr Opin Rheumatol.* 2015;27:319-325.
  - 21 44. Kump PK, Krause R, Allerberger F et al. Faecal microbiota transplantation-the Austrian approach. *Clin Microbiol Infect.* 2014;20:1106-1111.
  - 23 45. Cammarota G, Pecere S, Ianiro G et al. Principles of DNA-Based Gut Microbiota Assessment and Therapeutic Efficacy of Fecal Microbiota Transplantation in Gastrointestinal Diseases. *Dig Dis.* 2016;34:279-285.
  - 26 46. Austin M, Mellow M, Tierney WM. Fecal microbiota transplantation in the treatment of Clostridium difficile infections. *Am J Med.* 2014;127:479-483.
  - 28 47. van NE, Vrieze A, Nieuwdorp M et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med.* 2013;368:407-415.
  - 30 48. Cammarota G, Masucci L, Ianiro G et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. *Aliment Pharmacol Ther.* 2015;41:835-843.
  - 33 49. Lee CH, Steiner T, Petrof EO et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. *JAMA.* 2016;315:142-149.
  - 36 50. Li YT, Cai HF, Wang ZH et al. Systematic review with meta-analysis: long-term outcomes of faecal microbiota transplantation for Clostridium difficile infection. *Aliment Pharmacol Ther.* 2016;43:445-457.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 51. Paramsothy S, Kamm MA, Kaakoush NO et al. Multidonor intensive faecal microbiota  
2 transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet*.  
3 2017;389:1218-1228.
- 4 52. Cui B, Feng Q, Wang H et al. Fecal microbiota transplantation through mid-gut for refractory  
5 Crohn's disease: Safety, feasibility and efficacy trial results. *J Gastroenterol Hepatol*. 2014.
- 6 53. Lund H, Brunnhuber K, Juhl C et al. Towards evidence based research. *BMJ*. 2016;355:i5440.
- 7 54. Ciccio F, Ferrante A, Triolo G. Intestinal dysbiosis and innate immune responses in axial  
8 spondyloarthritis. *Curr Opin Rheumatol*. 2016;28:352-358.
- 9 55. Bravo-Blas A, Wessel H, Milling S. Microbiota and arthritis: correlations or cause? *Curr Opin  
10 Rheumatol*. 2016;28:161-167.
- 11 56. Kabeerdoss J, Sandhya P, Danda D. Gut inflammation and microbiome in spondyloarthritis.  
12 *Rheumatol Int*. 2016;36:457-468.
- 13 57. Costello ME, Robinson PC, Benham H et al. The intestinal microbiome in human disease and how it  
14 relates to arthritis and spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2015;29:202-212.
- 15 58. Bazso A, Szodoray P, Suto G et al. Importance of intestinal microenvironment in development of  
16 arthritis. A systematic review. *Immunol Res*. 2015;61:172-176.
- 17 59. Taneja V. Arthritis susceptibility and the gut microbiome. *FEBS Lett*. 2014;588:4244-4249.
- 18 60. Rosenbaum JT, Lin P, Asquith M et al. Does the microbiome play a causal role in spondyloarthritis?  
19 *Clin Rheumatol*. 2014;33:763-767.
- 20 61. Aggarwal A, Sarangi AN, Gaur P et al. Gut microbiome in children with enthesitis-related arthritis in  
21 a developing country and the effect of probiotic administration. *Clin Exp Immunol*. 2017;187:480-  
22 489.
- 23 62. Berntson L, Hedlund-Treutiger I, Alving K. Anti-inflammatory effect of exclusive enteral nutrition in  
24 patients with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2016;34:941-945.
- 25 63. Zamani B, Golkar HR, Farshbaf S et al. Clinical and metabolic response to probiotic supplementation  
26 in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Int J  
27 Rheum Dis*. 2016;19:869-879.
- 28 64. Taylor W, Gladman D, Helliwell P et al. Classification criteria for psoriatic arthritis: development of  
29 new criteria from a large international study. *Arthritis Rheum*. 2006;54:2665-2673.
- 30 65. Thorsen H, Hansen TM, McKenna SP et al. Adaptation into Danish of the Stanford Health  
31 Assessment Questionnaire (HAQ) and the Rheumatoid Arthritis Quality of Life Scale (RAQoL). *Scand  
32 J Rheumatol*. 2001;30:103-109.
- 33 66. Brodszky V, Pentek M, Balint PV et al. Comparison of the Psoriatic Arthritis Quality of Life (PsAQoL)  
34 questionnaire, the functional status (HAQ) and utility (EQ-5D) measures in psoriatic arthritis: results  
35 from a cross-sectional survey. *Scand J Rheumatol*. 2010;39:303-309.
- 36 67. Zachariae R, Zachariae C, Ibsen H et al. Dermatology life quality index: data from Danish inpatients  
37 and outpatients. *Acta Derm Venereol*. 2000;80:272-276.

- 1  
2 1 68. Fransen J, Antoni C, Mease PJ et al. Performance of response criteria for assessing peripheral  
3 2 arthritis in patients with psoriatic arthritis: analysis of data from randomised controlled trials of two  
4 3 tumour necrosis factor inhibitors. *Ann Rheum Dis*. 2006;65:1373-1378.  
5  
6 4 69. Felson DT, Anderson JJ, Boers M et al. American College of Rheumatology. Preliminary definition of  
7 5 improvement in rheumatoid arthritis. *Arthritis Rheum*. 1995;38:727-735.  
8  
9 6 70. Felson DT, Anderson JJ, Lange ML et al. Should improvement in rheumatoid arthritis clinical trials  
10 7 be defined as fifty percent or seventy percent improvement in core set measures, rather than  
11 8 twenty percent? *Arthritis Rheum*. 1998;41:1564-1570.  
12  
13 9 71. Faria JR, Araao AR, Jimenez LM et al. Inter-rater concordance study of the PASI (Psoriasis Area and  
14 10 Severity Index). *An Bras Dermatol*. 2010;85:625-629.  
15  
16 11 72. Jensen OK, Callesen J, Nielsen MG et al. Reproducibility of tender point examination in chronic low  
17 12 back pain patients as measured by intrarater and inter-rater reliability and agreement: a validation  
18 13 study. *BMJ Open*. 2013;3.  
19  
20 14 73. Mishra A, Makharia GK. Techniques of functional and motility test: how to perform and interpret  
21 15 intestinal permeability. *J Neurogastroenterol Motil*. 2012;18:443-447.  
22  
23 16 74. Klingberg E, Carlsten H, Hilme E et al. Calprotectin in ankylosing spondylitis--frequently elevated in  
24 17 feces, but normal in serum. *Scand J Gastroenterol*. 2012;47:435-444.  
25  
26 18 75. Kelly CR, Kahn S, Kashyap P et al. Update on Fecal Microbiota Transplantation 2015: Indications,  
27 19 Methodologies, Mechanisms, and Outlook. *Gastroenterology*. 2015;149:223-237.  
28  
29 20 76. Wang S, Xu M, Wang W et al. Systematic Review: Adverse Events of Fecal Microbiota  
30 21 Transplantation. *PLoS One*. 2016;11:e0161174.  
31  
32 22 77. Rossen NG, MacDonald JK, de Vries EM et al. Fecal microbiota transplantation as novel therapy in  
33 23 gastroenterology: A systematic review. *World J Gastroenterol*. 2015;21:5359-5371.  
34  
35 24 78. Girotra M, Garg S, Anand R et al. Fecal Microbiota Transplantation for Recurrent Clostridium  
36 25 difficile Infection in the Elderly: Long-Term Outcomes and Microbiota Changes. *Dig Dis Sci*.  
37 26 2016;61:3007-3015.  
38  
39 27 79. Gweon TG, Kim J, Lim CH et al. Fecal Microbiota Transplantation Using Upper Gastrointestinal Tract  
40 28 for the Treatment of Refractory or Severe Complicated Clostridium difficile Infection in Elderly  
41 29 Patients in Poor Medical Condition: The First Study in an Asian Country. *Gastroenterol Res Pract*.  
42 30 2016;2016:2687605.  
43  
44 31 80. Agrawal M, Aroniadis OC, Brandt LJ et al. The Long-term Efficacy and Safety of Fecal Microbiota  
45 32 Transplant for Recurrent, Severe, and Complicated Clostridium difficile Infection in 146 Elderly  
46 33 Individuals. *J Clin Gastroenterol*. 2016;50:403-407.  
47  
48 34 81. Di BS, Gouliouris T, Petrosillo N. Fecal microbiota transplantation (FMT) for Clostridium difficile  
49 35 infection: focus on immunocompromised patients. *J Infect Chemother*. 2015;21:230-237.  
50  
51 36 82. Webb BJ, Brunner A, Ford CD et al. Fecal microbiota transplantation for recurrent Clostridium  
52 37 difficile infection in hematopoietic stem cell transplant recipients. *Transpl Infect Dis*. 2016.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 1 83. Harris PA, Taylor R, Thielke R et al. Research electronic data capture (REDCap)--a metadata-driven  
3 2 methodology and workflow process for providing translational research informatics support. *J*  
4 3 *Biomed Inform.* 2009;42:377-381.  
5  
6 4 84. Thaïss CA, Zmora N, Levy M et al. The microbiome and innate immunity. *Nature.* 2016;535:65-74.  
7  
8 5 85. McLean MH, Dieguez D, Jr., Miller LM et al. Does the microbiota play a role in the pathogenesis of  
9 6 autoimmune diseases? *Gut.* 2015;64:332-341.  
10  
11 7 86. Longman RS, Yang Y, Diehl GE et al. Microbiota: host interactions in mucosal homeostasis and  
12 8 systemic autoimmunity. *Cold Spring Harb Symp Quant Biol.* 2013;78:193-201.  
13  
14 9 87. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin*  
15 10 *Gastroenterol.* 2015;31:69-75.  
16  
17 11 88. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune  
18 12 system. *Science.* 2012;336:1268-1273.  
19  
20 13 89. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health  
21 14 and disease. *Nat Rev Immunol.* 2009;9:313-323.  
22  
23 15 90. Van de Wiele T, Van Praet JT, Marzorati M et al. How the microbiota shapes rheumatic diseases.  
24 16 *Nat Rev Rheumatol.* 2016;12:398-411.  
25  
26 17 91. Butto LF, Haller D. Dysbiosis in intestinal inflammation: Cause or consequence. *Int J Med Microbiol.*  
27 18 2016.  
28  
29 19 92. Moayyedi P, Surette MG, Kim PT et al. Fecal Microbiota Transplantation Induces Remission in  
30 20 Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology.*  
31 21 2015;149:102-109.  
32  
33 22 93. Rossen NG, Fuentes S, van der Spek MJ et al. Findings From a Randomized Controlled Trial of Fecal  
34 23 Transplantation for Patients With Ulcerative Colitis. *Gastroenterology.* 2015;149:110-118.  
35  
36 24 94. Smolen JS, Schols M, Braun J et al. Treating axial spondyloarthritis and peripheral spondyloarthritis,  
37 25 especially psoriatic arthritis, to target: 2017 update of recommendations by an international task  
38 26 force. *Ann Rheum Dis.* 2017.  
39  
40 27 95. Zhernakova A, Kurilshikov A, Bonder MJ et al. Population-based metagenomics analysis reveals  
41 28 markers for gut microbiome composition and diversity. *Science.* 2016;352:565-569.  
42  
43 29 96. Falony G, Joossens M, Vieira-Silva S et al. Population-level analysis of gut microbiome variation.  
44 30 *Science.* 2016;352:560-564.  
45  
46 31 97. Forslund K, Hildebrand F, Nielsen T et al. Disentangling type 2 diabetes and metformin treatment  
47 32 signatures in the human gut microbiota. *Nature.* 2015;528:262-266.  
48  
49 33 98. Zhang X, Zhang D, Jia H et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis  
50 34 and partly normalized after treatment. *Nat Med.* 2015;21:895-905.  
51  
52 35 99. Woodworth MH, Neish EM, Miller NS et al. Laboratory Testing of Donors and Stool for Fecal  
53 36 Microbiota Transplantation for Recurrent *C. difficile* Infection. *J Clin Microbiol.* 2017.  
54  
55 37 100. Costello SP, Tucker EC, La BJ et al. Establishing a Fecal Microbiota Transplant Service for the  
56 38 Treatment of *Clostridium difficile* Infection. *Clin Infect Dis.* 2016;62:908-914.  
57  
58  
59  
60

- 1  
2 1 101. Cammarota G, Ianiro G, Tilg H et al. European consensus conference on faecal microbiota  
3 2 transplantation in clinical practice. *Gut*. 2017;66:569-580.  
4  
5 3 102. Chu ND, Smith MB, Perrotta AR et al. Profiling Living Bacteria Informs Preparation of Fecal  
6 4 Microbiota Transplantations. *PLoS One*. 2017;12:e0170922.  
7  
8 5 103. Satokari R, Mattila E, Kainulainen V et al. Simple faecal preparation and efficacy of frozen inoculum  
9 6 in faecal microbiota transplantation for recurrent *Clostridium difficile* infection--an observational  
10 7 cohort study. *Aliment Pharmacol Ther*. 2015;41:46-53.  
11  
12 8 104. Li J, Jia H, Cai X et al. An integrated catalog of reference genes in the human gut microbiome. *Nat*  
13 9 *Biotechnol*. 2014;32:834-841.  
14  
15 10 105. Arumugam M, Raes J, Pelletier E et al. Enterotypes of the human gut microbiome. *Nature*.  
16 11 2011;473:174-180.  
17  
18 12 106. Kazerouni A, Wein LM. Exploring the Efficacy of Pooled Stools in Fecal Microbiota Transplantation  
19 13 for Microbiota-Associated Chronic Diseases. *PLoS One*. 2017;12:e0163956.  
20  
21 14 107. Vandeputte D, Falony G, Vieira-Silva S et al. Stool consistency is strongly associated with gut  
22 15 microbiota richness and composition, enterotypes and bacterial growth rates. *Gut*. 2016;65:57-62.  
23  
24 16 108. Ley RE. The gene-microbe link. *Nature*. 2015;518:S7.  
25  
26 17 109. Li SS, Zhu A, Benes V et al. Durable coexistence of donor and recipient strains after fecal microbiota  
27 18 transplantation. *Science*. 2016;352:586-589.  
28  
29 19 110. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut  
30 20 microbiome. *Nat Rev Microbiol*. 2011;9:279-290.  
31  
32 21 111. Markle JG, Frank DN, Mortin-Toth S et al. Sex differences in the gut microbiome drive hormone-  
33 22 dependent regulation of autoimmunity. *Science*. 2013;339:1084-1088.  
34  
35 23 112. Xiao L, Estelle J, Kiilerich P et al. A reference gene catalogue of the pig gut microbiome. *Nat*  
36 24 *Microbiol*. 2016;16161.  
37  
38 25 113. Markle JG, Frank DN, Adeli K et al. Microbiome manipulation modifies sex-specific risk for  
39 26 autoimmunity. *Gut Microbes*. 2014;5:485-493.  
40  
41 27 114. Mills S, Shanahan F, Stanton C et al. Movers and shakers: influence of bacteriophages in shaping  
42 28 the mammalian gut microbiota. *Gut Microbes*. 2013;4:4-16.  
43  
44 29 115. Pfeiffer JK, Virgin HW. Viral immunity. Transkingdom control of viral infection and immunity in the  
45 30 mammalian intestine. *Science*. 2016;351.  
46  
47 31 116. Underhill DM, Pearlman E. Immune Interactions with Pathogenic and Commensal Fungi: A Two-  
48 32 Way Street. *Immunity*. 2015;43:845-858.  
49  
50 33 117. Castelino M, Eyre S, Moat J et al. The skin microbiome in psoriatic arthritis: methodology  
51 34 development and pilot data. *Lancet*. 2015;385 Suppl 1:S27.  
52  
53 35 118. Scher JU, Joshua V, Artacho A et al. The lung microbiota in early rheumatoid arthritis and  
54 36 autoimmunity. *Microbiome*. 2016;4:60.  
55  
56 37  
57 38  
58  
59  
60

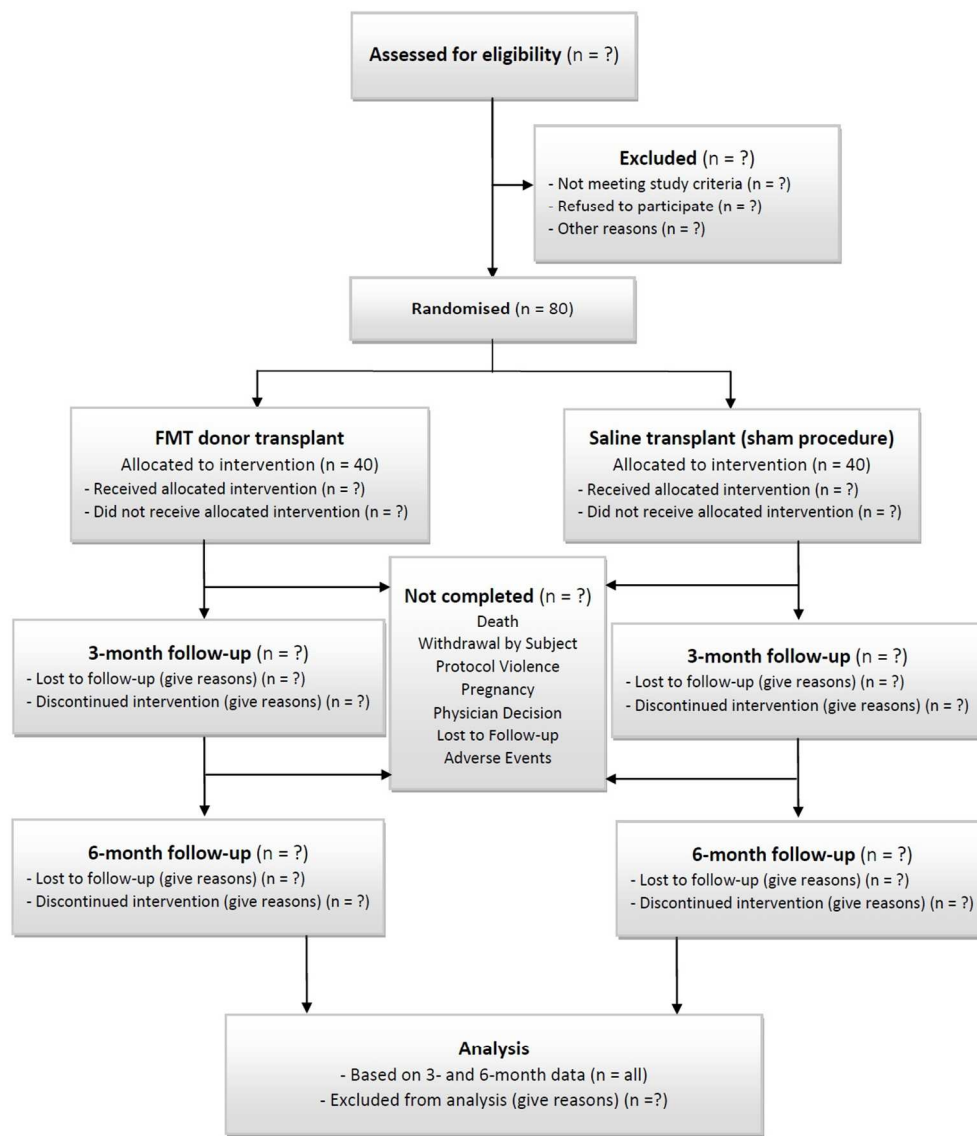


Figure. 1. Flow diagram of the randomised, placebo-controlled trial

174x201mm (192 x 192 DPI)



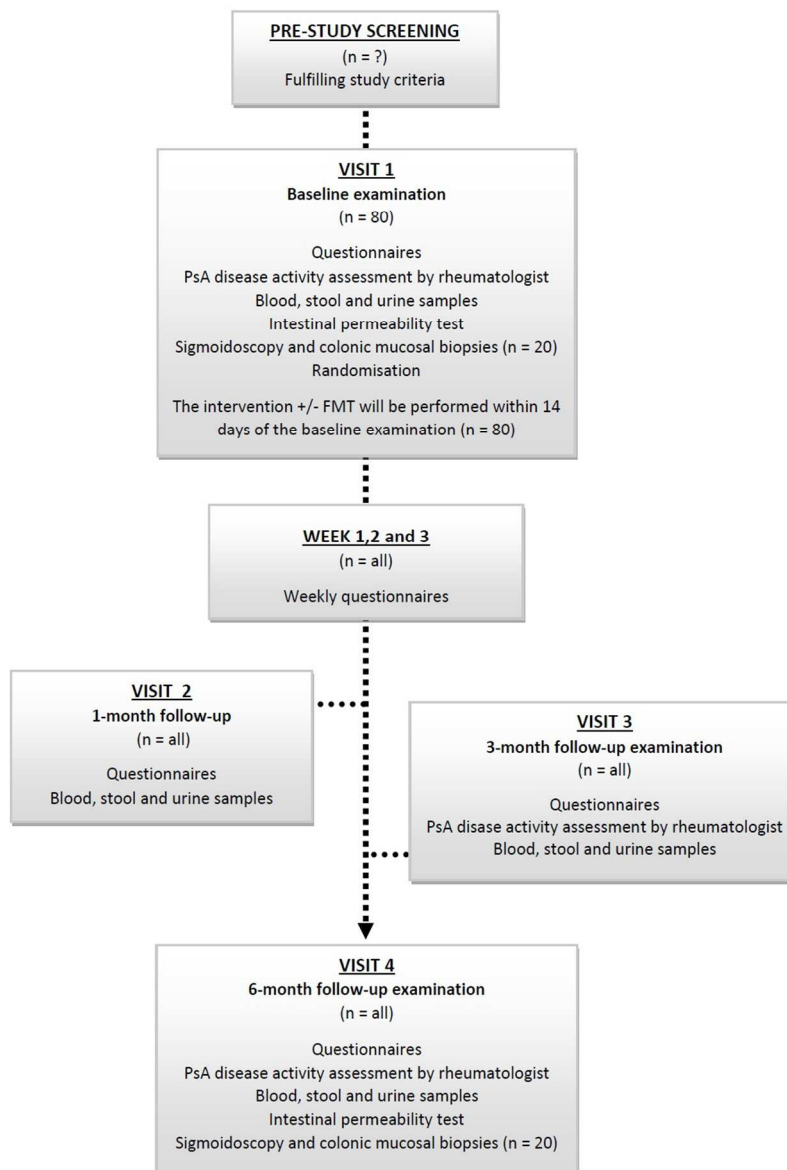


Figure 2. Participation timeline and general characteristics of each visit

151x203mm (192 x 192 DPI)

1  
2  
3  
4  
5  
6  
7 cand.med.  
8 Maja Skov Kragstnæs  
9 Odense Universitetshospital  
10 Reumatologisk afdeling C  
11 Sdr. Boulevard 29  
12 5000 Odense C  
13

**De Videnskabetiske Komitéer  
for Region Syddanmark**

komite@rsyd.dk

14 25. juni 2015

15 Projekt-ID: S-20150080  
16 HLP/bss

17 **Forskningsprojekt:**  
18 **Fæces-mikrobiom-transplantation hos patienter med perifer psoriasisigt:**  
19 **Et 6-måneders randomiseret, placebo-kontrolleret studie. Eudract nr.: ?**  
20

21 Den Videnskabetiske Komité for Region Syddanmark har på sit møde den 17. juni 2015  
22 behandlet ovennævnte forskningsprojekt og truffet følgende:

23 **Afgørelse**

24 Komiteen har godkendt projektet på vilkår i henhold til lov nr. 593 af 14. juni 2011 om  
25 videnskabetisk behandling af sundhedsvidenskabelige forskningsprojekter  
26  
27  
28

29 279x179mm (192 x 192 DPI)  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



# CONSORT 2010 checklist of information to include when reporting a randomised trial\*

Section/Topic	Item No	Checklist item	Reported on page No
<b>Title and abstract</b>			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
<b>Introduction</b>			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
<b>Methods</b>			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	8-9
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9-10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	10-11
Sample size	7a	How sample size was determined	12-13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
<b>Randomisation:</b>			
Sequence generation	8a	Method used to generate the random allocation sequence	13
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	13
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	13
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	13
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	13

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	13-14
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	14
<b>Results</b>			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	6
	13b	For each group, losses and exclusions after randomisation, together with reasons	6
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	-
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	12
<b>Discussion</b>			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	17-20
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	17-18
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
<b>Other information</b>			
Registration	23	Registration number and name of trial registry	20
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	20

\*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see [www.consort-statement.org](http://www.consort-statement.org).

# BMJ Open

## Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised placebo-controlled trial

### The FLORA trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019231.R1
Article Type:	Protocol
Date Submitted by the Author:	16-Dec-2017
Complete List of Authors:	Kragsnaes, Maja; Odense University Hospital, Department of Rheumatology; University of Southern Denmark, Odense Patient data Explorative Network (OPEN), Department of Clinical Institute Kjeldsen, Jens; Odense University Hospital, Department of Gastroenterology Horn, Hans; Odense University Hospital, Department of Rheumatology Munk, Heidi; Odense University Hospital, Department of Rheumatology Pedersen, Finn; Odense University Hospital, Department of Gastroenterology Holt, Hanne; Odense University Hospital, Department of Clinical Microbiology Pedersen, Jens Kristian; Odense University Hospital, Department of Rheumatology Holm, Dorte; Odense University Hospital, Department of Clinical Immunology Glerup, Henning; Silkeborg Regional Hospital, Diagnostic Centre Andersen, Vibeke; Hospital of Southern Jutland, IRS-Centre Sonderjylland; University of Southern Denmark, Institute of Molecular Medicine Fredberg, Ulrich; Silkeborg Regional Hospital, Diagnostic Centre Kristiansen, Karsten; University of Copenhagen, Laboratory of Genomics and Molecular Biomedicine, Department of Biology; BGI Christensen, Robin; Frederiksberg and Bispebjerg Hospital, Musculoskeletal Statistics Unit, Parker Institute Ellingsen, Torkell; Odense University Hospital, Department of Rheumatology
<b>Primary Subject Heading</b>:	Rheumatology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Psoriasis < DERMATOLOGY, Clinical trials < THERAPEUTICS, Faecal microbiota transplantation, Intestinal microbiota, Psoriatic arthritis

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

SCHOLARONE™  
Manuscripts

For peer review only

1  
2  
3 1  
4  
5 2  
6  
7 3 Efficacy and safety of faecal microbiota transplantation in  
8 patients with psoriatic arthritis:  
9 4  
10 protocol for a 6-month, double-blind, randomised placebo-  
11 5  
12 controlled trial  
13 6  
14  
15 7  
16  
17 8  
18 9

19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**The FLORA trial**

10 *Kragsnaes MS<sup>1,2\*</sup>, Kjeldsen J<sup>3</sup>, Horn HC<sup>1</sup>, Munk HL<sup>1</sup>, Pedersen FM<sup>3</sup>, Holt HM<sup>4</sup>, Pedersen JK<sup>1</sup>, Holm*  
11 *DK<sup>5</sup>, Glerup H<sup>6</sup>, Andersen V<sup>7,8</sup>, Fredberg U<sup>6</sup>, Kristiansen K<sup>9,10</sup>, Christensen R<sup>11</sup>, Ellingsen T<sup>1\*\*</sup>.*

13 <sup>1</sup> Department of Rheumatology, Odense University Hospital, Denmark.

14 <sup>2</sup> Odense Patient data Explorative Network (OPEN), Department of Clinical Institute, University of Southern Denmark.

15 <sup>3</sup> Department of Gastroenterology, Odense University Hospital, Denmark.

16 <sup>4</sup> Department of Clinical Microbiology, Odense University Hospital, Denmark.

17 <sup>5</sup> Department of Clinical Immunology, Odense University Hospital, Denmark.

18 <sup>6</sup> Diagnostic Centre, Silkeborg Regional Hospital, Denmark.

19 <sup>7</sup> IRS-Centre Sonderjylland, Hospital of Southern Jutland, Denmark.

20 <sup>8</sup> Institute of Molecular Medicine, University of Southern Denmark.

21 <sup>9</sup> Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Denmark.

22 <sup>10</sup> Institute of Metagenomics, BGI-Shenzhen, Shenzhen, China.

23 <sup>11</sup> Musculoskeletal Statistics Unit, The Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

24  
25 \* Corresponding author email address: [maja.kragsnaes@dadlnet.dk](mailto:maja.kragsnaes@dadlnet.dk).

26 \*\* Sponsor email address: [torkell.ellingsen@rsyd.dk](mailto:torkell.ellingsen@rsyd.dk).

27  
28 Protocol article version: 02.

29 Date: Dec 15, 2017.

## 1 ABSTRACT

2 **Introduction:** An unbalanced intestinal microbiota may mediate activation of the inflammatory  
3 pathways seen in psoriatic arthritis (PsA). A randomised, placebo-controlled trial of faecal  
4 microbiota transplantation (FMT) infused into the small intestine of PsA patients with active  
5 peripheral disease who are non-responsive to methotrexate (MTX) treatment will be conducted.  
6 The objective is to explore clinical aspects associated with FMT performed in PsA patients.

7  
8 **Methods and analysis:** The FLORA trial is a randomised, two-centre stratified, double-blind  
9 (patient, care provider and outcome assessor), placebo-controlled, parallel-group study. Eighty  
10 patients will be included and randomised (1:1) to either placebo (saline) or FMT provided from an  
11 anonymous healthy donor. Throughout the study, both groups will continue the weekly self-  
12 administered subcutaneous (s.c.) MTX treatment, remaining on the pre-inclusion dosage (15-25  
13 mg/week). The clinical measures of psoriasis and PsA disease activity used include the Health  
14 Assessment Questionnaire (2-page DI-HAQ), the Dermatology Quality of Life Index (DLQI), the  
15 Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index, the Psoriasis Area  
16 Severity Index (PASI), a dactylitis digit count, a swollen/tender joint count (66/68), plasma C-  
17 reactive protein as well as visual analogue scales for pain, fatigue, and patient and physician global  
18 assessments. The primary endpoint is the proportion of patients who experience treatment failure  
19 during the 6-month trial period. The number of adverse events will be registered throughout the  
20 study.

21  
22 **Ethics and dissemination:** This is a proof-of-concept clinical trial and will be performed in  
23 agreement with Good Clinical Practice (GCP) standards. Approvals have been obtained from the  
24 local Ethics Committee (DK-S-20150080) and the Danish Data Protection Agency (15/41684). The  
25 study has commenced in May 2017. Dissemination will be through presentations at national and  
26 international conferences and through publications in international peer-reviewed journal(s).

27  
28 **Trial registration number at ClinicalTrials.gov:** NCT03058900

### 29 30 **Strengths and limitations of this study**

- 31 • This is a double-blind, randomised, placebo-controlled trial.
- 32 • Subcutaneously administered MTX treatment.
- 33 • The primary endpoint is based on shared decision-making between patient and physician.
- 34 • No feasibility data regarding FMT in rheumatic patients were available when the trial was  
35 designed.
- 36 • A limitation of the study is that the content of the faecal transplant suspension cannot be  
37 fully standardised.



## 1 INTRODUCTION

2 Emerging data suggest a causal relationship between the intestinal microbiota and  
3 spondyloarthritis (SpA), thus, linking dysbiosis of the complex microbial communities with SpA  
4 pathogenesis.<sup>1-5</sup> Psoriatic arthritis (PsA) is one of five SpA categories in adults which also include  
5 ankylosing spondylitis, undifferentiated arthritis, reactive arthritis and arthritis associated with  
6 inflammatory bowel disease. While the association between the gut and the latter two disorders is  
7 well established,<sup>6</sup> only very recently, studies evaluating the faecal microbiota and the presence of  
8 subclinical gut inflammation in PsA patients have coupled this disease to a perturbation of the  
9 intestinal microbiota composition.<sup>7-12</sup>

10 PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum  
11 and a varied disease course.<sup>13</sup> The clinical manifestations include peripheral arthritis, enthesitis  
12 and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis,  
13 and dactylitis.<sup>14</sup> Nearly half of the patients with both early and established PsA also present with  
14 extra-musculoskeletal manifestations which can include bowel (16%), ocular, cardiovascular or  
15 urogenital involvement.<sup>15</sup> Without disease modifying intervention, 40-60% of PsA patients will  
16 develop erosive and deforming joint damage within a few years of disease onset.<sup>16</sup> Methotrexate  
17 (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug  
18 (csDMARD) for initial therapy.<sup>17</sup> However, the evidence for MTX in PsA is poor, and a substantial  
19 number of patients does not benefit from such treatment.<sup>18</sup> Currently, other treatment options  
20 may include biological agents such as tumour necrosis factor (TNF- $\alpha$ ) inhibitors aiming to block  
21 some of the downstream molecular pathways driving the disease.<sup>19</sup> Still, these drugs do not target  
22 the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and  
23 environmental factors.<sup>20</sup> The interplay between these complex aetiological factors has yet to be  
24 fully understood.<sup>21,22</sup>

25 The classic pathophysiological concept of PsA is that it is an autoimmune disease of  
26 the skin and joints and that the pathological processes at both sites are driven by inflammatory  
27 responses involving the innate immune system, natural killer cells, T cells, and the expression of  
28 pro-inflammatory cytokines, including TNF- $\alpha$ , interleukin (IL)-1, interferon- $\gamma$ , IL-6, IL-12, IL-15, IL-18  
29 and the IL-17/IL-23 axis.<sup>23-27</sup> However, although microbial agents including dormant bacteria,  
30 mycobacteria, bacterial products and viral antigens have been implicated as potential  
31 initiators,<sup>28,29</sup> the true pathophysiological factors triggering the dysregulated immunological  
32 cascade underlying the disease remain to be identified.

33 Intriguingly, it has recently been suggested that mucosal sites exposed to a high load  
34 of bacterial antigens, in particular the gastrointestinal tract, may represent the initial site of  
35 immunological tolerance break in PsA.<sup>30</sup> Indeed, under normal conditions the host and the  
36 microbiota live in harmony and benefit from their mutualistic relationship. However, alterations of  
37 the normal intestinal microbiota can affect mucosal immunity which, in turn, can induce local  
38 inflammation and elicit systemic effects at distant sites.<sup>31</sup> Mechanisms through which the  
39 intestinal microbiota may be involved in the pathogenesis of PsA include an abnormal activation of  
40 the gut-associated lymphoid tissue,<sup>32</sup> a decrease in regulatory T cell activity,<sup>33</sup> and/or an altered  
41 mucosal permeability thus compromising the capacity of the intestine to provide adequate  
42 containment of luminal microorganisms and molecules.<sup>34,35</sup> In support of these theories, several  
43 studies have documented subclinical gut inflammation in PsA patients.<sup>36-41</sup> Moreover, a recent

1 study reported that several intestinal bacteria including *Akkermansia* and *Ruminococcus* were  
2 practically absent in PsA patients. These commensal bacteria are, in fact, known to play an  
3 important role in maintaining gut homeostasis.<sup>42</sup>

### 4 5 **Rationale**

6 If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen  
7 in PsA,<sup>8</sup> modifying the intestinal microbiota could be a novel treatment strategy for this disease.<sup>1-  
8 3,43</sup> Faecal microbiota transplantation (FMT) is currently being used to restore the balance of the  
9 intestinal flora.<sup>44,45</sup> Particularly, this procedure has demonstrated more than 90% clinical  
10 resolution of recurrent or refractory *Clostridium difficile* infections.<sup>46-50</sup> Also, multiple FMTs seem  
11 to be able to induce remission in patients with inflammatory bowel disease (IBD).<sup>51</sup> Due to these  
12 results, FMT is now being tested as a potential novel treatment for other gastrointestinal and  
13 extra-intestinal diseases.<sup>52</sup> To the best of our knowledge, no study has yet ascertained the efficacy  
14 and safety of FMT in patients with inflammatory rheumatic diseases.

### 15 16 **Evidence-based research**

17 To avoid waste of research no new studies should be initiated without a systematic review of the  
18 existing evidence.<sup>53</sup> We performed a pragmatic search in the biomedical literature via Pubmed  
19 combining different related MeSH terms: ("Microbiota"[Mesh] OR "Fecal Microbiota  
20 Transplantation"[Mesh] OR "Faecal Microbiota Transplantation"[Mesh] OR "Gastrointestinal  
21 Microbiome"[Mesh]) AND (arthritis[tiab] OR "Arthritis"[Mesh] OR "Arthritis, Psoriatic"[Mesh] OR  
22 "Arthritis, Reactive"[Mesh] OR "Spondylarthritis"[Mesh] OR "Arthritis, Gouty"[Mesh] OR "Arthritis,  
23 Rheumatoid"[Mesh] OR "Psoriasis"[Mesh]). From the search revealing 122 citations it became  
24 clear that the majority of papers were reviews/editorials (74 [61%]), where the overall conclusion  
25 was that the main challenges are to uncover the cause-effect relationship between the intestinal  
26 microbiota and rheumatic diseases, and to investigate the potential of microbiome-targeting  
27 strategies.<sup>1,3,5,6,20,32,43,54-60</sup> Also from the published literature it became evident that to date only  
28 nine clinical interventional studies trying to modify the intestinal microbiota in arthritis patients  
29 have been performed: One study in SpA patients (n = 63),<sup>61</sup> and one study in enthesitis-related  
30 arthritis (n = 8) reported no beneficial effects of probiotic therapy,<sup>62</sup> whereas one study in juvenile  
31 idiopathic arthritis testing exclusive enteral nutrition administration (n = 7) found a moderate anti-  
32 inflammatory effect on active joints.<sup>63</sup> Five placebo-controlled trials of probiotic therapy in  
33 rheumatoid arthritis patients<sup>64-68</sup> (sample size between 26 and 60 patients) reported mixed  
34 results.<sup>69</sup> However, two of these studies demonstrated positive clinical effects of probiotic therapy  
35 which included improvement in HAQ-DI pain scale,<sup>65</sup> improvement in the Disease Activity Score of  
36 28 joints (DAS-28), and improvement on the C-reactive protein concentrations.<sup>66</sup> No clinical trials  
37 performing FMT on arthritic patients were identified.

### 38 39 **Objective**

40 The objective of this randomised trial is to explore whether FMT is more effective than placebo in  
41 reducing disease activity in PsA patients with active peripheral arthritis concomitantly treated with  
42 weekly subcutaneously administered MTX. In addition, extensive bacteria taxonomic and

1 metagenomic analyses will be performed on faecal samples before and after the FMT to get an  
2 indication of the functional capacity of the intestinal microbiota.

## 4 METHODS AND ANALYSIS

### 5 Trial design

6 This is a randomised – patient, physician and outcome-assessor blinded, placebo-controlled, 6-  
7 month trial, which will be followed by an open-label extension period for a minimum of 2 years.  
8 Patients will be randomly assigned in a 1:1 ratio to receive FMT or placebo (sham procedure).  
9 Outcome assessment will be based on follow-up by a rheumatologist and is scheduled to occur  
10 after 3 and 6 months (with the latter being the primary end-point evaluation), see Figure 1 and  
11 Figure 2.

### 13 Participants

14 Recruitment will take place at Danish rheumatology outpatient clinics, and patients fulfilling the  
15 eligibility criteria will be offered participation. No treatment with biologics within 6 months, and  
16 no systemic and/or local intra-articular or peritendinous steroid injections, or non-MTX csDMARD  
17 treatment, or antibiotics are allowed within 3 months prior to inclusion. Non-Steroidal Anti-  
18 Inflammatory Drugs (NSAIDs) must be paused within 14 days of study inclusion. Patients, who do  
19 not wish to participate, will be characterised by sex and age. The recruitment has commenced in  
20 May 2017 and will continue until 2019.

### 22 Psoriatic arthritis patients

23 A total of 80 PsA patients will be enrolled, and they will have to meet the following eligibility  
24 criteria:

#### 26 *Inclusion criteria:*

- 27 • Diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR).<sup>70</sup>
- 28 • Presence of active peripheral arthritis defined as  $\geq 3$  swollen joints.
- 29 • Subcutaneously administered MTX treatment ( $\geq 15\text{mg/week}$  (maximal tolerable dosage))  
30 for a minimum of 3 months prior to study inclusion.
- 31 • Age 18 to 70 years.

#### 33 *Exclusion criteria:*

- 34 • Other inflammatory rheumatic diseases than PsA.
- 35 • Current axial disease activity or severe peripheral joint activity demanding immediate  
36 change of treatment or contraindicating placebo treatment for 6 months.
- 37 • Inflammatory bowel disease, coeliac disease, food allergy, or other intestinal diseases.
- 38 • Current cancer or severe chronic infections.

- 1 • History of severe MTX toxicity or allergic reactions.
- 2 • Biological treatment within 6 months prior to inclusion.
- 3 • Non-MTX DMARD treatment within 3 months prior to inclusion.
- 4 • Systemic and/or local intra-articular or peritendinous steroid injections within 3 months
- 5 prior to inclusion.
- 6 • NSAIDs within 14 days prior to inclusion.
- 7 • Antibiotics within 3 months prior to inclusion.
- 8 • Pregnant or breastfeeding women.
- 9 • Not wishing to participate or unsuited for project evaluation.

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

### Stool donors

The stool donor corps will consist of four anonymous (to the recipient) donors who must be healthy as assessed by a screening questionnaire, and be active members of the Danish blood donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m<sup>2</sup>, and an average alcohol intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie diets), and must not be in a stressful life period. Before joining the stool donor corps, each potential donor will go through a screening process including stool analyses for faecal calprotectin and enteric pathogens (*Aeromonas*, *Campylobacter*, *C. difficile*, diarrhoeagenic *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, and multidrug-resistant bacteria, parasites including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium* (DNA) and *Giardia* (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-reactive protein (CRP) (acceptable level: < 6.0 mg/L), white blood cell count (acceptable range: 3.50-8.80 10<sup>9</sup>/L), haemoglobin (acceptable range: 8.3-10.5 mmol/L), albumin (acceptable range: 36-50 g/L), alanine aminotransferase (ALAT) (acceptable range: 10-70 U/L), glomerular filtration rate (eGFR) (acceptable level: > 59 mL/min), and coeliac disease, and blood test for infectious agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM), hepatitis A, B, C and E, tuberculosis (QuantiFERON<sup>®</sup> TB-Gold test), syphilis, human immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies), and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing the screening tests, the donor will donate stool for the next month after which, the donor will have to pass the screening programme once more before the stool can be released for transplantation.

51

52

53

54

55

56

57

58

59

60

### Interventions

#### Overall study interventions

1 The FMT will be an add-on strategy for PsA patients with active joint disease despite ongoing  
2 treatment with weekly subcutaneously administered MTX. Therefore, all enrolled PsA patients will  
3 continue their MTX treatment throughout the study, and they will remain on the same individual  
4 dosage that they received at the time of study inclusion (a minimum of 15 mg/week cf. the patient  
5 inclusion criteria) in addition to folic acid supplement. Paracetamol and tramadol in recommended  
6 dosages are allowed during the trial but no NSAIDs can be taken.

#### 7 8 *Active and sham comparator*

9 Patients will be randomised into two groups with an allocation ratio of active-to-placebo  
10 treatment of 1:1. The active comparator group (n = 40) will have an FMT with healthy donor  
11 faeces-suspension (250 mL) containing 50 g donor faeces, saline (NaCl 0.9%) and glycerol (10%),  
12 whereas, the sham comparator group (n = 40) will be treated with an identical appearing sham  
13 procedure where the transplant solution will consist of 250 mL brown coloured (brown food  
14 colourant) isotonic saline (NaCl 0.9%).

#### 15 16 *Preparing the FMT suspension*

17 Donors will collect faeces at home and transport it in a cooling bag to the study site within 1 hour.  
18 Faeces will be sieved to remove particulate material, followed by dilution in sterile saline (0.9%  
19 NaCl) and 10% glycerol. The FMT suspension will be stored at - 80 °C until use. On the day of the  
20 FMT transplantation, the transplant suspension (250 mL) will be thawed to 37 °C and subsequently  
21 apportioned into five 50 mL syringes.

#### 22 23 *FMT procedure*

24 The FMT will take place within 14 days (preferably 7 days) of the baseline clinical examination. The  
25 evening prior to the FMT, patients will take one dose (40 mg) of oral proton-pump inhibitor. They  
26 will meet at the Department of Gastroenterology after a six-hour fast. A total of 250 mL transplant  
27 suspension (active or placebo) will be installed in the duodenum using an oral-duodenal tube. The  
28 correct placement of the tube will be confirmed using gastroscopic guidance.

#### 29 30 *Treatment strategy for non-responders*

31 Patients who present with increased or unacceptable disease activity during the 6-month trial  
32 period will, depending on the clinical presentation, be offered another treatment strategy which  
33 may include local intra-articular steroid injections, change to another csDMARD or biological  
34 treatment. If the patient accepts such treatment changes, this will be characterised as FMT  
35 treatment failure according to the primary outcome definition (one intra-articular steroid injection  
36 is allowed).

#### 37 38 *MTX toxicity and drop-outs*

39 Blood tests for MTX toxicity will be performed in accordance with our current clinical practice. In  
40 case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other  
41 diseases that contraindicate MTX treatment, MTX dosage will be decreased or the treatment will  
42 be paused. These patients will remain in the study (unless their condition contraindicates this),

1 and they will be analysed as members of the treatment group to which they were randomised  
2 using intention-to-treat-type analyses.  
3

#### 4 *Collection of faecal samples and metagenomics analysis*

5 Fresh faecal samples will be collected by the patient at home using an EasySampler® stool  
6 collection kit within 24 hours prior to the study visit. Samples will be stored in the patient's freezer  
7 until transport to the study site. During transport, samples will be kept on ice in a cooling bag.  
8 Upon arrival to the study site, samples will immediately be transferred to the biobank and stored  
9 at -80°C. Bacterial DNA will be extracted from the faecal samples following established standard  
10 protocols including bead beating using a NucleoSpin soil kit (Macherey-Nagel, Germany) according  
11 to manufacturer's instructions. DNA will be sequenced using the BGISEQ-500 Platform which was  
12 recently benchmarked against the Illumina platforms showing excellent intra-platform  
13 reproducibility and less GC bias than observed using the Illumina platforms (Fang et al. Submitted  
14 for publication). The faecal metagenomics bioinformatics analyses will be performed using  
15 comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics  
16 species,<sup>71,72</sup> taxonomic annotation, and extensive functional analyses based on metagenomic  
17 species which provides a superior dataset compared to the conventional analyses based on the  
18 total gene pool.<sup>73</sup>

#### 19 *Intestinal permeability test*

20 After an overnight fasting, patients will provide a urine sample before ingesting 100 mL water  
21 containing 10 g of lactulose and 5 g of D-mannitol. All the urine passed in the subsequent 5 hours  
22 will be collected into a 2 L plastic container containing 1 mL of chlorohexidine (20 mg/mL) as a  
23 preservative. After 3- and 5 hours, the volume of the urine will be measured and a small volume  
24 (10 mL) will be preserved and stored at -80°C until analysis. No food or drinking (except for water)  
25 will be allowed during the test.<sup>74,75</sup>

### 26 **Outcomes**

#### 27 *Primary outcome measure:*

28 Treatment failure [Time Frame: 6 months (+/- 14 days)]

29 Proportion of patients in each group who experience treatment failure according to shared  
30 decision making between patient and rheumatologist defined as at least one of the following:

- 31 ○ Need for more than one intra-articular glucocorticoid injection due to disease  
32 activity.
- 33 ○ Need for change to other csDMARDs (e.g., oral leflunomide or sulfasalazin)  
34 according to the updated Danish treatment guideline due to disease activity.
- 35 ○ Need for biologic treatment according to the updated Danish treatment guideline  
36 due to severe disease activity.

#### 37 *Secondary outcome measures:*

- 1  
2 1 Change from baseline in the Short Health Assessment Questionnaire (2-page HAQ)<sup>76,77</sup>  
3 [Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14  
4 days)]  
5 3  
6 4  
7  
8 5 Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire<sup>78</sup> [Time Frame: 1  
9 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
10 6  
11 7  
12 8 Changes from baseline in patient reported gastrointestinal side effects [Time Frame: 1 week, 2  
13 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
14 9  
15 10  
16 11 Other non-gastrointestinal patient reported side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4  
17 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
18 12  
19 13  
20 14 Proportion of patients in each group achieving the American College of Rheumatology (ACR)<sup>79</sup>  
21 Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
22 15  
23 16 I. ACR20 response criteria<sup>80</sup>  
24 17 II. ACR50 response criteria<sup>81</sup>  
25 18 III. ACR70 response criteria<sup>81</sup>  
26 19  
27 20 Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)<sup>79</sup>  
28 [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
29 21  
30 22  
31 23 Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis  
32 Index<sup>68</sup> in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7  
33 days), 6 months (+/- 14 days)]  
34 24  
35 25  
36 26  
37 27 Change from baseline in the Psoriasis Area Severity Index (PASI)<sup>82</sup> in the subset of patients who  
38 have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days) ]  
39 28  
40 29  
41 30 Change from baseline in the number of digits affected with dactylitis in the subset of patients who  
42 have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
43 31  
44 32  
45 33 Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]  
46 34  
47 35 Number of adverse events in each group leading to discontinuation [Time Frame: 6 months (+/- 14  
48 days)]  
49 36  
50 37  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 1 Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14  
3 2 days)]  
4 3

5 4 *Tertiary (exploratory secondary) outcomes:* Proportion of patients in each group achieving changes  
6 5 in plasma CRP, changes in tender point count,<sup>83</sup> changes in faecal bacteria composition and  
7 6 metabolism, changes in intestinal permeability, changes in plasma orosomucoid, changes in  
8 7 plasma and faecal calprotectin,<sup>84</sup> changes in serum 1,25-dihydroxyvitamin D, changes in  
9 8 cardiovascular risk factors including Body Mass Index (BMI), blood pressure, plasma triglyceride,  
10 9 plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, and HbA<sub>1</sub>C levels,  
11 10 changes in specific circulating inflammatory markers (i.e. cytokines, adipokines, and chemokines),  
12 11 and macroscopic and microscopic inflammatory changes of the colonic mucosa, see Table 1.  
13 12

### 13 **Safety**

14 14 The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild  
15 15 diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the  
16 16 procedure.<sup>85</sup> A recent systematic review on the adverse events of FMT identified 50 relevant  
17 17 studies with a total of 1,089 patients. In this review, the incidences of serious adverse events  
18 18 (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The  
19 19 SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%),  
20 20 death (0.3%), auto-immune diseases and FMT procedure related injury.<sup>86</sup> Although most of the  
21 21 patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection  
22 22 and/or IBD), these findings warrant caution when performing FMT; especially when introducing  
23 23 the procedure in a new patient population. In addition, the potential long term side effects  
24 24 following FMT remains largely unknown.<sup>87</sup> Still, when strict donor screening is conducted and the  
25 25 procedure is performed by experienced practitioners, FMT is in general considered safe, and even  
26 26 elderly patients with a poor medical condition and multiple comorbidities as well as  
27 27 immunosuppressed patients have been proven to tolerate the FMT procedure well.<sup>88-92</sup>

28 28 In the present study, we will carefully monitor and evaluate safety by means of open  
29 29 assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be  
30 30 monitored until resolution, stabilisation or until it has been shown that the study intervention is  
31 31 not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events,  
32 32 version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events.  
33 33 Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool  
34 34 type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a  
35 35 week for the first month following the randomised intervention. Routine blood screening for MTX  
36 36 toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by  
37 37 the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject  
38 38 incidence rates of all treatment-emergent AE will be tabulated by system organ class and  
39 39 preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant  
40 40 treatment-emergent adverse events, will also be provided. For the long-term extension portion of  
41 41 this study, exposure adjusted event rates will be summarised.



## Sample size and power considerations

When designing this trial, no prior data for FMT efficacy in rheumatic patients were available. However, we found it reasonable to assume that if rheumatic patients should be willing to receive FMT as a future standardised treatment, the procedure should at least provide an effect size well beyond a moderate effect size. Consequently, we decided that at least twice as many PsA patients in the sham group should be treatment failures compared to the FMT group if the procedure should be considered clinically relevant. For a comparison of two independent binomial proportions using the Pearson's chi-squared statistic with a Chi-square approximation (a two-sided significance level of 0.05), a sample size of 40 PsA patients per group has a power of 90% (0.895) if we assume that the proportions of treatment failures are 35% (FMT-active group) and 70% (FMT-sham group), respectively. Consequently, the inclusion of 80 PsA patients allocated (1:1) to two treatment arms is believed to be sufficient to reveal any difference of clinical importance between treatment groups (i.e., an NNT <3 patients).

Assuming that there will be some attrition during the 6-month trial period, we also estimated how much drop-out would be possible while still having a reasonable statistical power (80%): a total sample size of 62 PsA patients assuming a comparable level of withdrawals (31 patients completing in each group) achieves a power of at least 0.8 with the proportion of treatment failures indicated above; i.e., even if we experience a drop-out rate of 20%, our trial will have 80% chance of detecting the intentional difference between groups.

Beyond the primary endpoint, a total sample size of 80 (with a balanced design) corresponds to a sufficient statistical power (82%) to detect a standardised mean difference of 0.65 SD units (i.e. Cohen's effect size) in any of the Patient-Reported Outcome Measures.

## Randomisation, allocation concealment and blinding

The randomisation has been conducted using central-computer randomisation. Patients are randomly allocated (1:1) to receive either a FMT or a placebo saline transplant (sham procedure). The randomisation lists were generated by the trial statistician and uploaded to the REDCap database by an independent data manager who is not involved in any other aspects of the trial. Eligible patients will - after signing informed consent - be assigned randomly in permuted blocks with varying sizes of 4 and 6, according to computer-generated random numbers (SAS programming via SAS PROC PLAN), to undergo either FMT or saline (sham) procedure using stratification for centre. The randomisation of each patient will be implemented by the local trial coordinator and allocation will be concealed as this is done independent of the pre-determined sequence generation (i.e. randomisation). The patients, care providers and outcome assessors will remain unaware of the group assignments, and only de-identified codes will be used to link participants to their data during the study to maintain their confidentiality. In case of exceptional circumstances when knowledge of the treatment allocation is essential for further management of the patient, the trial secretary will reveal the assigned intervention to the treating doctor.

1  
2 1 However, patients, trial care providers and outcome assessors will remain blinded as far as  
3 possible. Cases of unblinding will be registered and reported.  
4

#### 5 3 6 7 4 **Data collection, management and confidentiality**

8  
9 5 Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central  
10 REDCap<sup>93</sup> database hosted by Odense Patient data Explorative Network (OPEN) at Odense  
11 University Hospital. Data obtained during the clinical examination will be entered directly into the  
12 database. Also, patient questionnaires will be fulfilled directly into the database. Access to the  
13 study data will be restricted, and a password system will be utilized to control access. All  
14 information about the patients' health and other private matters is covered by confidentiality. The  
15 authorisation from the Danish Data Protection Agency has been secured.  
16  
17  
18  
19  
20

#### 21 13 **Statistical methods**

22  
23 14 The full analysis set will consist of all randomised participants (i.e. the intention to treat [ITT]  
24 population): Participants will be analysed according to their randomised treatment group; i.e. the  
25 ITT has the consequence that participants allocated to a treatment group will be followed up,  
26 assessed and analysed as members of that group irrespective of their compliance to the planned  
27 treatment. The safety analysis set will include all patients who were randomly assigned to a study  
28 group and had exposure to a transplant (independent of group). Descriptive statistics will be  
29 provided for demographics and baseline characteristics. The summary statistics of continuous  
30 variables will include: N, mean, standard deviation, median, interquartiles, and range. All  
31 summaries presenting frequencies and incidences will include counts, percentages, and the total  
32 number of participants in the corresponding arm.  
33

34  
35  
36 24 The pre-specified efficacy analyses will be based on data from the full-analysis set,  
37 which include all patients who underwent randomisation, have had their baseline measurement  
38 performed, and who have received the initial transplant (independent of group). Although proper  
39 random assignment prevents selection bias, it does not guarantee that the groups will be  
40 equivalent at baseline. Any differences in baseline characteristics are, however, the result of  
41 chance rather than bias;<sup>94</sup> thus, the study groups will be evaluated (and presented) at baseline for  
42 important demographic and clinical characteristics so that readers can assess how similar they are.  
43 However, only cohort studies can be subject to selection bias and confounding due to differences  
44 in baseline characteristics between the intervention and comparison groups.<sup>95</sup>  
45

46  
47  
48 33 Our strategy for ITT analysis with incomplete observations will be based on the  
49 recommendations from White et al<sup>96</sup>:

- 50 35 1: Attempt to follow up all randomised participants, even if they withdraw from allocated  
51 36 treatment.
- 52 37 2: Perform a main analysis of all observed data (data as observed).
- 53 38 3: Perform sensitivity analyses to explore the effect of departures from the assumption made in  
54 39 the main analysis (Baseline Observation Carried Forward [BOCF] imputations, repeated measures  
55 40 mixed models, and multiple imputations).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 This results in the following steps: Missing values will be imputed with the use of a  
2 non-responder imputation by use of the BOCF method for measurements made after baseline.  
3 Thus, missing data for dichotomous endpoints will also be imputed using a conservative “null  
4 responder” imputation, assuming the patient did not have any benefit from being enrolled in the  
5 trial (e.g., for the primary endpoint we will assume that the patient had a treatment failure which  
6 is valid based on clinical judgement even if data is not missing at random [NMAR]). Other sensitivity  
7 analyses will be including “worst” and “best” case imputation, repeated-measures and multiple-  
8 imputation analyses, using model-based approaches; repeated measures linear mixed models will  
9 also be used to model the potential group-dependent trajectories over time (i.e. Repeated Mixed  
10 Models and Multiple Imputation are valid if data is assumed Missing at Random [MAR]).

11 Categorical data for dichotomous end points will be analysed with the use of logistic  
12 regression with the model including treatment and centre as class effects. For continuous  
13 outcome measures analysis of covariance (ANCOVA) models will be used to analyse mean changes  
14 in continuous end points. All models will include treatment, centre, with the baseline value of the  
15 relevant variable as covariates.

16 Additionally, completer analyses will be performed on those who complete 6 months  
17 of treatment. During follow-up, any medical treatments which could potentially modify the  
18 intestinal microbiota including antibiotics will be reported, but will not affect the statistical  
19 analysis. Statistical estimates will be calculated as odds ratios (OR) for the dichotomous variables  
20 and difference between means for continuous outcomes reported with 95% confidence intervals  
21 (95% CI). Two-sided 95% CIs and P-values for primary, secondary and exploratory outcomes will be  
22 computed and will not be adjusted for multiplicity, but will be interpreted cautiously as this is an  
23 exploratory trial per se.

24 Pre-specified exploratory analyses: Stratified analyses will investigate whether the  
25 treatment effect varies with I) the faecal microbiota analyses performed at follow-up compared  
26 with baseline (+/- long-term changes in the intestinal microbiota and intestinal inflammation); and  
27 II) the demographic match (sex, age) between the stool donor and the recipient. Non-responders  
28 will represent the outcome group not fulfilling the primary outcome measure. Differences in  
29 demographics and baseline disease activity between this treatment-failure subpopulation and the  
30 remaining group will be examined to identify potential prognostic factors for poor responders.  
31 Patients not participating in the follow-up examination will be classified as "drop-outs", and if  
32 possible, the reason for not participating will be registered.

33 The faecal metagenomics bioinformatics analyses will be performed using  
34 comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics  
35 species,<sup>71,72</sup> taxonomic annotation, and extensive functional analyses based on metagenomic  
36 species which provides a superior dataset compared to the conventional analyses based on the  
37 total gene pool.<sup>73</sup> To identify possible associations, metagenome analysis will be correlated to all  
38 clinical parameter. We will use an L1 restricted LASSO procedure to determine the optimal  
39 number of features to be tested as described. Analysis of correlations between microbiota  
40 taxonomic or functional features, community diversity indices and sample metadata variables will  
41 be performed using Spearman correlation tests corrected for multiple tests using the Benjamini-

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 Hochberg false discovery rate control procedure. To control for confounders, we will use blocked  
2 Spearman tests as implemented in COIN.<sup>97,98</sup>  
3 Data will be analysed with the STATA statistical package (version 15; StataCorp LP),  
4 and SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA).

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2017-019231 on 27 April 2018. Downloaded from <http://bmjopen.bmj.com/> on April 17, 2024 by guest. Protected by copyright.

Activity/assessment	Pre-study screening	Visit 1 Baseline	Week 1, 2 and 3	Visit 2 1 month	Visit 3 3 months	Visit 4 6 months
Patients	n = ?	n = 80	n = all	n = all	n = all	n = all
Screening log	x					
Inclusion/exclusion form	x					
Consent form		x				
Randomisation		x				
Study-composed questionnaire		x	x	x	x	x
Patient global (VAS 0-100 mm)		x	x	x	x	x
Patient fatigue (VAS 0-100 mm)		x	x	x	x	x
Patient pain (VAS 0-100 mm)		x	x	x	x	x
HAQ		x	x	x	x	x
BASDAI		x			x	x
BASFAI		x			x	x
DLQI		x	x	x	x	x
Gastrointestinal symptom diary		x	x	x	x	x
Eating habits questionnaire		x				
Clinical examination:						
- Height (m)		x				
- Weight (kg)		x			x	x
- Blood pressure (mmHg)		x			x	x
- Psoriasis Area Severity Index		x			x	x
- SPARCC Enthesitis Score		x			x	x
- Swollen joint count (66)		x			x	x
- Tender joint count (68)		x			x	x
- Doctors global (VAS 0-100 mm)		x			x	x
- BASMI		x			x	x
- Tender point count		x			x	x
Interview (AEs)				x	x	x
Blood sample analysis:						
- C-reactive protein (mg/L)		x		x	x	x
- Orosomucoid (g/L)		x		x	x	x
- Calprotectin		x		x	x	x
- 1,25-dihydroxyvitamin D (nmol/L)		x		x	x	x
- TSH (miu/L)		x				x
- Hgb (mmol/L)		x				x
- Triglyceride (mmol/L)		x				x
- LDL-cholesterol (mmol/L)		x				x
- HDL-cholesterol (mmol/L)		x				x
- Total-cholesterol (mmol/L)		x				x
- HbA <sub>1c</sub> (mmol/mol)		x				x
- HLA-B27 status (+/-)		x				
- Serology tests for <i>Yersinia</i> , <i>Campylobacter</i> , <i>Salmonella</i> (+/-)		x				
Faecal calprotectin		x		x	x	x
Faecal microbiota analysis		x		x	x	x
Sigmoidoscopy and mucosa biopsy		x				x
Stool, blood, and urine samples (biobank)		x		x	x	x
Intestinal permeability test		x				x
Intervention (+/- FMT)		x				
Serious adverse event forms				x		

**Table 1.** Protocol schedule of forms and procedures

## 1 ETHICS AND DISSEMINATION

2 This study is designed as a proof-of-concept clinical trial and will be performed in agreement with  
3 GCP-standards, and in accordance with the ethical standards of the responsible committee on  
4 human experimentation (institutional and national) and with the Helsinki Declaration (64th, 2013).  
5 The relevance of the study, the design and the recruitment strategy were evaluated with three  
6 patient research partners (PRPs), and alterations especially in primary outcome and recruitment  
7 strategy were embedded. Furthermore, a minimum of two PRPs (participating in the study) will be  
8 involved in the discussion regarding the progress of the recruitment phase and results, and will be  
9 offered the opportunity to comment on the manuscript draft. The Regional Committees on Health  
10 Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency  
11 (15/41684) have approved the study protocol. The trial has been registered with ClinicalTrials.gov  
12 (NCT03058900) and important protocol modifications will be updated here. The Danish Health and  
13 Medicines Authority does not classify the FMT procedure as a medical intervention, and has had  
14 no objection to the use of FMT for this study and patient category. Thus, no GCP auditing is legally  
15 required. A report describing any potential side effects and adverse events will be submitted to  
16 the Ethics Committee yearly. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be  
17 reported to the Ethics Committee within 7 days. Based on these reports, the Ethics committee can  
18 determine to terminate the trial early. The Danish Patient Compensation Association provides  
19 compensations for patients injured in connection to medical clinical trials.

20 Although the Danish Health Authorities, for the time being, do not classify donor  
21 faecal microbiota as tissue, all steps of the stool donor recruitment, stool donation and FMT  
22 preparation will be in accordance with the Danish Tissue Law to ensure that the quality and safety  
23 standards laid down in the Danish Legislation BEK nr 764 of May 26, 2015 (implementing Directive  
24 2004/23/EC) are met. Four stool donors will be recruited from the South Danish Transfusion  
25 Service & Tissue Centre, Department of Clinical Immunology, Odense University Hospital, and they  
26 will be carefully screened for potentially transmissible infections and other conditions associated  
27 with gut microbiota function before their stool can be released for FMT. Being a stool donor is  
28 voluntary, and no compensation fee will be given. Furthermore, to ensure donor traceability, each  
29 patient in the active treatment arm will only receive microbiota from one donor. Also, frozen  
30 samples will be clearly labelled with a unique donation code based on the ISBT 128 coding and  
31 labelling system, and the release of the final product will adhere to the standards for tissue and  
32 blood donation.

33 Due to the well-documented risk of permanent joint destruction and occurrence of  
34 extra-articular manifestations in the PsA disease course, identification of new treatment modalities  
35 and biomarkers is essential to help the physician to slow down the disease development or  
36 ultimately to prevent it. All PsA patients participating in this study have significant activity in their  
37 joint disease despite treatment with the current guideline treatment and first-line drug, MTX, for  
38 this condition. This patient population will therefore benefit greatly from new treatment options.  
39 Consequently, when weighing the pros and cons, this trial should be performed from a scientific and  
40 ethical perspective.

41 Dissemination will occur through presentations at national and international  
42 conferences and publications in international peer-reviewed journal(s).

## DISCUSSION

Recent years have seen growing recognition of the complexity of the role of the microbiota in shaping the immune system and its potential effects for health and disease.<sup>22,99,100</sup> In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.<sup>101-104</sup> Intriguingly, an abnormal intestinal bacteria composition has been observed in PsA patients, and this association has fostered theories linking intestinal dysbiosis and PsA joint inflammation.<sup>105</sup> Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic diseases are causal related,<sup>55</sup> and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.<sup>54,106</sup> We expect that this double-blind, randomised, placebo-controlled trial will shed new light on this highly relevant topic.

This is the first time that the efficacy and safety of FMT is being investigated in MTX immunosuppressed patients with rheumatic diseases. Subsequently, no data on the feasibility of conducting FMTs in the rheumatological setting is, so far, available. Nor do we know whether one FMT will be sufficient, or whether it should be repeated shortly after the first intervention to normalise the alterations of the intestinal microbiota, which would expectedly enhance any potential anti-inflammatory effects. In the present proof-of-concept clinical trial, the FMT procedure is considered an add-on to the current guideline intervention and first-line drug, MTX. Therefore, from a pragmatic and ethical perspective, we have decided to perform only one FMT (or sham procedure) in each patient even if we are well aware that this approach may not be adequate to achieve long-lasting effects. Indeed, in patients with inflammatory bowel diseases it appears that performing a frequent dosing-regime repeating the FMT procedure up to five times a week for eight weeks provided the best results.<sup>51,107,108</sup> Hence, in contrast to the treatment of *C. difficile* infections where the microbiota is pushed past the point of homeostasis and can be restored following only one FMT,<sup>47</sup> the chronic nature of PsA and other autoimmune and inflammatory diseases, and the somewhat lesser degree of intestinal dysbiosis, may make the host microbiota more resistant to long-lasting modifications. Nevertheless, we hope that the FMT procedure in the present study will be sufficient to boost the effects of MTX so that the participants who are all MTX-non-responders prior to study enrolment will achieve disease control without needing to add or switch to other non-MTX medication.

In the present trial, the primary outcome measure is defined as the occurrence of treatment failure according to shared decision making between patient and physician evaluated at 6 months following the randomised intervention (FMT versus sham procedure). Shared decision making is a process in which both patient and health professional make a decision, taking into account the best evidence of available treatment options and the patient's values and preferences. This approach is considered a key element in the management of rheumatic diseases.<sup>109</sup> As both patients and the treating rheumatologists are blinded to the randomised intervention, the shared decision making will be unaffected by the type of transplant suspension (active or placebo) installed at baseline. Nevertheless, we acknowledge that our assumption that twice as many PsA patients in the sham group will be treatment failures is ambitious, and that we might miss a smaller and less clinically significant treatment effect of the FMT-procedure. In this

1  
2 1 case, we hope that our secondary outcome measures will be able to detect potential trends of  
3 2 positive effects in PsA subdomains such as enthesitis score, dactylitis count, and PASI skin score. In  
4 3 addition to the primary endpoint evaluation at 6 months, patients will be asked to fill out a weekly  
5 4 questionnaire regarding side effects as well as skin and arthritis symptoms during the first month  
6 5 following the randomised intervention to reveal any short-term effects on patient-reported  
7 6 outcomes.

8 7 Next, only patients with active peripheral PsA will be included. One reason for this is  
9 8 that this will be the first time that FMT is performed on rheumatic patients. Therefore, it seems  
10 9 reasonable only to enrol patients who have had inadequate effect from the initial guideline  
11 10 treatment (MTX), and consequently, on an individual basis could benefit the most from  
12 11 participating in new experimental clinical trials. Also, since patients need to have at least three  
13 12 swollen joints, we expect that we will be able to detect treatment effects of clinical importance.  
14 13 The fact that we do not include recent onset treatment naive patients will, of course, limit our  
15 14 ability to generalise our findings unto the entire PsA patient population. Indeed, in a recent  
16 15 randomised controlled trial of FMT in ulcerative colitis patients, participants with a recent  
17 16 diagnosis (< 1 year) were statistically significantly more likely to respond to FMT compared with  
18 17 those with longer disease duration.<sup>107</sup> That patients will have to subcutaneously administer MTX  
19 18 for at least three months prior to study enrolment will ensure that low intestinal MTX absorption  
20 19 is excluded as a potential effect modifier for the poor MTX response. In addition, as many drugs,  
21 20 including MTX, seem to affect the intestinal microbiological milieu,<sup>110-113</sup> bypassing the intestine  
22 21 during MTX administration will ensure that no local non-disease related effects on the intestinal  
23 22 microbiota will occur.

24 23 A great challenge when conducting a trial of FMT is that for the present being there  
25 24 is a lack of both national and international recommendations guiding the regulation and the best  
26 25 clinical practices for donor screening, stool sample handling and preparation of the FMT  
27 26 suspension.<sup>114-116</sup> Indeed, the variability in faecal bacterial communities can complicate or  
28 27 undermine treatment efficacy. This variability stems from both biological variation and variation  
29 28 introduced by sample handling. A recent study reported that oxygen exposure degraded faecal  
30 29 bacterial communities, whereas freeze-thaw cycles and lag time between donor defecation and  
31 30 transplant preparation had much more limited effects.<sup>117</sup> Given that many intestinal bacteria are  
32 31 obligate anaerobe, including many beneficial bacteria potentially possessing anti-inflammatory  
33 32 effects, exposure to oxygen during the preparation of FMT may potentially compromise the  
34 33 therapeutic value of FMT in PsA and other inflammatory diseases. Therefore, although frozen  
35 34 faecal preparations of stool suspended into physiological saline and glycerol have proven just as  
36 35 effective as fresh stool in treating *C. difficile* infections,<sup>118</sup> the optimal transplant preparation  
37 36 method in treating inflammatory diseases remains to be established.

38 37 Our stool handling setup is in line with the prevailing practice, which includes mixing  
39 38 and filtration of the stool suspension and adding saline and glycerol as a cryopreservative before  
40 39 storage at -80 °C.<sup>116</sup> In addition, we have sought to limit the oxygen exposure during transport by  
41 40 placing the donor stool within a plastic bag, which is subsequently put into a tightly closed small  
42 41 plastic container. Supplementary, during preparation the solution will not be homogenized for  
43 42 more than 10 seconds. Nevertheless, the lack of optimal anaerobe conditions during stool



1 handling could possibly undermine the therapeutic potential of our FMT procedure. Furthermore,  
2 although we aim to use 50 g of faeces for each transplant, we acknowledge that the exact weight  
3 between donations could vary with an estimated +/- 5 g. Also, due to the wide variability in  
4 microbial content in stool between donations, the content cannot be fully standardized, and may  
5 likely differ between each FMT procedure. However, to meet this challenge we will collect and  
6 store samples from each donation which will enable us to determine the microbiota composition  
7 of each donation in case some donations prove more effective than others.

8 Stool donor selection is another critical issue that needs to be addressed. The  
9 composition of the normal microbiota composition has only recently been mapped,<sup>119</sup> and the  
10 existence of a limited number of well-balanced host-microbial symbiotic states, where one or  
11 more bacteria species are considered the main functional driver(s), have been identified using  
12 clustering of metagenomic sequences.<sup>120</sup> Still, the most favourable donor microbiota composition  
13 for treating inflammatory diseases has yet to be determined. Therefore, it also remains to be  
14 established whether donors with a high stool bacteria diversity should be preferred over isolation  
15 of specific bacteria, or if pooled stool samples from several donors outperforms a single-donor  
16 transplant.<sup>51,121</sup> We have chosen to use only single donations from four different anonymous stool  
17 donors to ensure donor traceability and to enable us to identify any individual donor-specific  
18 microbial effects. Also, since host intrinsic-, environmental-, and dietary factors as well as  
19 pharmaceutical drugs have been associated with gut bacteria composition and  
20 functionality,<sup>110,111,122,123</sup> the donors must eat a balanced diet, not be overweight or take any  
21 medications or be physical or psychological stressed, smoke or consume alcohol during the  
22 donation period to limit the risk of transferring "abnormal" microbiota to the recipients. These  
23 donor criteria have been set for safety reasons, and we acknowledge, that this could potentially  
24 limit the inter-donor microbiota diversity due to shared lifestyle characteristics.

25 Another factor to keep in mind is the concept of matching donor and recipient, which  
26 may be of importance for enhancing the colonisation capabilities of the donor microbial  
27 communities. In fact, Rossen et al<sup>108</sup> did find that in patients with ulcerative colitis, the microbiota  
28 of FMT responders shifted to their respective donors, whereas non-responders did not. Li et al<sup>124</sup>  
29 reported that donor bacteria strains established extensively in the recipient and persisted for at  
30 least 3 months with a negligible decline of donor-strain populations detected between 45 days  
31 and 3 months following FMT in metabolic syndrome patients. However, they also found that  
32 recipients receiving the same donor transplant displayed varying degrees of microbiota transfer,  
33 indicating individual patterns of microbiome resistance and donor-recipient compatibilities. In  
34 addition, host genetics is known to effect the gut microbiota,<sup>125</sup> and animal models have shown  
35 that sex<sup>126</sup> and age<sup>127</sup> also can be potentially modifiers of the gut bacteria composition. These  
36 observations may prove to be of importance for the outcome of FMT in inflammatory diseases.<sup>128</sup>  
37 However, whether sex- and/or age-matching between donor and recipient is crucial for a  
38 successful FMT in humans remains to be enlighten. Therefore, in the present study, no donor-  
39 recipient matching will be conducted. However, a subgroup analysis will be performed to reveal  
40 any trend that could indicate better results in sex- or age-match cases.

41 Furthermore, as the interactions between the microbiota and the host are influenced  
42 by cooperation and competition between pathogenic and commensal microbes and multiple

1  
2 1 environmental variables, the lifestyle of the recipient following the FMT may be of importance.  
3 2 Nevertheless, due to the uncertainty of how to define the optimal lifestyle and the lack of  
4 3 knowledge on how different lifestyle factors may interfere with the microbiota, we have decided  
5 4 that the patients in the present study will not have to adhere to any predefined lifestyle "regime"  
6 5 or diet following the randomised intervention. However, every participant will fulfil an eating habit  
7 6 questionnaire at the beginning of the trial.

8 7 Finally, non-bacteria microorganisms such as bacteriophages, viruses and fungi may  
9 8 also be of importance when targeting components of the microbiota or host cells for therapeutic  
10 9 purposes.<sup>129-131</sup> Other complicating factors may include the composition of other microbiological  
11 10 niches such as the oral, lung, genitourinary, and skin microbiota.<sup>132,133</sup> Indeed, the latter could  
12 11 likely prove to be of significance in patients with skin psoriasis. However, these factors will not be  
13 12 assessed in the present study.

14 13 In conclusion, this trial has the potential to substantially expand the growing body of  
15 14 literature on the role of the intestinal microbiota in general and PsA in particular. Thereby we  
16 15 anticipate that this study will enhance our understanding of cause and effect. The results of this  
17 16 study, when completed, may be exploited for biomarker discovery, and for diagnostic and  
18 17 therapeutic purposes.

## 19 **AUTHORS' CONTRIBUTION**

20 20 T. Ellingsen, M.S. Kragstnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for  
21 21 the study. T. Ellingsen and M.S. Kragstnaes are the principal investigators and wrote the first study  
22 22 protocol draft. T. Ellingsen and M.S. Kragstnaes were responsible for all communication with the  
23 23 scientific ethical committee, the Danish Data Protection Agency and the National Health Board. T  
24 24 Ellingsen is the responsible party and sponsor. M.S. Kragstnaes, T. Ellingsen, H.C. Horn, J.K.  
25 25 Pedersen, H.L. Munk, and U. Fredberg sat up the inclusion and exclusion criteria for the psoriatic  
26 26 arthritis patients, and the latter five rheumatologists are conducting the clinical examinations. J.  
27 27 Kjeldsen, F.M. Pedersen, and H. Glerup discussed and planned the FMT procedure, and they are  
28 28 conducting the FMTs and the sigmoidoscopies (obtaining mucosae tissue samples). D.K. Holm and  
29 29 H.M. Holt helped set up the donor screening programme, and they were responsible for  
30 30 conducting this programme and performing the microbiological and immunological tests. V.  
31 31 Andersen and K. Kristiansen are responsible for the omics and microbiome analyses, and  
32 32 have advised on how the tissue collection should be performed and what kind of tissue would be  
33 33 relevant to collect. R. Christensen has written the statistical analysis plan and will be responsible  
34 34 for the final statistical analyses. In conclusion, all participants designated as authors have  
35 35 contributed to the conception and design of the study, and they have critically either drafted or  
36 36 revised the first draft of the study protocol and the protocol paper. Also, all authors have  
37 37 approved the final version before submission.

## 39 **REGISTRATION**

40 40 The trial has been registered with ClinicalTrials.gov (NCT03058900).

## 1 **FUNDING STATEMENT**

2 This work was supported by the Region of Southern Denmark, Odense University Hospital, the  
3 Danish Regions, University of Southern Denmark, the Danish Rheumatism Association, the Danish  
4 Psoriasis Research Fund, and Odense Patient Explorative data Network (OPEN). Musculoskeletal  
5 Statistics Unit at the Parker Institute, Bispebjerg and Frederiksberg Hospital (R. Christensen), is  
6 supported by a core grant from the Oak Foundation (OCAY-13-309). K. Kristiansen is supported by  
7 BGI-Research, BGI-Shenzhen, China.

## 9 **COMPETING INTEREST STATEMENT**

10 None of the team members of this research project has declared any potential conflict of interest.

## 12 **ACKNOWLEDGEMENTS**

13 Great thanks to Lene Albjerg, biomedical laboratory technologist and quality control manager at  
14 the Dept. of Clinical Immunology, Odense University Hospital, Denmark, for her expertise and  
15 dedicated work regarding the practical handling of the FMT suspension.

## References

1. Scher JU, Littman DR, Abramson SB. Microbiome in Inflammatory Arthritis and Human Rheumatic Diseases. *Arthritis Rheumatol*. 2016;68:35-45.
2. Asquith M, Elewaut D, Lin P et al. The role of the gut and microbes in the pathogenesis of spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2014;28:687-702.
3. Stoll ML. Gut microbes, immunity, and spondyloarthritis. *Clin Immunol*. 2015;159:134-142.
4. Costello ME, Ciccia F, Willner D et al. Intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol*. 2014.
5. Yang L, Wang L, Wang X et al. A Possible Role of Intestinal Microbiota in the Pathogenesis of Ankylosing Spondylitis. *Int J Mol Sci*. 2016;17.
6. Manasson J, Scher JU. Spondyloarthritis and the microbiome: new insights from an ancient hypothesis. *Curr Rheumatol Rep*. 2015;17:10.
7. De WK, Debusschere K, Beeckman S et al. Integrating the pathogenesis of spondyloarthritis: gut and joint united? *Curr Opin Rheumatol*. 2015;27:189-196.
8. Eppinga H, Konstantinov SR, Peppelenbosch MP et al. The microbiome and psoriatic arthritis. *Curr Rheumatol Rep*. 2014;16:407.
9. Coit P, Sawalha AH. The human microbiome in rheumatic autoimmune diseases: A comprehensive review. *Clin Immunol*. 2016;170:70-79.
10. Ciccia F, Ferrante A, Guggino G et al. The role of the gastrointestinal tract in the pathogenesis of rheumatic diseases. *Best Pract Res Clin Rheumatol*. 2016;30:889-900.
11. Tito RY, Cypers H, Joossens M et al. Brief Report: Dialister as a Microbial Marker of Disease Activity in Spondyloarthritis. *Arthritis Rheumatol*. 2017;69:114-121.
12. Eppinga H, Sperna Weiland CJ, Thio HB et al. Similar Depletion of Protective Faecalibacterium prausnitzii in Psoriasis and Inflammatory Bowel Disease, but not in Hidradenitis Suppurativa. *J Crohns Colitis*. 2016.
13. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. *N Engl J Med*. 2017;376:2095-2096.
14. Terslev L, Naredo E, Iagnocco A et al. Defining enthesitis in spondyloarthritis by ultrasound: results of a Delphi process and of a reliability reading exercise. *Arthritis Care Res (Hoboken)*. 2014;66:741-748.
15. Peluso R, Iervolino S, Vitiello M et al. Extra-articular manifestations in psoriatic arthritis patients. *Clin Rheumatol*. 2014.
16. Gladman DD. Psoriatic arthritis. *Dermatol Ther*. 2009;22:40-55.
17. Gossec L, Coates LC, De WM et al. Management of psoriatic arthritis in 2016: a comparison of EULAR and GRAPPA recommendations. *Nat Rev Rheumatol*. 2016;12:743-750.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
18. Kingsley GH, Kowalczyk A, Taylor H et al. A randomized placebo-controlled trial of methotrexate in psoriatic arthritis. *Rheumatology (Oxford)*. 2012;51:1368-1377.
  19. Gossec L, Smolen JS, Ramiro S et al. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis*. 2016;75:499-510.
  20. Asquith M, Rosenbaum JT. The interaction between host genetics and the microbiome in the pathogenesis of spondyloarthropathies. *Curr Opin Rheumatol*. 2016;28:405-412.
  21. Benham H, Robinson PC, Baillet AC et al. Role of genetics in infection-associated arthritis. *Best Pract Res Clin Rheumatol*. 2015;29:213-225.
  22. Shamriz O, Mizrahi H, Werbner M et al. Microbiota at the crossroads of autoimmunity. *Autoimmun Rev*. 2016;15:859-869.
  23. Lories RJ, de VK. Is psoriatic arthritis a result of abnormalities in acquired or innate immunity? *Curr Rheumatol Rep*. 2012;14:375-382.
  24. Mortezaei M, Thiele R, Ritchlin C. The joint in psoriatic arthritis. *Clin Exp Rheumatol*. 2015;33:20-25.
  25. Acosta Felquer ML, Fitzgerald O. Peripheral joint involvement in psoriatic arthritis patients. *Clin Exp Rheumatol*. 2015;33:26-30.
  26. Matt P, Lindqvist U, Kleinau S. Up-regulation of CD64-expressing monocytes with impaired FcγR function reflects disease activity in polyarticular psoriatic arthritis. *Scand J Rheumatol*. 2015;44:464-473.
  27. Al-Mossawi MH, Ridley A, Kiedel S et al. The role of natural killer cells, gamma delta T-cells and other innate immune cells in spondyloarthritis. *Curr Opin Rheumatol*. 2013;25:434-439.
  28. Ryan C, Korman NJ, Gelfand JM et al. Research gaps in psoriasis: opportunities for future studies. *J Am Acad Dermatol*. 2014;70:146-167.
  29. Berthelot JM, de la Cochetiere MF, Potel G et al. Evidence supporting a role for dormant bacteria in the pathogenesis of spondylarthritis. *Joint Bone Spine*. 2013;80:135-140.
  30. Abdollahi-Roodsaz S, Abramson SB, Scher JU. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nat Rev Rheumatol*. 2016;12:446-455.
  31. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature*. 2016;535:75-84.
  32. Ciccia F, Rizzo A, Triolo G. Subclinical gut inflammation in ankylosing spondylitis. *Curr Opin Rheumatol*. 2016;28:89-96.
  33. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med*. 2009;361:888-898.
  34. Ciccia F, Guggino G, Rizzo A et al. Dysbiosis and zonulin upregulation alter gut epithelial and vascular barriers in patients with ankylosing spondylitis. *Ann Rheum Dis*. 2017.
  35. Pianta A, Arvikar SL, Strle K et al. Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. *J Clin Invest*. 2017.

- 1  
2 1 36. Scher JU, Ubeda C, Artacho A et al. Decreased bacterial diversity characterizes an altered gut  
3 2 microbiota in psoriatic arthritis and resembles dysbiosis of inflammatory bowel disease. *Arthritis*  
4 3 *Rheumatol.* 2014.  
5  
6 4 37. Lindqvist U, Kristjansson G, Pihl-Lundin I et al. Patients with psoriatic arthritis have an increased  
7 5 number of lymphocytes in the duodenal mucosa in comparison with patients with psoriasis  
8 6 vulgaris. *J Rheumatol.* 2006;33:924-927.  
9  
10 7 38. Scarpa R, Manguso F, D'Arienzo A et al. Microscopic inflammatory changes in colon of patients with  
11 8 both active psoriasis and psoriatic arthritis without bowel symptoms. *J Rheumatol.* 2000;27:1241-  
12 9 1246.  
13  
14 10 39. Van PL, Van den Bosch F, Mielants H et al. Mucosal inflammation in spondylarthritides: past,  
15 11 present, and future. *Curr Rheumatol Rep.* 2011;13:409-415.  
16  
17 12 40. Schatteman L, Mielants H, Veys EM et al. Gut inflammation in psoriatic arthritis: a prospective  
18 13 ileocolonoscopy study. *J Rheumatol.* 1995;22:680-683.  
19  
20 14 41. Ciccia F, Guggino G, Ferrante A et al. Interleukin-9 Overexpression and Th9 Polarization  
21 15 Characterize the Inflamed Gut, the Synovial Tissue, and the Peripheral Blood of Patients With  
22 16 Psoriatic Arthritis. *Arthritis Rheumatol.* 2016;68:1922-1931.  
23  
24 17 42. Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis and human  
25 18 diseases. *BMC Immunol.* 2017;18:2.  
26  
27 19 43. Gill T, Asquith M, Rosenbaum JT et al. The intestinal microbiome in spondyloarthritis. *Curr Opin*  
28 20 *Rheumatol.* 2015;27:319-325.  
29  
30 21 44. Kump PK, Krause R, Allerberger F et al. Faecal microbiota transplantation-the Austrian approach.  
31 22 *Clin Microbiol Infect.* 2014;20:1106-1111.  
32  
33 23 45. Cammarota G, Pecere S, Ianiro G et al. Principles of DNA-Based Gut Microbiota Assessment and  
34 24 Therapeutic Efficacy of Fecal Microbiota Transplantation in Gastrointestinal Diseases. *Dig Dis.*  
35 25 2016;34:279-285.  
36  
37 26 46. Austin M, Mellow M, Tierney WM. Fecal microbiota transplantation in the treatment of *Clostridium*  
38 27 *difficile* infections. *Am J Med.* 2014;127:479-483.  
39  
40 28 47. van NE, Vrieze A, Nieuwdorp M et al. Duodenal infusion of donor feces for recurrent *Clostridium*  
41 29 *difficile*. *N Engl J Med.* 2013;368:407-415.  
42  
43 30 48. Cammarota G, Masucci L, Ianiro G et al. Randomised clinical trial: faecal microbiota transplantation  
44 31 by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection.  
45 32 *Aliment Pharmacol Ther.* 2015;41:835-843.  
46  
47 33 49. Lee CH, Steiner T, Petrof EO et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical  
48 34 Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection: A Randomized  
49 35 Clinical Trial. *JAMA.* 2016;315:142-149.  
50  
51 36 50. Li YT, Cai HF, Wang ZH et al. Systematic review with meta-analysis: long-term outcomes of faecal  
52 37 microbiota transplantation for *Clostridium difficile* infection. *Aliment Pharmacol Ther.* 2016;43:445-  
53 38 457.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 51. Paramsothy S, Kamm MA, Kaakoush NO et al. Multidonor intensive faecal microbiota  
2 transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet*.  
3 2017;389:1218-1228.
- 4 52. Cui B, Feng Q, Wang H et al. Fecal microbiota transplantation through mid-gut for refractory  
5 Crohn's disease: Safety, feasibility and efficacy trial results. *J Gastroenterol Hepatol*. 2014.
- 6 53. Lund H, Brunnhuber K, Juhl C et al. Towards evidence based research. *BMJ*. 2016;355:i5440.
- 7 54. Ciccia F, Ferrante A, Triolo G. Intestinal dysbiosis and innate immune responses in axial  
8 spondyloarthritis. *Curr Opin Rheumatol*. 2016;28:352-358.
- 9 55. Bravo-Blas A, Wessel H, Milling S. Microbiota and arthritis: correlations or cause? *Curr Opin*  
10 *Rheumatol*. 2016;28:161-167.
- 11 56. Kabeerdoss J, Sandhya P, Danda D. Gut inflammation and microbiome in spondyloarthritis.  
12 *Rheumatol Int*. 2016;36:457-468.
- 13 57. Costello ME, Robinson PC, Benham H et al. The intestinal microbiome in human disease and how it  
14 relates to arthritis and spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2015;29:202-212.
- 15 58. Bazso A, Szodoray P, Suto G et al. Importance of intestinal microenvironment in development of  
16 arthritis. A systematic review. *Immunol Res*. 2015;61:172-176.
- 17 59. Taneja V. Arthritis susceptibility and the gut microbiome. *FEBS Lett*. 2014;588:4244-4249.
- 18 60. Rosenbaum JT, Lin P, Asquith M et al. Does the microbiome play a causal role in spondyloarthritis?  
19 *Clin Rheumatol*. 2014;33:763-767.
- 20 61. Jenks K, Stebbings S, Burton J et al. Probiotic therapy for the treatment of spondyloarthritis: a  
21 randomized controlled trial. *J Rheumatol*. 2010;37:2118-2125.
- 22 62. Aggarwal A, Sarangi AN, Gaur P et al. Gut microbiome in children with enthesitis-related arthritis in  
23 a developing country and the effect of probiotic administration. *Clin Exp Immunol*. 2017;187:480-  
24 489.
- 25 63. Berntson L, Hedlund-Treutiger I, Alving K. Anti-inflammatory effect of exclusive enteral nutrition in  
26 patients with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2016;34:941-945.
- 27 64. Hatakka K, Martio J, Korpela M et al. Effects of probiotic therapy on the activity and activation of  
28 mild rheumatoid arthritis--a pilot study. *Scand J Rheumatol*. 2003;32:211-215.
- 29 65. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms  
30 of rheumatoid arthritis according to a randomized, controlled trial. *BMC Complement Altern Med*.  
31 2010;10:1.
- 32 66. Zamani B, Golkar HR, Farshbaf S et al. Clinical and metabolic response to probiotic supplementation  
33 in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Int J*  
34 *Rheum Dis*. 2016;19:869-879.
- 35 67. Pineda ML, Thompson SF, Summers K et al. A randomized, double-blinded, placebo-controlled pilot  
36 study of probiotics in active rheumatoid arthritis. *Med Sci Monit*. 2011;17:CR347-CR354.

- 1  
2 1 68. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E et al. Effects of Lactobacillus casei  
3 2 supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a  
4 3 randomized double-blind clinical trial. *Int J Rheum Dis*. 2014;17:519-527.  
5  
6 4 69. Schorpion A, Kolasinski SL. Can Probiotic Supplements Improve Outcomes in Rheumatoid Arthritis?  
7 5 *Curr Rheumatol Rep*. 2017;19:73.  
8  
9 6 70. Taylor W, Gladman D, Helliwell P et al. Classification criteria for psoriatic arthritis: development of  
10 7 new criteria from a large international study. *Arthritis Rheum*. 2006;54:2665-2673.  
11  
12 8 71. Qin J, Li Y, Cai Z et al. A metagenome-wide association study of gut microbiota in type 2 diabetes.  
13 9 *Nature*. 2012;490:55-60.  
14  
15 10 72. Nielsen HB, Almeida M, Juncker AS et al. Identification and assembly of genomes and genetic  
16 11 elements in complex metagenomic samples without using reference genomes. *Nat Biotechnol*.  
17 12 2014;32:822-828.  
18  
19 13 73. Li J, Jia H, Cai X et al. An integrated catalog of reference genes in the human gut microbiome. *Nat*  
20 14 *Biotechnol*. 2014;32:834-841.  
21  
22 15 74. Mishra A, Makharia GK. Techniques of functional and motility test: how to perform and interpret  
23 16 intestinal permeability. *J Neurogastroenterol Motil*. 2012;18:443-447.  
24  
25 17 75. Sequeira IR, Lentle RG, Kruger MC et al. Standardising the lactulose mannitol test of gut  
26 18 permeability to minimise error and promote comparability. *PLoS One*. 2014;9:e99256.  
27  
28 19 76. Thorsen H, Hansen TM, McKenna SP et al. Adaptation into Danish of the Stanford Health  
29 20 Assessment Questionnaire (HAQ) and the Rheumatoid Arthritis Quality of Life Scale (RAQoL). *Scand*  
30 21 *J Rheumatol*. 2001;30:103-109.  
31  
32 22 77. Brodzky V, Pentek M, Balint PV et al. Comparison of the Psoriatic Arthritis Quality of Life (PsAQoL)  
33 23 questionnaire, the functional status (HAQ) and utility (EQ-5D) measures in psoriatic arthritis: results  
34 24 from a cross-sectional survey. *Scand J Rheumatol*. 2010;39:303-309.  
35  
36 25 78. Zachariae R, Zachariae C, Ibsen H et al. Dermatology life quality index: data from Danish inpatients  
37 26 and outpatients. *Acta Derm Venereol*. 2000;80:272-276.  
38  
39 27 79. Fransen J, Antoni C, Mease PJ et al. Performance of response criteria for assessing peripheral  
40 28 arthritis in patients with psoriatic arthritis: analysis of data from randomised controlled trials of two  
41 29 tumour necrosis factor inhibitors. *Ann Rheum Dis*. 2006;65:1373-1378.  
42  
43 30 80. Felson DT, Anderson JJ, Boers M et al. American College of Rheumatology. Preliminary definition of  
44 31 improvement in rheumatoid arthritis. *Arthritis Rheum*. 1995;38:727-735.  
45  
46 32 81. Felson DT, Anderson JJ, Lange ML et al. Should improvement in rheumatoid arthritis clinical trials  
47 33 be defined as fifty percent or seventy percent improvement in core set measures, rather than  
48 34 twenty percent? *Arthritis Rheum*. 1998;41:1564-1570.  
49  
50 35 82. Faria JR, Aarao AR, Jimenez LM et al. Inter-rater concordance study of the PASI (Psoriasis Area and  
51 36 Severity Index). *An Bras Dermatol*. 2010;85:625-629.  
52  
53 37 83. Jensen OK, Callesen J, Nielsen MG et al. Reproducibility of tender point examination in chronic low  
54 38 back pain patients as measured by intrarater and inter-rater reliability and agreement: a validation  
55 39 study. *BMJ Open*. 2013;3.



- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 84. Klingberg E, Carlsten H, Hilme E et al. Calprotectin in ankylosing spondylitis--frequently elevated in feces, but normal in serum. *Scand J Gastroenterol*. 2012;47:435-444.
- 2  
3 85. Kelly CR, Kahn S, Kashyap P et al. Update on Fecal Microbiota Transplantation 2015: Indications, Methodologies, Mechanisms, and Outlook. *Gastroenterology*. 2015;149:223-237.
- 4  
5 86. Wang S, Xu M, Wang W et al. Systematic Review: Adverse Events of Fecal Microbiota Transplantation. *PLoS One*. 2016;11:e0161174.
- 6  
7 87. Rossen NG, MacDonald JK, de Vries EM et al. Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. *World J Gastroenterol*. 2015;21:5359-5371.
- 8  
9 88. Girotra M, Garg S, Anand R et al. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection in the Elderly: Long-Term Outcomes and Microbiota Changes. *Dig Dis Sci*. 2016;61:3007-3015.
- 10  
11 89. Gweon TG, Kim J, Lim CH et al. Fecal Microbiota Transplantation Using Upper Gastrointestinal Tract for the Treatment of Refractory or Severe Complicated Clostridium difficile Infection in Elderly Patients in Poor Medical Condition: The First Study in an Asian Country. *Gastroenterol Res Pract*. 2016;2016:2687605.
- 12  
13 90. Agrawal M, Aroniadis OC, Brandt LJ et al. The Long-term Efficacy and Safety of Fecal Microbiota Transplant for Recurrent, Severe, and Complicated Clostridium difficile Infection in 146 Elderly Individuals. *J Clin Gastroenterol*. 2016;50:403-407.
- 14  
15 91. Di BS, Gouliouris T, Petrosillo N. Fecal microbiota transplantation (FMT) for Clostridium difficile infection: focus on immunocompromised patients. *J Infect Chemother*. 2015;21:230-237.
- 16  
17 92. Webb BJ, Brunner A, Ford CD et al. Fecal microbiota transplantation for recurrent Clostridium difficile infection in hematopoietic stem cell transplant recipients. *Transpl Infect Dis*. 2016.
- 18  
19 93. Harris PA, Taylor R, Thielke R et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377-381.
- 20  
21 94. Altman DG, Dore CJ. Randomisation and baseline comparisons in clinical trials. *Lancet*. 1990;335:149-153.
- 22  
23 95. Normand SL, Sykora K, Li P et al. Readers guide to critical appraisal of cohort studies: 3. Analytical strategies to reduce confounding. *BMJ*. 2005;330:1021-1023.
- 24  
25 96. White IR, Horton NJ, Carpenter J et al. Strategy for intention to treat analysis in randomised trials with missing outcome data. *BMJ*. 2011;342:d40.
- 26  
27 97. Liu R, Hong J, Xu X et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med*. 2017;23:859-868.
- 28  
29 98. Pedersen HK, Gudmundsdottir V, Nielsen HB et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*. 2016;535:376-381.
- 30  
31 99. Thaiss CA, Zmora N, Levy M et al. The microbiome and innate immunity. *Nature*. 2016;535:65-74.
- 32  
33 100. McLean MH, Dieguez D, Jr., Miller LM et al. Does the microbiota play a role in the pathogenesis of autoimmune diseases? *Gut*. 2015;64:332-341.

- 1  
2 1 101. Longman RS, Yang Y, Diehl GE et al. Microbiota: host interactions in mucosal homeostasis and  
3 2 systemic autoimmunity. *Cold Spring Harb Symp Quant Biol.* 2013;78:193-201.  
4  
5 3 102. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin*  
6 4 *Gastroenterol.* 2015;31:69-75.  
7  
8 5 103. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune  
9 6 system. *Science.* 2012;336:1268-1273.  
10  
11 7 104. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health  
12 8 and disease. *Nat Rev Immunol.* 2009;9:313-323.  
13  
14 9 105. Van de Wiele T, Van Praet JT, Marzorati M et al. How the microbiota shapes rheumatic diseases.  
15 10 *Nat Rev Rheumatol.* 2016;12:398-411.  
16  
17 11 106. Butto LF, Haller D. Dysbiosis in intestinal inflammation: Cause or consequence. *Int J Med Microbiol.*  
18 12 2016.  
19  
20 13 107. Moayyedi P, Surette MG, Kim PT et al. Fecal Microbiota Transplantation Induces Remission in  
21 14 Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology.*  
22 15 2015;149:102-109.  
23  
24 16 108. Rossen NG, Fuentes S, van der Spek MJ et al. Findings From a Randomized Controlled Trial of Fecal  
25 17 Transplantation for Patients With Ulcerative Colitis. *Gastroenterology.* 2015;149:110-118.  
26  
27 18 109. Smolen JS, Schols M, Braun J et al. Treating axial spondyloarthritis and peripheral spondyloarthritis,  
28 19 especially psoriatic arthritis, to target: 2017 update of recommendations by an international task  
29 20 force. *Ann Rheum Dis.* 2017.  
30  
31 21 110. Zhernakova A, Kurilshikov A, Bonder MJ et al. Population-based metagenomics analysis reveals  
32 22 markers for gut microbiome composition and diversity. *Science.* 2016;352:565-569.  
33  
34 23 111. Falony G, Joossens M, Vieira-Silva S et al. Population-level analysis of gut microbiome variation.  
35 24 *Science.* 2016;352:560-564.  
36  
37 25 112. Forslund K, Hildebrand F, Nielsen T et al. Disentangling type 2 diabetes and metformin treatment  
38 26 signatures in the human gut microbiota. *Nature.* 2015;528:262-266.  
39  
40 27 113. Zhang X, Zhang D, Jia H et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis  
41 28 and partly normalized after treatment. *Nat Med.* 2015;21:895-905.  
42  
43 29 114. Woodworth MH, Neish EM, Miller NS et al. Laboratory Testing of Donors and Stool for Fecal  
44 30 Microbiota Transplantation for Recurrent *C. difficile* Infection. *J Clin Microbiol.* 2017.  
45  
46 31 115. Costello SP, Tucker EC, La BJ et al. Establishing a Fecal Microbiota Transplant Service for the  
47 32 Treatment of *Clostridium difficile* Infection. *Clin Infect Dis.* 2016;62:908-914.  
48  
49 33 116. Cammarota G, Ianiro G, Tilg H et al. European consensus conference on faecal microbiota  
50 34 transplantation in clinical practice. *Gut.* 2017;66:569-580.  
51  
52 35 117. Chu ND, Smith MB, Perrotta AR et al. Profiling Living Bacteria Informs Preparation of Fecal  
53 36 Microbiota Transplantations. *PLoS One.* 2017;12:e0170922.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 118. Satokari R, Mattila E, Kainulainen V et al. Simple faecal preparation and efficacy of frozen inoculum  
2 in faecal microbiota transplantation for recurrent *Clostridium difficile* infection--an observational  
3 cohort study. *Aliment Pharmacol Ther.* 2015;41:46-53.
- 4 119. Li J, Jia H, Cai X et al. An integrated catalog of reference genes in the human gut microbiome. *Nat*  
5 *Biotechnol.* 2014;32:834-841.
- 6 120. Arumugam M, Raes J, Pelletier E et al. Enterotypes of the human gut microbiome. *Nature.*  
7 2011;473:174-180.
- 8 121. Kazerouni A, Wein LM. Exploring the Efficacy of Pooled Stools in Fecal Microbiota Transplantation  
9 for Microbiota-Associated Chronic Diseases. *PLoS One.* 2017;12:e0163956.
- 10 122. Vandeputte D, Falony G, Vieira-Silva S et al. Stool consistency is strongly associated with gut  
11 microbiota richness and composition, enterotypes and bacterial growth rates. *Gut.* 2016;65:57-62.
- 12 123. Ley RE. The gene-microbe link. *Nature.* 2015;518:57.
- 13 124. Li SS, Zhu A, Benes V et al. Durable coexistence of donor and recipient strains after fecal microbiota  
14 transplantation. *Science.* 2016;352:586-589.
- 15 125. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut  
16 microbiome. *Nat Rev Microbiol.* 2011;9:279-290.
- 17 126. Markle JG, Frank DN, Mortin-Toth S et al. Sex differences in the gut microbiome drive hormone-  
18 dependent regulation of autoimmunity. *Science.* 2013;339:1084-1088.
- 19 127. Xiao L, Estelle J, Kiilerich P et al. A reference gene catalogue of the pig gut microbiome. *Nat*  
20 *Microbiol.* 2016;16161.
- 21 128. Markle JG, Frank DN, Adeli K et al. Microbiome manipulation modifies sex-specific risk for  
22 autoimmunity. *Gut Microbes.* 2014;5:485-493.
- 23 129. Mills S, Shanahan F, Stanton C et al. Movers and shakers: influence of bacteriophages in shaping  
24 the mammalian gut microbiota. *Gut Microbes.* 2013;4:4-16.
- 25 130. Pfeiffer JK, Virgin HW. Viral immunity. Transkingdom control of viral infection and immunity in the  
26 mammalian intestine. *Science.* 2016;351.
- 27 131. Underhill DM, Pearlman E. Immune Interactions with Pathogenic and Commensal Fungi: A Two-  
28 Way Street. *Immunity.* 2015;43:845-858.
- 29 132. Castelino M, Eyre S, Moat J et al. The skin microbiome in psoriatic arthritis: methodology  
30 development and pilot data. *Lancet.* 2015;385 Suppl 1:S27.
- 31 133. Scher JU, Joshua V, Artacho A et al. The lung microbiota in early rheumatoid arthritis and  
32 autoimmunity. *Microbiome.* 2016;4:60.
- 33  
34  
35  
36

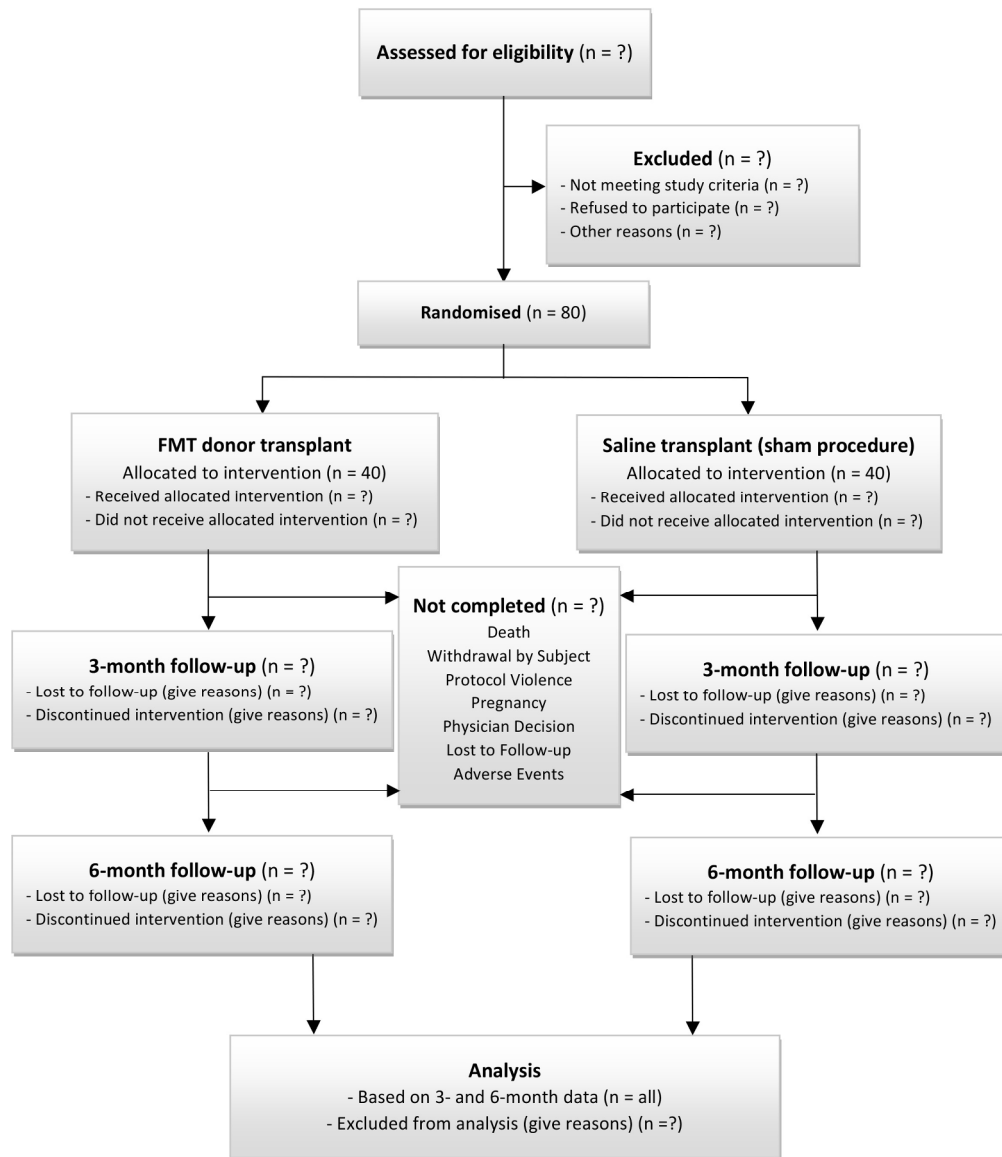


Figure 1. Flow diagram of the randomised, placebo-controlled trial.

198x236mm (300 x 300 DPI)

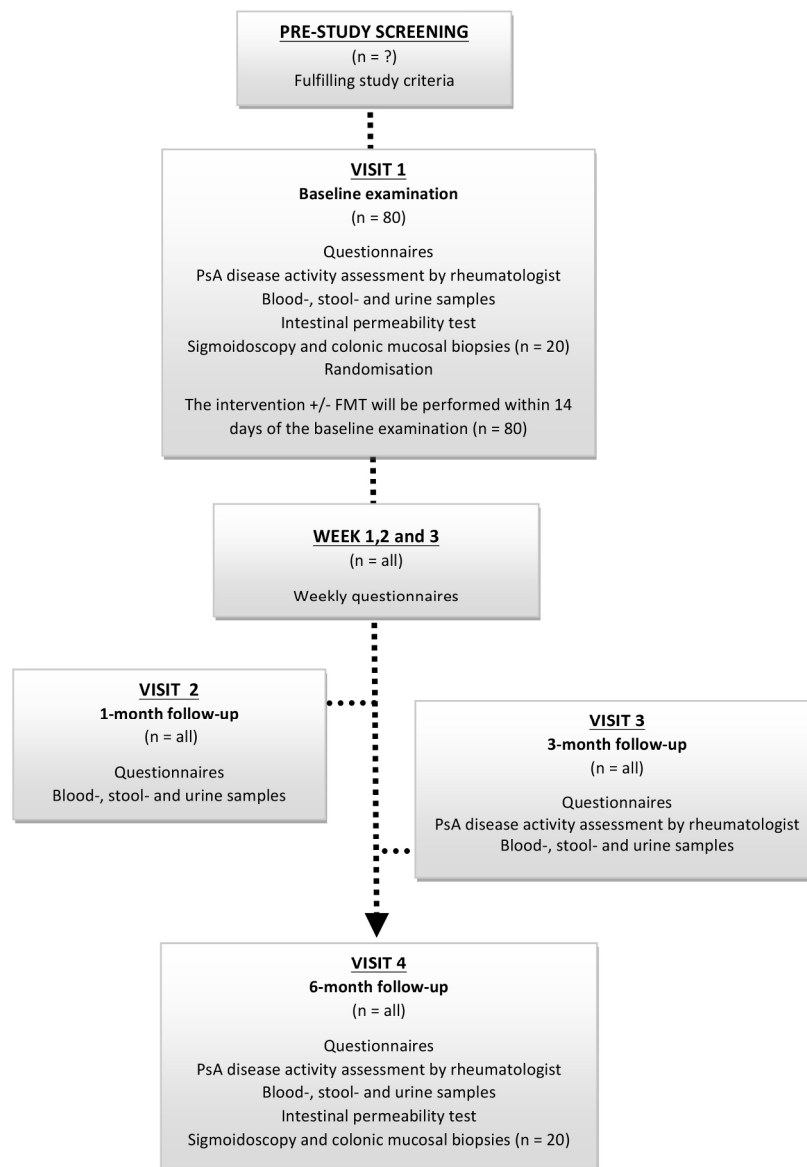


Figure 2. Participation timeline and characteristics of each visit.

160x237mm (300 x 300 DPI)



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

## SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	<u>1</u>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<u>2</u>
	2b	All items from the World Health Organization Trial Registration Data Set	<u>1-23</u>
Protocol version	3	Date and version identifier	<u>1</u>
Funding	4	Sources and types of financial, material, and other support	<u>23</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>1 and 22</u>
	5b	Name and contact information for the trial sponsor	<u>1</u>
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	<u>22</u>
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	<u>22</u>

## 1 Introduction

2			
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
4	rationale		<u>3-4</u>
5		6b	Explanation for choice of comparators
6			<u>4</u>
7	Objectives	7	Specific objectives or hypotheses
8			<u>4-5</u>
9	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, or single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
10			<u>5</u>
11			
12			
13			
14	<b>Methods: Participants, interventions, and outcomes</b>		
15			
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
17			<u>8</u>
18	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
19			<u>8-9</u>
20	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
21			<u>9-10</u>
22		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
23			<u>10</u>
24		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
25			<u>Not applicable</u>
26		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
27			<u>8 and 9</u>
28	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
29			<u>11-12</u>
30	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
31			<u>7</u>
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			

1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	<u>13-14</u>
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>8</u>
5				
6	<b>Methods: Assignment of interventions (for controlled trials)</b>			
7	<b>Allocation:</b>			
8				
9				
10	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	<u>14</u>
11				
12				
13				
14				
15				
16	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	<u>14</u>
17				
18				
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>14</u>
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>14</u>
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>14</u>
28				
29				
30				
31	<b>Methods: Data collection, management, and analysis</b>			
32				
33	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	<u>14</u>
34				
35				
36				
37				
38				
39		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	<u>16</u>
40				
41				
42				
43				
44				
45				
46				



1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	<u>14</u>
2				
3				
4				
5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	<u>15</u>
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>15-16</u>
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	<u>15</u>
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	<u>18</u>
17				
18				
19				
20				
21				
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	<u>18</u>
23				
24				
25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	<u>13</u>
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<u>18</u>
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>18</u>
35				
36				
37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	<u>18</u>
38				
39				
40				
41				
42				
43				
44				
45				
46				

1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	<u>14</u>
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>Not applicable</u>
5				
6				
7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	<u>14</u>
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>23</u>
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	<u>Not applicable</u>
14				
15				
16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	<u>18</u>
17				
18				
19				
20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	<u>18</u>
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>22</u>
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Not applicable</u>
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>18</u>
32				
33				
34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<u>10-11</u>
35				
36				

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by/4.0/)" license.

# BMJ Open

## Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised, placebo-controlled trial

### The FLORA trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019231.R2
Article Type:	Protocol
Date Submitted by the Author:	13-Feb-2018
Complete List of Authors:	Kragsnaes, Maja; Odense University Hospital, Department of Rheumatology; University of Southern Denmark, Odense Patient data Explorative Network (OPEN), Department of Clinical Institute Kjeldsen, Jens; Odense University Hospital, Department of Gastroenterology Horn, Hans; Odense University Hospital, Department of Rheumatology Munk, Heidi; Odense University Hospital, Department of Rheumatology Pedersen, Finn; Odense University Hospital, Department of Gastroenterology Holt, Hanne; Odense University Hospital, Department of Clinical Microbiology Pedersen, Jens Kristian; Odense University Hospital, Department of Rheumatology Holm, Dorte; Odense University Hospital, Department of Clinical Immunology Glerup, Henning; Silkeborg Regional Hospital, Diagnostic Centre Andersen, Vibeke; Hospital of Southern Jutland, IRS-Centre Sonderjylland; University of Southern Denmark, Institute of Molecular Medicine Fredberg, Ulrich; Silkeborg Regional Hospital, Diagnostic Centre Kristiansen, Karsten; University of Copenhagen, Laboratory of Genomics and Molecular Biomedicine, Department of Biology; BGI Christensen, Robin; Frederiksberg and Bispebjerg Hospital, Musculoskeletal Statistics Unit, Parker Institute Ellingsen, Torkell; Odense University Hospital, Department of Rheumatology
<b>Primary Subject Heading</b>:	Rheumatology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Psoriasis < DERMATOLOGY, Clinical trials < THERAPEUTICS, Faecal microbiota transplantation, Intestinal microbiota, Psoriatic arthritis

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

SCHOLARONE™  
Manuscripts

For peer review only

1  
2  
3 1  
4  
5 2  
6  
7 3 Efficacy and safety of faecal microbiota transplantation in  
8  
9 4 patients with psoriatic arthritis:  
10  
11 5 protocol for a 6-month, double-blind, randomised,  
12  
13 6 placebo-controlled trial  
14  
15 7

16  
17 8 The FLORA trial  
18  
19 9

20 10 *Kragsnaes MS<sup>1,2\*</sup>, Kjeldsen J<sup>3</sup>, Horn HC<sup>1</sup>, Munk HL<sup>1</sup>, Pedersen FM<sup>3</sup>, Holt HM<sup>4</sup>, Pedersen JK<sup>1</sup>, Holm*  
21 11 *DK<sup>5</sup>, Glerup H<sup>6</sup>, Andersen V<sup>7,8</sup>, Fredberg U<sup>6</sup>, Kristiansen K<sup>9,10</sup>, Christensen R<sup>11</sup>, Ellingsen T<sup>1\*\*</sup>.*

22  
23 12  
24  
25 13 <sup>1</sup> Department of Rheumatology, Odense University Hospital, Denmark.

26 14 <sup>2</sup> Odense Patient data Explorative Network (OPEN), Department of Clinical Institute, University of Southern Denmark.

27 15 <sup>3</sup> Department of Gastroenterology, Odense University Hospital, Denmark.

28 16 <sup>4</sup> Department of Clinical Microbiology, Odense University Hospital, Denmark.

29 17 <sup>5</sup> Department of Clinical Immunology, Odense University Hospital, Denmark.

30 18 <sup>6</sup> Diagnostic Centre, Silkeborg Regional Hospital, Denmark.

31 19 <sup>7</sup> IRS-Centre Sonderjylland, Hospital of Southern Jutland, Denmark.

32 20 <sup>8</sup> Institute of Molecular Medicine, University of Southern Denmark.

33 21 <sup>9</sup> Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Denmark.

34 22 <sup>10</sup> Institute of Metagenomics, BGI-Shenzhen, Shenzhen, China.

35 23 <sup>11</sup> Musculoskeletal Statistics Unit, The Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

36 24  
37 25 \* Corresponding author email address: [maja.kragsnaes@dadlnet.dk](mailto:maja.kragsnaes@dadlnet.dk).

38 26 \*\* Sponsor email address: [torkell.ellingsen@rsyd.dk](mailto:torkell.ellingsen@rsyd.dk).

39  
40 27  
41 28 Protocol manuscript version: 04.

42  
43 29 Date: Feb 13, 2018.

## 1 ABSTRACT

2 **Introduction:** An unbalanced intestinal microbiota may mediate activation of the inflammatory  
3 pathways seen in psoriatic arthritis (PsA). A randomised, placebo-controlled trial of faecal  
4 microbiota transplantation (FMT) infused into the small intestine of PsA patients with active  
5 peripheral disease who are non-responsive to methotrexate (MTX) treatment will be conducted.  
6 The objective is to explore clinical aspects associated with FMT performed in PsA patients.

7  
8 **Methods and analysis:** The FLORA trial is a randomised, two-centre stratified, double-blind  
9 (patient, care provider and outcome assessor), placebo-controlled, parallel-group study. Eighty  
10 patients will be included and randomised (1:1) to either placebo (saline) or FMT provided from an  
11 anonymous healthy donor. Throughout the study, both groups will continue the weekly self-  
12 administered subcutaneous (s.c.) MTX treatment, remaining on the pre-inclusion dosage (15-25  
13 mg/week). The clinical measures of psoriasis and PsA disease activity used include the Health  
14 Assessment Questionnaire (2-page DI-HAQ), the Dermatology Quality of Life Index (DLQI), the  
15 Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index, the Psoriasis Area  
16 Severity Index (PASI), a dactylitis digit count, a swollen/tender joint count (66/68), plasma C-  
17 reactive protein as well as visual analogue scales for pain, fatigue, and patient and physician global  
18 assessments. The primary endpoint is the proportion of patients who experience treatment failure  
19 during the 6-month trial period. The number of adverse events will be registered throughout the  
20 study.

21  
22 **Ethics and dissemination:** This is a proof-of-concept clinical trial and will be performed in  
23 agreement with Good Clinical Practice (GCP) standards. Approvals have been obtained from the  
24 local Ethics Committee (DK-S-20150080) and the Danish Data Protection Agency (15/41684). The  
25 study has commenced in May 2017. Dissemination will be through presentations at national and  
26 international conferences and through publications in international peer-reviewed journal(s).

27  
28 **Trial registration number at ClinicalTrials.gov:** NCT03058900

### 29 30 **Strengths and limitations of this study**

- 31 • This is a double-blind, randomised, placebo-controlled trial.
- 32 • Subcutaneously administered MTX treatment.
- 33 • The primary endpoint is based on shared decision-making between patient and physician.
- 34 • No feasibility data regarding FMT in rheumatic patients were available when the trial was  
35 designed.
- 36 • A limitation of the study is that the content of the faecal transplant suspension cannot be  
37 fully standardised.

## 1 INTRODUCTION

2 Emerging data suggest a causal relationship between the intestinal microbiota and  
3 spondyloarthritis (SpA), thus, linking dysbiosis of the complex microbial communities with SpA  
4 pathogenesis.<sup>1-5</sup> Psoriatic arthritis (PsA) is one of five SpA categories in adults which also include  
5 ankylosing spondylitis, undifferentiated arthritis, reactive arthritis and arthritis associated with  
6 inflammatory bowel disease. While the association between the gut and the latter two disorders is  
7 well established,<sup>6</sup> only very recently, studies evaluating the faecal microbiota and the presence of  
8 subclinical gut inflammation in PsA patients have coupled this disease to a perturbation of the  
9 intestinal microbiota composition.<sup>7-12</sup>

10 PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum  
11 and a varied disease course.<sup>13</sup> The clinical manifestations include peripheral arthritis, enthesitis  
12 and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis,  
13 and dactylitis.<sup>14</sup> Nearly half of the patients with both early and established PsA also present with  
14 extra-musculoskeletal manifestations which can include bowel (16%), ocular, cardiovascular or  
15 urogenital involvement.<sup>15</sup> Without disease modifying intervention, 40-60% of PsA patients will  
16 develop erosive and deforming joint damage within a few years of disease onset.<sup>16</sup> Methotrexate  
17 (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug  
18 (csDMARD) for initial therapy.<sup>17</sup> However, the evidence for MTX in PsA is poor, and a substantial  
19 number of patients does not benefit from such treatment.<sup>18</sup> Currently, other treatment options  
20 may include biological agents such as tumour necrosis factor (TNF- $\alpha$ ) inhibitors aiming to block  
21 some of the downstream molecular pathways driving the disease.<sup>19</sup> Still, these drugs do not target  
22 the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and  
23 environmental factors.<sup>20</sup> The interplay between these complex aetiological factors has yet to be  
24 fully understood.<sup>21,22</sup>

25 The classic pathophysiological concept of PsA is that it is an autoimmune disease of  
26 the skin and joints and that the pathological processes at both sites are driven by inflammatory  
27 responses involving the innate immune system, natural killer cells, T cells, and the expression of  
28 pro-inflammatory cytokines, including TNF- $\alpha$ , interleukin (IL)-1, interferon- $\gamma$ , IL-6, IL-12, IL-15, IL-18  
29 and the IL-17/IL-23 axis.<sup>23-27</sup> However, although microbial agents including dormant bacteria,  
30 mycobacteria, bacterial products and viral antigens have been implicated as potential  
31 initiators,<sup>28,29</sup> the true pathophysiological factors triggering the dysregulated immunological  
32 cascade underlying the disease remain to be identified.

33 Intriguingly, it has recently been suggested that mucosal sites exposed to a high load  
34 of bacterial antigens, in particular the gastrointestinal tract, may represent the initial site of  
35 immunological tolerance break in PsA.<sup>30</sup> Indeed, under normal conditions the host and the  
36 microbiota live in harmony and benefit from their mutualistic relationship. However, alterations of  
37 the normal intestinal microbiota can affect mucosal immunity which, in turn, can induce local  
38 inflammation and elicit systemic effects at distant sites.<sup>31</sup> Mechanisms through which the  
39 intestinal microbiota may be involved in the pathogenesis of PsA include an abnormal activation of  
40 the gut-associated lymphoid tissue,<sup>32</sup> a decrease in regulatory T cell activity,<sup>33</sup> and/or an altered  
41 mucosal permeability thus compromising the capacity of the intestine to provide adequate  
42 containment of luminal microorganisms and molecules.<sup>34,35</sup> In support of these theories, several  
43 studies have documented subclinical gut inflammation in PsA patients.<sup>36-41</sup> Moreover, a recent

1 study reported that several intestinal bacteria including *Akkermansia* and *Ruminococcus* were  
2 practically absent in PsA patients. These commensal bacteria are, in fact, known to play an  
3 important role in maintaining gut homeostasis.<sup>42</sup>

### 4 5 **Rationale**

6 If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen  
7 in PsA,<sup>8</sup> modifying the intestinal microbiota could be a novel treatment strategy for this disease.<sup>1-  
8 3,43</sup> Faecal microbiota transplantation (FMT) is currently being used to restore the balance of the  
9 intestinal flora.<sup>44,45</sup> Particularly, this procedure has demonstrated more than 90% clinical  
10 resolution of recurrent or refractory *Clostridium difficile* infections.<sup>46-50</sup> Also, multiple FMTs seem  
11 to be able to induce remission in patients with inflammatory bowel disease (IBD).<sup>51</sup> Due to these  
12 results, FMT is now being tested as a potential novel treatment for other gastrointestinal and  
13 extra-intestinal diseases.<sup>52</sup> To the best of our knowledge, no study has yet ascertained the efficacy  
14 and safety of FMT in patients with inflammatory rheumatic diseases.

### 15 16 **Evidence-based research**

17 To avoid waste of research no new studies should be initiated without a systematic review of the  
18 existing evidence.<sup>53</sup> We performed a pragmatic search in the biomedical literature via Pubmed  
19 combining different related MeSH terms: ("Microbiota"[Mesh] OR "Fecal Microbiota  
20 Transplantation"[Mesh] OR "Faecal Microbiota Transplantation"[Mesh] OR "Gastrointestinal  
21 Microbiome"[Mesh]) AND (arthritis[tiab] OR "Arthritis"[Mesh] OR "Arthritis, Psoriatic"[Mesh] OR  
22 "Arthritis, Reactive"[Mesh] OR "Spondylarthritis"[Mesh] OR "Arthritis, Gouty"[Mesh] OR "Arthritis,  
23 Rheumatoid"[Mesh] OR "Psoriasis"[Mesh]). From the search revealing 122 citations it became  
24 clear that the majority of papers were reviews/editorials (74 [61%]), where the overall conclusion  
25 was that the main challenges are to uncover the cause-effect relationship between the intestinal  
26 microbiota and rheumatic diseases, and to investigate the potential of microbiome-targeting  
27 strategies.<sup>1,3,5,6,20,32,43,54-60</sup> Also from the published literature it became evident that to date only  
28 nine clinical interventional studies trying to modify the intestinal microbiota in arthritis patients  
29 have been performed: One study in SpA patients (n = 63),<sup>61</sup> and one study in enthesitis-related  
30 arthritis (n = 8) reported no beneficial effects of probiotic therapy,<sup>62</sup> whereas one study in juvenile  
31 idiopathic arthritis testing exclusive enteral nutrition administration (n = 7) found a moderate anti-  
32 inflammatory effect on active joints.<sup>63</sup> Five placebo-controlled trials of probiotic therapy in  
33 rheumatoid arthritis patients<sup>64-68</sup> (sample size between 26 and 60 patients) reported mixed  
34 results.<sup>69</sup> However, two of these studies demonstrated positive clinical effects of probiotic therapy  
35 which included improvement in HAQ-DI pain scale,<sup>65</sup> improvement in the Disease Activity Score of  
36 28 joints (DAS-28), and improvement on the C-reactive protein concentrations.<sup>66</sup> No clinical trials  
37 performing FMT on arthritic patients were identified.

### 38 39 **Objective**

40 The objective of this randomised trial is to explore whether FMT is more effective than placebo in  
41 reducing disease activity in PsA patients with active peripheral arthritis concomitantly treated with  
42 weekly subcutaneously administered MTX. In addition, extensive bacteria taxonomic and



1 metagenomic analyses will be performed on faecal samples before and after the FMT to get an  
2 indication of the functional capacity of the intestinal microbiota.

## 4 METHODS AND ANALYSIS

### 5 Trial design

6 This is a randomised – patient, physician and outcome-assessor blinded, placebo-controlled, 6-  
7 month trial, which will be followed by an open-label extension period for a minimum of 2 years.  
8 Patients will be randomly assigned in a 1:1 ratio to receive FMT or placebo (sham procedure).  
9 Outcome assessment will be based on follow-up by a rheumatologist and is scheduled to occur  
10 after 3 and 6 months (with the latter being the primary end-point evaluation), see Figure 1 and  
11 Figure 2.

### 13 Participants

14 Recruitment will take place at Danish rheumatology outpatient clinics, and patients fulfilling the  
15 eligibility criteria will be offered participation. No treatment with biologics within 6 months, and  
16 no systemic and/or local intra-articular or peritendinous steroid injections, or non-MTX csDMARD  
17 treatment, or antibiotics are allowed within 3 months prior to inclusion. Non-Steroidal Anti-  
18 Inflammatory Drugs (NSAIDs) must be paused within 14 days of study inclusion. Patients, who do  
19 not wish to participate, will be characterised by sex and age. The recruitment has commenced in  
20 May 2017 and will continue until 2019.

### 22 Psoriatic arthritis patients

23 A total of 80 PsA patients will be enrolled, and they will have to meet the following eligibility  
24 criteria:

#### 26 *Inclusion criteria:*

- 27 • Diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR).<sup>70</sup>
- 28 • Presence of active peripheral arthritis defined as  $\geq 3$  swollen joints.
- 29 • Subcutaneously administered MTX treatment ( $\geq 15\text{mg/week}$  (maximal tolerable dosage))  
30 for a minimum of 3 months prior to study inclusion.
- 31 • Age 18 to 70 years.

#### 33 *Exclusion criteria:*

- 34 • Other inflammatory rheumatic diseases than PsA.
- 35 • Current axial disease activity or severe peripheral joint activity demanding immediate  
36 change of treatment or contraindicating placebo treatment for 6 months.
- 37 • Inflammatory bowel disease, coeliac disease, food allergy, or other intestinal diseases.
- 38 • Current cancer or severe chronic infections.

- 1 • History of severe MTX toxicity or allergic reactions.
- 2 • Biological treatment within 6 months prior to inclusion.
- 3 • Non-MTX DMARD treatment within 3 months prior to inclusion.
- 4 • Systemic and/or local intra-articular or peritendinous steroid injections within 3 months
- 5 prior to inclusion.
- 6 • NSAIDs within 14 days prior to inclusion.
- 7 • Antibiotics within 3 months prior to inclusion.
- 8 • Pregnant or breastfeeding women.
- 9 • Not wishing to participate or unsuited for project evaluation.

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

### Stool donors

The stool donor corps will consist of four anonymous (to the recipient) donors who must be healthy as assessed by a screening questionnaire, and be active members of the Danish blood donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m<sup>2</sup>, and an average alcohol intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie diets), and must not be in a stressful life period. Before joining the stool donor corps, each potential donor will go through a screening process including stool analyses for faecal calprotectin and enteric pathogens (*Aeromonas*, *Campylobacter*, *C. difficile*, diarrhoeagenic *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, and multidrug-resistant bacteria, parasites including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium* (DNA) and *Giardia* (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-reactive protein (CRP) (acceptable level: < 6.0 mg/L), white blood cell count (acceptable range: 3.50-8.80 10<sup>9</sup>/L), haemoglobin (acceptable range: 8.3-10.5 mmol/L), albumin (acceptable range: 36-50 g/L), alanine aminotransferase (ALAT) (acceptable range: 10-70 U/L), glomerular filtration rate (eGFR) (acceptable level: > 59 mL/min), and coeliac disease, and blood test for infectious agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM), hepatitis A, B, C and E, tuberculosis (QuantiFERON<sup>®</sup> TB-Gold test), syphilis, human immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies), and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing the screening tests, the donor will donate stool for the next month after which, the donor will have to pass the screening programme once more before the stool can be released for transplantation.

51

52

53

54

55

56

57

58

59

60

### Interventions

#### Overall study interventions

1 The FMT will be an add-on strategy for PsA patients with active joint disease despite ongoing  
2 treatment with weekly subcutaneously administered MTX. Therefore, all enrolled PsA patients will  
3 continue their MTX treatment throughout the study, and they will remain on the same individual  
4 dosage that they received at the time of study inclusion (a minimum of 15 mg/week cf. the patient  
5 inclusion criteria) in addition to folic acid supplement. Paracetamol and tramadol in recommended  
6 dosages are allowed during the trial but no NSAIDs can be taken.

#### 7 8 *Active and sham comparator*

9 Patients will be randomised into two groups with an allocation ratio of active-to-placebo  
10 treatment of 1:1. The active comparator group (n = 40) will have an FMT with healthy donor  
11 faeces-suspension (250 mL) containing 50 g donor faeces, saline (NaCl 0.9%) and glycerol (10%),  
12 whereas, the sham comparator group (n = 40) will be treated with an identical appearing sham  
13 procedure where the transplant solution will consist of 250 mL brown coloured (brown food  
14 colourant) isotonic saline (NaCl 0.9%).

#### 15 16 *Preparing the FMT suspension*

17 Donors will collect faeces at home and transport it in a cooling bag to the study site within 1 hour.  
18 Faeces will be sieved to remove particulate material, followed by dilution in sterile saline (0.9%  
19 NaCl) and 10% glycerol. The FMT suspension will be stored at - 80 °C until use. On the day of the  
20 FMT transplantation, the transplant suspension (250 mL) will be thawed to 37 °C and subsequently  
21 apportioned into five 50 mL syringes.

#### 22 23 *FMT procedure*

24 The FMT will take place within 14 days (preferably 7 days) of the baseline clinical examination. The  
25 evening prior to the FMT, patients will take one dose (40 mg) of oral proton-pump inhibitor. They  
26 will meet at the Department of Gastroenterology after a six-hour fast. A total of 250 mL transplant  
27 suspension (active or placebo) will be installed in the duodenum using an oral-duodenal tube. The  
28 correct placement of the tube will be confirmed using gastroscopic guidance.

#### 29 30 *Treatment strategy for non-responders*

31 Patients who present with increased or unacceptable disease activity during the 6-month trial  
32 period will, depending on the clinical presentation, be offered another treatment strategy which  
33 may include local intra-articular steroid injections, change to another csDMARD or biological  
34 treatment. If the patient accepts such treatment changes, this will be characterised as FMT  
35 treatment failure according to the primary outcome definition (one intra-articular steroid injection  
36 is allowed).

#### 37 38 *MTX toxicity and drop-outs*

39 Blood tests for MTX toxicity will be performed in accordance with our current clinical practice. In  
40 case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other  
41 diseases that contraindicate MTX treatment, MTX dosage will be decreased or the treatment will  
42 be paused. These patients will remain in the study (unless their condition contraindicates this),

1 and they will be analysed as members of the treatment group to which they were randomised  
2 using intention-to-treat-type analyses.

#### 3 4 *Collection of faecal samples and metagenomics analysis*

5 Fresh faecal samples will be collected by the patient at home using an EasySampler® stool  
6 collection kit within 24 hours prior to the study visit. Samples will be stored in the patient's freezer  
7 until transport to the study site. During transport, samples will be kept on ice in a cooling bag.  
8 Upon arrival to the study site, samples will immediately be transferred to the biobank and stored  
9 at -80°C. Bacterial DNA will be extracted from the faecal samples following established standard  
10 protocols including bead beating using a NucleoSpin soil kit (Macherey-Nagel, Germany) according  
11 to manufacturer's instructions. DNA will be sequenced using the BGISEQ-500 Platform which was  
12 recently benchmarked against the Illumina platforms showing excellent intra-platform  
13 reproducibility and less GC bias than observed using the Illumina platforms.<sup>71</sup> The faecal  
14 metagenomics bioinformatics analyses will be performed using comprehensive pipelines including  
15 the assembly of metagenomics linkage groups/metagenomics species,<sup>72,73</sup> taxonomic annotation,  
16 and extensive functional analyses based on metagenomic species which provides a superior  
17 dataset compared to the conventional analyses based on the total gene pool.<sup>74</sup>

#### 18 19 *Intestinal permeability test*

20 After an overnight fasting, patients will provide a urine sample before ingesting 100 mL water  
21 containing 10 g of lactulose and 5 g of D-mannitol. All the urine passed in the subsequent 5 hours  
22 will be collected into a 2 L plastic container containing 1 mL of chlorohexidine (20 mg/mL) as a  
23 preservative. After 3- and 5 hours, the volume of the urine will be measured and a small volume  
24 (10 mL) will be preserved and stored at -80°C until analysis. No food or drinking (except for water)  
25 will be allowed during the test.<sup>75,76</sup>

#### 26 27 **Outcomes**

##### 28 *Primary outcome measure:*

29 Treatment failure [Time Frame: 6 months (+/- 14 days)]

30 Proportion of patients in each group who experience treatment failure according to shared  
31 decision making between patient and rheumatologist defined as at least one of the following:

- 32 ○ Need for more than one intra-articular glucocorticoid injection due to disease  
33 activity.
- 34 ○ Need for change to other csDMARDs (e.g., oral leflunomide or sulfasalazin)  
35 according to the updated Danish treatment guideline due to disease activity.
- 36 ○ Need for biologic treatment according to the updated Danish treatment guideline  
37 due to severe disease activity.

##### 38 39 *Secondary outcome measures:*

40 Change from baseline in the Short Health Assessment Questionnaire (2-page HAQ)<sup>77,78</sup>

41 [Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14  
42 days)]

1  
2  
3  
4 2 Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire<sup>79</sup> [Time Frame: 1  
5 3 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
6  
7 4

8 5 Changes from baseline in patient reported gastrointestinal side effects [Time Frame: 1 week, 2  
9 6 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
10  
11 7

12 8 Other non-gastrointestinal patient reported side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4  
13 9 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]  
14  
15 10

16 11 Proportion of patients in each group achieving the American College of Rheumatology (ACR)<sup>80</sup>  
17 12 Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

18 13 I. ACR20 response criteria<sup>81</sup>

19 14 II. ACR50 response criteria<sup>82</sup>

20 15 III. ACR70 response criteria<sup>82</sup>  
21  
22  
23  
24  
25  
26 16

27 17 Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)<sup>80</sup>  
28 18 [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
29  
30 19

31 20 Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis  
32 21 Index<sup>68</sup> in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7  
33 22 days), 6 months (+/- 14 days)]  
34  
35 23

36 24 Change from baseline in the Psoriasis Area Severity Index (PASI)<sup>83</sup> in the subset of patients who  
37 25 have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days) ]  
38  
39 26

40 27 Change from baseline in the number of digits affected with dactylitis in the subset of patients who  
41 28 have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]  
42  
43 29

44 30 Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]  
45  
46 31

47 32 Number of adverse events in each group leading to discontinuation [Time Frame: 6 months (+/- 14  
48 33 days)]  
49  
50 34

51 35 Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14  
52 36 days)]  
53  
54 37  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 *Tertiary (exploratory secondary) outcomes:* Proportion of patients in each group achieving changes  
2 in plasma CRP, changes in tender point count,<sup>84</sup> changes in faecal bacteria composition and  
3 metabolism, changes in intestinal permeability, changes in plasma orosomucoid, changes in  
4 plasma and faecal calprotectin,<sup>85</sup> changes in serum 1,25-dihydroxyvitamin D, changes in  
5 cardiovascular risk factors including Body Mass Index (BMI), blood pressure, plasma triglyceride,  
6 plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, and HbA<sub>1</sub>C levels,  
7 changes in specific circulating inflammatory markers (i.e. cytokines, adipokines, and chemokines),  
8 and macroscopic and microscopic inflammatory changes of the colonic mucosa, see Table 1.  
9

## 10 **Safety**

11 The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild  
12 diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the  
13 procedure.<sup>86</sup> A recent systematic review on the adverse events of FMT identified 50 relevant  
14 studies with a total of 1,089 patients. In this review, the incidences of serious adverse events  
15 (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The  
16 SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%),  
17 death (0.3%), auto-immune diseases and FMT procedure related injury.<sup>87</sup> Although most of the  
18 patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection  
19 and/or IBD), these findings warrant caution when performing FMT; especially when introducing  
20 the procedure in a new patient population. In addition, the potential long term side effects  
21 following FMT remains largely unknown.<sup>88</sup> Still, when strict donor screening is conducted and the  
22 procedure is performed by experienced practitioners, FMT is in general considered safe, and even  
23 elderly patients with a poor medical condition and multiple comorbidities as well as  
24 immunosuppressed patients have been proven to tolerate the FMT procedure well.<sup>89-93</sup>

25 In the present study, we will carefully monitor and evaluate safety by means of open  
26 assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be  
27 monitored until resolution, stabilisation or until it has been shown that the study intervention is  
28 not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events,  
29 version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events.  
30 Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool  
31 type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a  
32 week for the first month following the randomised intervention. Routine blood screening for MTX  
33 toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by  
34 the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject  
35 incidence rates of all treatment-emergent AE will be tabulated by system organ class and  
36 preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant  
37 treatment-emergent adverse events, will also be provided. For the long-term extension portion of  
38 this study, exposure adjusted event rates will be summarised.  
39

## 40 **Sample size and power considerations**

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 When designing this trial, no prior data for FMT efficacy in rheumatic patients were available.  
2 However, we found it reasonable to assume that if rheumatic patients should be willing to receive  
3 FMT as a future standardised treatment, the procedure should at least provide an effect size well  
4 beyond a moderate effect size. Consequently, we decided that at least twice as many PsA patients  
5 in the sham group should be treatment failures compared to the FMT group if the procedure  
6 should be considered clinical relevant. For a comparison of two independent binomial proportions  
7 using the Pearson's chi-squared statistic with a Chi-square approximation (a two-sided significance  
8 level of 0.05), a sample size of 40 PsA patients per group has a power of 90% (0.895) if we assume  
9 that the proportions of treatment failures are 35% (FMT-active group) and 70% (FMT-sham  
10 group), respectively. Consequently, the inclusion of 80 PsA patients allocated (1:1) to two  
11 treatment arms is believed to be sufficient to reveal any difference of clinical importance between  
12 treatment groups (i.e., an NNT <3 patients).

13 Assuming that there will be some attrition during the 6-month trial period, we also  
14 estimated how much drop-out would be possible while still having a reasonable statistical power  
15 (80%): a total sample size of 62 PsA patients assuming a comparable level of withdrawals (31  
16 patients completing in each group) achieves a power of at least 0.8 with the proportion of  
17 treatment failures indicated above; i.e., even if we experience a drop-out rate of 20%, our trial will  
18 have 80% chance of detecting the intentional difference between groups.

19 Beyond the primary endpoint, a total sample size of 80 (with a balanced design)  
20 corresponds to a sufficient statistical power (82%) to detect a standardised mean difference of  
21 0.65 SD units (i.e. Cohen's effect size) in any of the Patient-Reported Outcome Measures.  
22

### 23 **Randomisation, allocation concealment and blinding**

24 The randomisation has been conducted using central-computer randomisation. Patients are  
25 randomly allocated (1:1) to receive either a FMT or a placebo saline transplant (sham procedure).  
26 The randomisation lists were generated by the trial statistician and uploaded to the REDCap  
27 database by an independent data manager who is not involved in any other aspects of the trial.  
28 Eligible patients will - after signing informed consent - be assigned randomly in permuted blocks  
29 with varying sizes of 4 and 6, according to computer-generated random numbers (SAS  
30 programming via SAS PROC PLAN), to undergo either FMT or saline (sham) procedure using  
31 stratification for centre. The randomisation of each patient will be implemented by the local trial  
32 coordinator and allocation will be concealed as this is done independent of the pre-determined  
33 sequence generation (i.e. randomisation). The patients, care providers and outcome assessors will  
34 remain unaware of the group assignments, and only de-identified codes will be used to link  
35 participants to their data during the study to maintain their confidentiality. In case of exceptional  
36 circumstances when knowledge of the treatment allocation is essential for further management of  
37 the patient, the trial secretary will reveal the assigned intervention to the treating doctor.  
38 However, patients, trial care providers and outcome assessors will remain blinded as far as  
39 possible. Cases of unblinding will be registered and reported.  
40

## 1 Data collection, management and confidentiality

2 Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central  
3 REDCap<sup>94</sup> database hosted by Odense Patient data Explorative Network (OPEN) at Odense  
4 University Hospital. Data obtained during the clinical examination will be entered directly into the  
5 database. Also, patient questionnaires will be fulfilled directly into the database. Access to the  
6 study data will be restricted, and a password system will be utilized to control access. All  
7 information about the patients' health and other private matters is covered by confidentiality. The  
8 authorisation from the Danish Data Protection Agency has been secured.

## 10 Statistical methods

11 The full analysis set will consist of all randomised participants (i.e. the intention to treat [ITT]  
12 population): Participants will be analysed according to their randomised treatment group; i.e. the  
13 ITT has the consequence that participants allocated to a treatment group will be followed up,  
14 assessed and analysed as members of that group irrespective of their compliance to the planned  
15 treatment. The safety analysis set will include all patients who were randomly assigned to a study  
16 group and had exposure to a transplant (independent of group). Descriptive statistics will be  
17 provided for demographics and baseline characteristics. The summary statistics of continuous  
18 variables will include: N, mean, standard deviation, median, interquartiles, and range. All  
19 summaries presenting frequencies and incidences will include counts, percentages, and the total  
20 number of participants in the corresponding arm.

21 The pre-specified efficacy analyses will be based on data from the full-analysis set,  
22 which include all patients who underwent randomisation, have had their baseline measurement  
23 performed, and who have received the initial transplant (independent of group). Although proper  
24 random assignment prevents selection bias, it does not guarantee that the groups will be  
25 equivalent at baseline. Any differences in baseline characteristics are, however, the result of  
26 chance rather than bias;<sup>95</sup> thus, the study groups will be evaluated (and presented) at baseline for  
27 important demographic and clinical characteristics so that readers can assess how similar they are.  
28 However, only cohort studies can be subject to selection bias and confounding due to differences  
29 in baseline characteristics between the intervention and comparison groups.<sup>96</sup>

30 Our strategy for ITT analysis with incomplete observations will be based on the  
31 recommendations from White et al<sup>97</sup>:

- 32 1: Attempt to follow up all randomised participants, even if they withdraw from allocated  
33 treatment.
- 34 2: Perform a main analysis of all observed data (data as observed).
- 35 3: Perform sensitivity analyses to explore the effect of departures from the assumption made in  
36 the main analysis (Baseline Observation Carried Forward [BOCF] imputations, repeated measures  
37 mixed models, and multiple imputations).

38 This results in the following steps: Missing values will be imputed with the use of a  
39 non-responder imputation by use of the BOCF method for measurements made after baseline.  
40 Thus, missing data for dichotomous endpoints will also be imputed using a conservative "null  
41 responder" imputation, assuming the patient did not have any benefit from being enrolled in the



1 trial (e.g., for the primary endpoint we will assume that the patient had a treatment failure which  
2 is valid based on clinical judgement even if data is not missing at random [NMAR]). Other sensitivity  
3 analyses will be including “worst” and “best” case imputation, repeated-measures and multiple-  
4 imputation analyses, using model-based approaches; repeated measures linear mixed models will  
5 also be used to model the potential group-dependent trajectories over time (i.e. Repeated Mixed  
6 Models and Multiple Imputation are valid if data is assumed Missing at Random [MAR]).

7 Categorical data for dichotomous end points will be analysed with the use of logistic  
8 regression with the model including treatment and centre as class effects. For continuous  
9 outcome measures analysis of covariance (ANCOVA) models will be used to analyse mean changes  
10 in continuous end points. All models will include treatment, centre, with the baseline value of the  
11 relevant variable as covariates.

12 Additionally, completer analyses will be performed on those who complete 6 months  
13 of treatment. During follow-up, any medical treatments which could potentially modify the  
14 intestinal microbiota including antibiotics will be reported, but will not affect the statistical  
15 analysis. Statistical estimates will be calculated as odds ratios (OR) for the dichotomous variables  
16 and difference between means for continuous outcomes reported with 95% confidence intervals  
17 (95% CI). Two-sided 95% CIs and P-values for primary, secondary and exploratory outcomes will be  
18 computed and will not be adjusted for multiplicity, but will be interpreted cautiously as this is an  
19 exploratory trial per se.

20 Pre-specified exploratory analyses: Stratified analyses will investigate whether the  
21 treatment effect varies with I) the faecal microbiota analyses performed at follow-up compared  
22 with baseline (+/- long-term changes in the intestinal microbiota and intestinal inflammation); and  
23 II) the demographic match (sex, age) between the stool donor and the recipient. Non-responders  
24 will represent the outcome group not fulfilling the primary outcome measure. Differences in  
25 demographics and baseline disease activity between this treatment-failure subpopulation and the  
26 remaining group will be examined to identify potential prognostic factors for poor responders.  
27 Patients not participating in the follow-up examination will be classified as "drop-outs", and if  
28 possible, the reason for not participating will be registered.

29 The faecal metagenomics bioinformatics analyses will be performed using  
30 comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics  
31 species,<sup>72,73</sup> taxonomic annotation, and extensive functional analyses based on metagenomic  
32 species which provides a superior dataset compared to the conventional analyses based on the  
33 total gene pool.<sup>74</sup> To identify possible associations, metagenome analysis will be correlated to all  
34 clinical parameter. We will use an L1 restricted LASSO procedure to determine the optimal  
35 number of features to be tested as described. Analysis of correlations between microbiota  
36 taxonomic or functional features, community diversity indices and sample metadata variables will  
37 be performed using Spearman correlation tests corrected for multiple tests using the Benjamini-  
38 Hochberg false discovery rate control procedure. To control for confounders, we will use blocked  
39 Spearman tests as implemented in COIN.<sup>98,99</sup>

40 Data will be analysed with the STATA statistical package (version 15; StataCorp LP),  
41 and SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA).

Activity/assessment	Pre-study screening	Visit 1 Baseline	Week 1, 2 and 3	Visit 2 1 month	Visit 3 3 months	Visit 4 6 months
Patients	n = ?	n = 80	n = all	n = all	n = all	n = all
Screening log	x					
Inclusion/exclusion form	x					
Consent form		x				
Randomisation		x				
Study-composed questionnaire		x	x	x	x	x
Patient global (VAS 0-100 mm)		x	x	x	x	x
Patient fatigue (VAS 0-100 mm)		x	x	x	x	x
Patient pain (VAS 0-100 mm)		x	x	x	x	x
HAQ		x	x	x	x	x
BASDAI		x			x	x
BASFAI		x			x	x
DLQI		x	x	x	x	x
Gastrointestinal symptom diary		x	x	x	x	x
Eating habits questionnaire		x				
Clinical examination:						
- Height (m)		x				
- Weight (kg)		x			x	x
- Blood pressure (mmHg)		x			x	x
- Psoriasis Area Severity Index		x			x	x
- SPARCC Enthesitis Score		x			x	x
- Swollen joint count (66)		x			x	x
- Tender joint count (68)		x			x	x
- Doctors global (VAS 0-100 mm)		x			x	x
- BASMI		x			x	x
- Tender point count		x			x	x
Interview (AEs)				x	x	x
Blood sample analysis:						
- C-reactive protein (mg/L)		x		x	x	x
- Orosomucoid (g/L)		x		x	x	x
- Calprotectin		x		x	x	x
- 1,25-dihydroxyvitamin D (nmol/L)		x		x	x	x
- TSH (miu/L)		x				x
- Hgb (mmol/L)		x				x
- Triglyceride (mmol/L)		x				x
- LDL-cholesterol (mmol/L)		x				x
- HDL-cholesterol (mmol/L)		x				x
- Total-cholesterol (mmol/L)		x				x
- HbA <sub>1c</sub> (mmol/mol)		x				x
- HLA-B27 status (+/-)		x				
- Serology tests for <i>Yersinia</i> , <i>Campylobacter</i> , <i>Salmonella</i> (+/-)		x				
Faecal calprotectin		x		x	x	x
Faecal microbiota analysis		x		x	x	x
Sigmoidoscopy and mucosa biopsy		x				x
Stool, blood, and urine samples (biobank)		x		x	x	x
Intestinal permeability test		x				x
Intervention (+/- FMT)		x				
Serious adverse event forms				x		

**Table 1.** Protocol schedule of forms and procedures.

## 1 ETHICS AND DISSEMINATION

2 This study is designed as a proof-of-concept clinical trial and will be performed in agreement with  
3 GCP-standards, and in accordance with the ethical standards of the responsible committee on  
4 human experimentation (institutional and national) and with the Helsinki Declaration (64th, 2013).  
5 The relevance of the study, the design and the recruitment strategy were evaluated with three  
6 patient research partners (PRPs), and alterations especially in primary outcome and recruitment  
7 strategy were embedded. Furthermore, a minimum of two PRPs (participating in the study) will be  
8 involved in the discussion regarding the progress of the recruitment phase and results, and will be  
9 offered the opportunity to comment on the manuscript draft. The Regional Committees on Health  
10 Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency  
11 (15/41684) have approved the study protocol. The trial has been registered with ClinicalTrials.gov  
12 (NCT03058900) and important protocol modifications will be updated here. The Danish Health and  
13 Medicines Authority does not classify the FMT procedure as a medical intervention, and has had  
14 no objection to the use of FMT for this study and patient category. Thus, no GCP auditing is legally  
15 required. A report describing any potential side effects and adverse events will be submitted to  
16 the Ethics Committee yearly. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be  
17 reported to the Ethics Committee within seven days. Based on these reports, the Ethics committee  
18 can determine to terminate the trial early. The Danish Patient Compensation Association provides  
19 compensations for patients injured in connection to medical clinical trials.

20 Although the Danish Health Authorities, for the time being, do not classify donor  
21 faecal microbiota as tissue, all steps of the stool donor recruitment, stool donation and FMT  
22 preparation will be in accordance with the Danish Tissue Law to ensure that the quality and safety  
23 standards laid down in the Danish Legislation BEK nr 764 of May 26, 2015 (implementing Directive  
24 2004/23/EC) are met. Four stool donors will be recruited from the South Danish Transfusion  
25 Service & Tissue Centre, Department of Clinical Immunology, Odense University Hospital, and they  
26 will be carefully screened for potentially transmissible infections and other conditions associated  
27 with gut microbiota function before their stool can be released for FMT. Being a stool donor is  
28 voluntary, and no compensation fee will be given. Furthermore, to ensure donor traceability, each  
29 patient in the active treatment arm will only receive microbiota from one donor. Also, frozen  
30 samples will be clearly labelled with a unique donation code based on the ISBT 128 coding and  
31 labelling system, and the release of the final product will adhere to the standards for tissue and  
32 blood donation.

33 Due to the well-documented risk of permanent joint destruction and occurrence of  
34 extra-articular manifestations in the PsA disease course, identification of new treatment modalities  
35 and biomarkers is essential to help the physician to slow down the disease development or  
36 ultimately to prevent it. All PsA patients participating in this study have significant activity in their  
37 joint disease despite treatment with the current guideline treatment and first-line drug, MTX, for  
38 this condition. This patient population will therefore benefit greatly from new treatment options.  
39 Consequently, when weighing the pros and cons, this trial should be performed from a scientific and  
40 ethical perspective.

41 Dissemination will occur through presentations at national and international  
42 conferences and publications in international peer-reviewed journal(s).

## DISCUSSION

Recent years have seen growing recognition of the complexity of the role of the microbiota in shaping the immune system and its potential effects for health and disease.<sup>22,100,101</sup> In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.<sup>102-105</sup> Intriguingly, an abnormal intestinal bacteria composition has been observed in PsA patients, and this association has fostered theories linking intestinal dysbiosis and PsA joint inflammation.<sup>106</sup> Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic diseases are causal related,<sup>55</sup> and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.<sup>54,107</sup> We expect that this double-blind, randomised, placebo-controlled trial will shed new light on this highly relevant topic.

This is the first time that the efficacy and safety of FMT is being investigated in MTX immunosuppressed patients with rheumatic diseases. Subsequently, no data on the feasibility of conducting FMTs in the rheumatological setting is, so far, available. Nor do we know whether one FMT will be sufficient, or whether it should be repeated shortly after the first intervention to normalise the alterations of the intestinal microbiota, which would expectedly enhance any potential anti-inflammatory effects. In the present proof-of-concept clinical trial, the FMT procedure is considered an add-on to the current guideline intervention and first-line drug, MTX. Therefore, from a pragmatic and ethical perspective, we have decided to perform only one FMT (or sham procedure) in each patient even if we are well aware that this approach may not be adequate to achieve long-lasting effects. Indeed, in patients with inflammatory bowel diseases it appears that performing a frequent dosing-regime repeating the FMT procedure up to five times a week for eight weeks provided the best results.<sup>51,108,109</sup> Hence, in contrast to the treatment of *C. difficile* infections where the microbiota is pushed past the point of homeostasis and can be restored following only one FMT,<sup>47</sup> the chronic nature of PsA and other autoimmune and inflammatory diseases, and the somewhat lesser degree of intestinal dysbiosis, may make the host microbiota more resistant to long-lasting modifications. Nevertheless, we hope that the FMT procedure in the present study will be sufficient to boost the effects of MTX so that the participants who are all MTX-non-responders prior to study enrolment will achieve disease control without needing to add or switch to other non-MTX medication.

In the present trial, the primary outcome measure is defined as the occurrence of treatment failure according to shared decision making between patient and physician evaluated at 6 months following the randomised intervention (FMT versus sham procedure). Shared decision making is a process in which both patient and health professional make a decision, taking into account the best evidence of available treatment options and the patient's values and preferences. This approach is considered a key element in the management of rheumatic diseases.<sup>110</sup> As both patients and the treating rheumatologists are blinded to the randomised intervention, the shared decision making will be unaffected by the type of transplant suspension (active or placebo) installed at baseline. Nevertheless, we acknowledge that our assumption that twice as many PsA patients in the sham group will be treatment failures is ambitious, and that we might miss a smaller and less clinically significant treatment effect of the FMT-procedure. In this

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 case, we hope that our secondary outcome measures will be able to detect potential trends of  
2 positive effects in PsA subdomains such as enthesitis score, dactylitis count, and PASI skin score. In  
3 addition to the primary endpoint evaluation at 6 months, patients will be asked to fill out a weekly  
4 questionnaire regarding side effects as well as skin and arthritis symptoms during the first month  
5 following the randomised intervention to reveal any short-term effects on patient-reported  
6 outcomes.

7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Next, only patients with active peripheral PsA will be included. One reason for this is  
that this will be the first time that FMT is performed on rheumatic patients. Therefore, it seems  
reasonable only to enrol patients who have had inadequate effect from the initial guideline  
treatment (MTX), and consequently, on an individual basis could benefit the most from  
participating in new experimental clinical trials. Also, since patients need to have at least three  
swollen joints, we expect that we will be able to detect treatment effects of clinical importance.  
The fact that we do not include recent onset treatment naive patients will, of course, limit our  
ability to generalise our findings unto the entire PsA patient population. Indeed, in a recent  
randomised controlled trial of FMT in ulcerative colitis patients, participants with a recent  
diagnosis (< 1 year) were statistically significantly more likely to respond to FMT compared with  
those with longer disease duration.<sup>108</sup> That patients will have to subcutaneously administer MTX  
for at least three months prior to study enrolment will ensure that low intestinal MTX absorption  
is excluded as a potential effect modifier for the poor MTX response. In addition, as many drugs,  
including MTX, seem to affect the intestinal microbiological milieu,<sup>111-114</sup> bypassing the intestine  
during MTX administration will ensure that no local non-disease related effects on the intestinal  
microbiota will occur.

A great challenge when conducting a trial of FMT is that for the present being there  
is a lack of both national and international recommendations guiding the regulation and the best  
clinical practices for donor screening, stool sample handling and preparation of the FMT  
suspension.<sup>115-117</sup> Indeed, the variability in faecal bacterial communities can complicate or  
undermine treatment efficacy. This variability stems from both biological variation and variation  
introduced by sample handling. A recent study reported that oxygen exposure degraded faecal  
bacterial communities, whereas freeze-thaw cycles and lag time between donor defecation and  
transplant preparation had much more limited effects.<sup>118</sup> Given that many intestinal bacteria are  
obligate anaerobe, including many beneficial bacteria potentially possessing anti-inflammatory  
effects, exposure to oxygen during the preparation of FMT may potentially compromise the  
therapeutic value of FMT in PsA and other inflammatory diseases. Therefore, although frozen  
faecal preparations of stool suspended into physiological saline and glycerol have proven just as  
effective as fresh stool in treating *C. difficile* infections,<sup>119</sup> the optimal transplant preparation  
method in treating inflammatory diseases remains to be established.

Our stool handling setup is in line with the prevailing practice, which includes mixing  
and filtration of the stool suspension and adding saline and glycerol as a cryopreservative before  
storage at -80 °C.<sup>117</sup> In addition, we have sought to limit the oxygen exposure during transport by  
placing the donor stool within a plastic bag, which is subsequently put into a tightly closed small  
plastic container. Supplementary, during preparation the solution will not be homogenized for  
more than 10 seconds. Nevertheless, the lack of optimal anaerobe conditions during stool

1  
2 1 handling could possibly undermine the therapeutic potential of our FMT procedure. Furthermore,  
3 2 although we aim to use 50 g of faeces for each transplant, we acknowledge that the exact weight  
4 3 between donations could vary with an estimated +/- 5 g. Also, due to the wide variability in  
5 4 microbial content in stool between donations, the content cannot be fully standardized, and may  
6 5 likely differ between each FMT procedure. However, to meet this challenge we will collect and  
7 6 store samples from each donation which will enable us to determine the microbiota composition  
8 7 of each donation in case some donations prove more effective than others.

9 8 Stool donor selection is another critical issue that needs to be addressed. The  
10 9 composition of the normal microbiota composition has only recently been mapped,<sup>120</sup> and the  
11 10 existence of a limited number of well-balanced host-microbial symbiotic states, where one or  
12 11 more bacteria species are considered the main functional driver(s), have been identified using  
13 12 clustering of metagenomic sequences.<sup>121</sup> Still, the most favourable donor microbiota composition  
14 13 for treating inflammatory diseases has yet to be determined. Therefore, it also remains to be  
15 14 established whether donors with a high stool bacteria diversity should be preferred over isolation  
16 15 of specific bacteria, or if pooled stool samples from several donors outperforms a single-donor  
17 16 transplant.<sup>51,122</sup> We have chosen to use only single donations from four different anonymous stool  
18 17 donors to ensure donor traceability and to enable us to identify any individual donor-specific  
19 18 microbial effects. Also, since host intrinsic-, environmental-, and dietary factors as well as  
20 19 pharmaceutical drugs have been associated with gut bacteria composition and  
21 20 functionality,<sup>111,112,123,124</sup> the donors must eat a balanced diet, not be overweight or take any  
22 21 medications or be physical or psychological stressed, smoke or consume alcohol during the  
23 22 donation period to limit the risk of transferring "abnormal" microbiota to the recipients. These  
24 23 donor criteria have been set for safety reasons, and we acknowledge, that this could potentially  
25 24 limit the inter-donor microbiota diversity due to shared lifestyle characteristics.

26 25 Another factor to keep in mind is the concept of matching donor and recipient, which  
27 26 may be of importance for enhancing the colonisation capabilities of the donor microbial  
28 27 communities. In fact, Rossen et al<sup>109</sup> did find that in patients with ulcerative colitis, the microbiota  
29 28 of FMT responders shifted to their respective donors, whereas non-responders did not. Li et al<sup>125</sup>  
30 29 reported that donor bacteria strains established extensively in the recipient and persisted for at  
31 30 least 3 months with a negligible decline of donor-strain populations detected between 45 days  
32 31 and 3 months following FMT in metabolic syndrome patients. However, they also found that  
33 32 recipients receiving the same donor transplant displayed varying degrees of microbiota transfer,  
34 33 indicating individual patterns of microbiome resistance and donor-recipient compatibilities. In  
35 34 addition, host genetics is known to effect the gut microbiota,<sup>126</sup> and animal models have shown  
36 35 that sex<sup>127</sup> and age<sup>128</sup> also can be potentially modifiers of the gut bacteria composition. These  
37 36 observations may prove to be of importance for the outcome of FMT in inflammatory diseases.<sup>129</sup>  
38 37 However, whether sex- and/or age-matching between donor and recipient is crucial for a  
39 38 successful FMT in humans remains to be enlighten. Therefore, in the present study, no donor-  
40 39 recipient matching will be conducted. However, a sub-group analysis will be performed to reveal  
41 40 any trend that could indicate better results in sex- or age-match cases.

42 41 Furthermore, as the interactions between the microbiota and the host are influenced  
43 42 by cooperation and competition between pathogenic and commensal microbes and multiple

1 environmental variables, the lifestyle of the recipient following the FMT may be of importance.  
2 Nevertheless, due to the uncertainty of how to define the optimal lifestyle and the lack of  
3 knowledge on how different lifestyle factors may interfere with the microbiota, we have decided  
4 that the patients in the present study will not have to adhere to any predefined lifestyle "regime"  
5 or diet following the randomised intervention. However, every participant will fulfil an eating habit  
6 questionnaire at the beginning of the trial.

7 Finally, non-bacteria microorganisms such as bacteriophages, viruses and fungi may  
8 also be of importance when targeting components of the microbiota or host cells for therapeutic  
9 purposes.<sup>130-132</sup> Other complicating factors may include the composition of other microbiological  
10 niches such as the oral, lung, genitourinary, and skin microbiota.<sup>133,134</sup> Indeed, the latter could  
11 likely prove to be of significance in patients with skin psoriasis. However, these factors will not be  
12 assessed in the present study.

13 In conclusion, this trial has the potential to substantially expand the growing body of  
14 literature on the role of the intestinal microbiota in general and PsA in particular. Thereby we  
15 anticipate that this study will enhance our understanding of cause and effect. The results of this  
16 study, when completed, may be exploited for biomarker discovery, and for diagnostic and  
17 therapeutic purposes.

## 19 **AUTHORS' CONTRIBUTION**

20 T. Ellingsen, M.S. Kragstnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for  
21 the study. T. Ellingsen and M.S. Kragstnaes are the principal investigators and wrote the first study  
22 protocol draft. T. Ellingsen and M.S. Kragstnaes were responsible for all communication with the  
23 scientific ethical committee, the Danish Data Protection Agency and the National Health Board. T  
24 Ellingsen is the responsible party and sponsor. M.S. Kragstnaes, T. Ellingsen, H.C. Horn, J.K.  
25 Pedersen, H.L. Munk, and U. Fredberg sat up the inclusion and exclusion criteria for the psoriatic  
26 arthritis patients, and the latter five rheumatologists are conducting the clinical examinations. J.  
27 Kjeldsen, F.M. Pedersen, and H. Glerup discussed and planned the FMT procedure, and they are  
28 conducting the FMTs and the sigmoidoscopies (obtaining mucosae tissue samples). D.K. Holm and  
29 H.M. Holt helped set up the donor screening programme, and they were responsible for  
30 conducting this programme and performing the microbiological and immunological tests. V.  
31 Andersen and K. Kristiansen are responsible for the omics and microbiome analyses, and  
32 have advised on how the tissue collection should be performed and what kind of tissue would be  
33 relevant to collect. R. Christensen has written the statistical analysis plan and will be responsible  
34 for the final statistical analyses. In conclusion, all participants designated as authors have  
35 contributed to the conception and design of the study, and they have critically either drafted or  
36 revised the first draft of the study protocol and the protocol paper. Also, all authors have  
37 approved the final version before submission.

## 39 **REGISTRATION**

40 The trial has been registered with ClinicalTrials.gov (NCT03058900).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 1 FUNDING STATEMENT

2 This work was supported by the Region of Southern Denmark, Odense University Hospital, the  
3 Danish Regions, University of Southern Denmark, the Danish Rheumatism Association, the Danish  
4 Psoriasis Research Fund, Odense Patient Explorative data Network (OPEN), and Fabrikant Vilhelm  
5 Pedersen and Hustrus Legat based on the recommendation of the Novo Nordisk Foundation.  
6 Musculoskeletal Statistics Unit at the Parker Institute, Bispebjerg and Frederiksberg Hospital (R.  
7 Christensen), is supported by a core grant from the Oak Foundation (OCAY-13-309). K. Kristiansen  
8 is supported by BGI-Research, BGI-Shenzhen, China.

## 10 COMPETING INTEREST STATEMENT

11 None of the team members of this research project has declared any potential conflict of interest.  
12

## 13 ACKNOWLEDGEMENTS

14 Great thanks to Lene Albjerg, biomedical laboratory technologist and quality control manager at  
15 the Dept. of Clinical Immunology, Odense University Hospital, Denmark, for her expertise and  
16 dedicated work regarding the practical handling of the FMT suspension.



## References

1. Scher JU, Littman DR, Abramson SB. Microbiome in Inflammatory Arthritis and Human Rheumatic Diseases. *Arthritis Rheumatol*. 2016;68:35-45.
2. Asquith M, Elewaut D, Lin P et al. The role of the gut and microbes in the pathogenesis of spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2014;28:687-702.
3. Stoll ML. Gut microbes, immunity, and spondyloarthritis. *Clin Immunol*. 2015;159:134-142.
4. Costello ME, Ciccia F, Willner D et al. Intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol*. 2014.
5. Yang L, Wang L, Wang X et al. A Possible Role of Intestinal Microbiota in the Pathogenesis of Ankylosing Spondylitis. *Int J Mol Sci*. 2016;17.
6. Manasson J, Scher JU. Spondyloarthritis and the microbiome: new insights from an ancient hypothesis. *Curr Rheumatol Rep*. 2015;17:10.
7. De WK, Debusschere K, Beeckman S et al. Integrating the pathogenesis of spondyloarthritis: gut and joint united? *Curr Opin Rheumatol*. 2015;27:189-196.
8. Eppinga H, Konstantinov SR, Peppelenbosch MP et al. The microbiome and psoriatic arthritis. *Curr Rheumatol Rep*. 2014;16:407.
9. Coit P, Sawalha AH. The human microbiome in rheumatic autoimmune diseases: A comprehensive review. *Clin Immunol*. 2016;170:70-79.
10. Ciccia F, Ferrante A, Guggino G et al. The role of the gastrointestinal tract in the pathogenesis of rheumatic diseases. *Best Pract Res Clin Rheumatol*. 2016;30:889-900.
11. Tito RY, Cypers H, Joossens M et al. Brief Report: Dialister as a Microbial Marker of Disease Activity in Spondyloarthritis. *Arthritis Rheumatol*. 2017;69:114-121.
12. Eppinga H, Sperna Weiland CJ, Thio HB et al. Similar Depletion of Protective Faecalibacterium prausnitzii in Psoriasis and Inflammatory Bowel Disease, but not in Hidradenitis Suppurativa. *J Crohns Colitis*. 2016.
13. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. *N Engl J Med*. 2017;376:2095-2096.
14. Terslev L, Naredo E, Iagnocco A et al. Defining enthesitis in spondyloarthritis by ultrasound: results of a Delphi process and of a reliability reading exercise. *Arthritis Care Res (Hoboken)*. 2014;66:741-748.
15. Peluso R, Iervolino S, Vitiello M et al. Extra-articular manifestations in psoriatic arthritis patients. *Clin Rheumatol*. 2014.
16. Gladman DD. Psoriatic arthritis. *Dermatol Ther*. 2009;22:40-55.
17. Gossec L, Coates LC, De WM et al. Management of psoriatic arthritis in 2016: a comparison of EULAR and GRAPPA recommendations. *Nat Rev Rheumatol*. 2016;12:743-750.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
18. Kingsley GH, Kowalczyk A, Taylor H et al. A randomized placebo-controlled trial of methotrexate in psoriatic arthritis. *Rheumatology (Oxford)*. 2012;51:1368-1377.
  19. Gossec L, Smolen JS, Ramiro S et al. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis*. 2016;75:499-510.
  20. Asquith M, Rosenbaum JT. The interaction between host genetics and the microbiome in the pathogenesis of spondyloarthropathies. *Curr Opin Rheumatol*. 2016;28:405-412.
  21. Benham H, Robinson PC, Baillet AC et al. Role of genetics in infection-associated arthritis. *Best Pract Res Clin Rheumatol*. 2015;29:213-225.
  22. Shamriz O, Mizrahi H, Werbner M et al. Microbiota at the crossroads of autoimmunity. *Autoimmun Rev*. 2016;15:859-869.
  23. Lories RJ, de VK. Is psoriatic arthritis a result of abnormalities in acquired or innate immunity? *Curr Rheumatol Rep*. 2012;14:375-382.
  24. Mortezaei M, Thiele R, Ritchlin C. The joint in psoriatic arthritis. *Clin Exp Rheumatol*. 2015;33:20-25.
  25. Acosta Felquer ML, Fitzgerald O. Peripheral joint involvement in psoriatic arthritis patients. *Clin Exp Rheumatol*. 2015;33:26-30.
  26. Matt P, Lindqvist U, Kleinau S. Up-regulation of CD64-expressing monocytes with impaired FcγR function reflects disease activity in polyarticular psoriatic arthritis. *Scand J Rheumatol*. 2015;44:464-473.
  27. Al-Mossawi MH, Ridley A, Kiedel S et al. The role of natural killer cells, gamma delta T-cells and other innate immune cells in spondyloarthritis. *Curr Opin Rheumatol*. 2013;25:434-439.
  28. Ryan C, Korman NJ, Gelfand JM et al. Research gaps in psoriasis: opportunities for future studies. *J Am Acad Dermatol*. 2014;70:146-167.
  29. Berthelot JM, de la Cochetiere MF, Potel G et al. Evidence supporting a role for dormant bacteria in the pathogenesis of spondylarthritis. *Joint Bone Spine*. 2013;80:135-140.
  30. Abdollahi-Roodsaz S, Abramson SB, Scher JU. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nat Rev Rheumatol*. 2016;12:446-455.
  31. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature*. 2016;535:75-84.
  32. Ciccia F, Rizzo A, Triolo G. Subclinical gut inflammation in ankylosing spondylitis. *Curr Opin Rheumatol*. 2016;28:89-96.
  33. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med*. 2009;361:888-898.
  34. Ciccia F, Guggino G, Rizzo A et al. Dysbiosis and zonulin upregulation alter gut epithelial and vascular barriers in patients with ankylosing spondylitis. *Ann Rheum Dis*. 2017.
  35. Pianta A, Arvikar SL, Strle K et al. Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. *J Clin Invest*. 2017.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 36. Scher JU, Ubeda C, Artacho A et al. Decreased bacterial diversity characterizes an altered gut  
2 microbiota in psoriatic arthritis and resembles dysbiosis of inflammatory bowel disease. *Arthritis*  
3 *Rheumatol.* 2014.  
4  
5  
6 4 37. Lindqvist U, Kristjansson G, Pihl-Lundin I et al. Patients with psoriatic arthritis have an increased  
7 number of lymphocytes in the duodenal mucosa in comparison with patients with psoriasis  
8 vulgaris. *J Rheumatol.* 2006;33:924-927.  
9  
10 7 38. Scarpa R, Manguso F, D'Arienzo A et al. Microscopic inflammatory changes in colon of patients with  
11 both active psoriasis and psoriatic arthritis without bowel symptoms. *J Rheumatol.* 2000;27:1241-  
12 1246.  
13  
14 10 39. Van PL, Van den Bosch F, Mielants H et al. Mucosal inflammation in spondylarthritides: past,  
15 present, and future. *Curr Rheumatol Rep.* 2011;13:409-415.  
16  
17 12 40. Schatteman L, Mielants H, Veys EM et al. Gut inflammation in psoriatic arthritis: a prospective  
18 ileocolonoscopy study. *J Rheumatol.* 1995;22:680-683.  
19  
20 14 41. Ciccia F, Guggino G, Ferrante A et al. Interleukin-9 Overexpression and Th9 Polarization  
21 Characterize the Inflamed Gut, the Synovial Tissue, and the Peripheral Blood of Patients With  
22 Psoriatic Arthritis. *Arthritis Rheumatol.* 2016;68:1922-1931.  
23  
24 17 42. Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis and human  
25 diseases. *BMC Immunol.* 2017;18:2.  
26  
27 19 43. Gill T, Asquith M, Rosenbaum JT et al. The intestinal microbiome in spondyloarthritis. *Curr Opin*  
28 *Rheumatol.* 2015;27:319-325.  
29  
30 21 44. Kump PK, Krause R, Allerberger F et al. Faecal microbiota transplantation-the Austrian approach.  
31 *Clin Microbiol Infect.* 2014;20:1106-1111.  
32  
33 23 45. Cammarota G, Pecere S, Ianiro G et al. Principles of DNA-Based Gut Microbiota Assessment and  
34 Therapeutic Efficacy of Fecal Microbiota Transplantation in Gastrointestinal Diseases. *Dig Dis.*  
35 2016;34:279-285.  
36  
37 26 46. Austin M, Mellow M, Tierney WM. Fecal microbiota transplantation in the treatment of *Clostridium*  
38 *difficile* infections. *Am J Med.* 2014;127:479-483.  
39  
40 28 47. van NE, Vrieze A, Nieuwdorp M et al. Duodenal infusion of donor feces for recurrent *Clostridium*  
41 *difficile*. *N Engl J Med.* 2013;368:407-415.  
42  
43 30 48. Cammarota G, Masucci L, Ianiro G et al. Randomised clinical trial: faecal microbiota transplantation  
44 by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection.  
45 *Aliment Pharmacol Ther.* 2015;41:835-843.  
46  
47 33 49. Lee CH, Steiner T, Petrof EO et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical  
48 Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection: A Randomized  
49 Clinical Trial. *JAMA.* 2016;315:142-149.  
50  
51 36 50. Li YT, Cai HF, Wang ZH et al. Systematic review with meta-analysis: long-term outcomes of faecal  
52 microbiota transplantation for *Clostridium difficile* infection. *Aliment Pharmacol Ther.* 2016;43:445-  
53 457.  
54  
55  
56  
57  
58  
59  
60

- 1  
2 1 51. Paramsothy S, Kamm MA, Kaakoush NO et al. Multidonor intensive faecal microbiota  
3 2 transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet*.  
4 3 2017;389:1218-1228.  
5  
6 4 52. Cui B, Feng Q, Wang H et al. Fecal microbiota transplantation through mid-gut for refractory  
7 5 Crohn's disease: Safety, feasibility and efficacy trial results. *J Gastroenterol Hepatol*. 2014.  
8  
9 6 53. Lund H, Brunnhuber K, Juhl C et al. Towards evidence based research. *BMJ*. 2016;355:i5440.  
10  
11 7 54. Ciccio F, Ferrante A, Triolo G. Intestinal dysbiosis and innate immune responses in axial  
12 8 spondyloarthritis. *Curr Opin Rheumatol*. 2016;28:352-358.  
13  
14 9 55. Bravo-Blas A, Wessel H, Milling S. Microbiota and arthritis: correlations or cause? *Curr Opin*  
15 10 *Rheumatol*. 2016;28:161-167.  
16  
17 11 56. Kabeerdoss J, Sandhya P, Danda D. Gut inflammation and microbiome in spondyloarthritis.  
18 12 *Rheumatol Int*. 2016;36:457-468.  
19  
20 13 57. Costello ME, Robinson PC, Benham H et al. The intestinal microbiome in human disease and how it  
21 14 relates to arthritis and spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2015;29:202-212.  
22  
23 15 58. Bazso A, Szodoray P, Suto G et al. Importance of intestinal microenvironment in development of  
24 16 arthritis. A systematic review. *Immunol Res*. 2015;61:172-176.  
25  
26 17 59. Taneja V. Arthritis susceptibility and the gut microbiome. *FEBS Lett*. 2014;588:4244-4249.  
27  
28 18 60. Rosenbaum JT, Lin P, Asquith M et al. Does the microbiome play a causal role in spondyloarthritis?  
29 19 *Clin Rheumatol*. 2014;33:763-767.  
30  
31 20 61. Jenks K, Stebbings S, Burton J et al. Probiotic therapy for the treatment of spondyloarthritis: a  
32 21 randomized controlled trial. *J Rheumatol*. 2010;37:2118-2125.  
33  
34 22 62. Aggarwal A, Sarangi AN, Gaur P et al. Gut microbiome in children with enthesitis-related arthritis in  
35 23 a developing country and the effect of probiotic administration. *Clin Exp Immunol*. 2017;187:480-  
36 24 489.  
37  
38 25 63. Berntson L, Hedlund-Treutiger I, Alving K. Anti-inflammatory effect of exclusive enteral nutrition in  
39 26 patients with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2016;34:941-945.  
40  
41 27 64. Hatakka K, Martio J, Korpela M et al. Effects of probiotic therapy on the activity and activation of  
42 28 mild rheumatoid arthritis--a pilot study. *Scand J Rheumatol*. 2003;32:211-215.  
43  
44 29 65. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms  
45 30 of rheumatoid arthritis according to a randomized, controlled trial. *BMC Complement Altern Med*.  
46 31 2010;10:1.  
47  
48 32 66. Zamani B, Golkar HR, Farshbaf S et al. Clinical and metabolic response to probiotic supplementation  
49 33 in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Int J*  
50 34 *Rheum Dis*. 2016;19:869-879.  
51  
52 35 67. Pineda ML, Thompson SF, Summers K et al. A randomized, double-blinded, placebo-controlled pilot  
53 36 study of probiotics in active rheumatoid arthritis. *Med Sci Monit*. 2011;17:CR347-CR354.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 68. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E et al. Effects of Lactobacillus casei  
2 supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a  
3 randomized double-blind clinical trial. *Int J Rheum Dis*. 2014;17:519-527.
- 4 69. Schorpion A, Kolasinski SL. Can Probiotic Supplements Improve Outcomes in Rheumatoid Arthritis?  
5 *Curr Rheumatol Rep*. 2017;19:73.
- 6 70. Taylor W, Gladman D, Helliwell P et al. Classification criteria for psoriatic arthritis: development of  
7 new criteria from a large international study. *Arthritis Rheum*. 2006;54:2665-2673.
- 8 71. Fang C, Zhong H, Lin Y et al. Assessment of the cPAS-based BGISEQ-500 platform for metagenomic  
9 sequencing. *Gigascience*. 2017.
- 10 72. Qin J, Li Y, Cai Z et al. A metagenome-wide association study of gut microbiota in type 2 diabetes.  
11 *Nature*. 2012;490:55-60.
- 12 73. Nielsen HB, Almeida M, Juncker AS et al. Identification and assembly of genomes and genetic  
13 elements in complex metagenomic samples without using reference genomes. *Nat Biotechnol*.  
14 2014;32:822-828.
- 15 74. Li J, Jia H, Cai X et al. An integrated catalog of reference genes in the human gut microbiome. *Nat*  
16 *Biotechnol*. 2014;32:834-841.
- 17 75. Mishra A, Makharia GK. Techniques of functional and motility test: how to perform and interpret  
18 intestinal permeability. *J Neurogastroenterol Motil*. 2012;18:443-447.
- 19 76. Sequeira IR, Lentle RG, Kruger MC et al. Standardising the lactulose mannitol test of gut  
20 permeability to minimise error and promote comparability. *PLoS One*. 2014;9:e99256.
- 21 77. Thorsen H, Hansen TM, McKenna SP et al. Adaptation into Danish of the Stanford Health  
22 Assessment Questionnaire (HAQ) and the Rheumatoid Arthritis Quality of Life Scale (RAQoL). *Scand*  
23 *J Rheumatol*. 2001;30:103-109.
- 24 78. Brodzky V, Pentek M, Balint PV et al. Comparison of the Psoriatic Arthritis Quality of Life (PsAQoL)  
25 questionnaire, the functional status (HAQ) and utility (EQ-5D) measures in psoriatic arthritis: results  
26 from a cross-sectional survey. *Scand J Rheumatol*. 2010;39:303-309.
- 27 79. Zachariae R, Zachariae C, Ibsen H et al. Dermatology life quality index: data from Danish inpatients  
28 and outpatients. *Acta Derm Venereol*. 2000;80:272-276.
- 29 80. Fransen J, Antoni C, Mease PJ et al. Performance of response criteria for assessing peripheral  
30 arthritis in patients with psoriatic arthritis: analysis of data from randomised controlled trials of two  
31 tumour necrosis factor inhibitors. *Ann Rheum Dis*. 2006;65:1373-1378.
- 32 81. Felson DT, Anderson JJ, Boers M et al. American College of Rheumatology. Preliminary definition of  
33 improvement in rheumatoid arthritis. *Arthritis Rheum*. 1995;38:727-735.
- 34 82. Felson DT, Anderson JJ, Lange ML et al. Should improvement in rheumatoid arthritis clinical trials  
35 be defined as fifty percent or seventy percent improvement in core set measures, rather than  
36 twenty percent? *Arthritis Rheum*. 1998;41:1564-1570.
- 37 83. Faria JR, Aarao AR, Jimenez LM et al. Inter-rater concordance study of the PASI (Psoriasis Area and  
38 Severity Index). *An Bras Dermatol*. 2010;85:625-629.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 84. Jensen OK, Callesen J, Nielsen MG et al. Reproducibility of tender point examination in chronic low back pain patients as measured by intrarater and inter-rater reliability and agreement: a validation study. *BMJ Open*. 2013;3.
- 4 85. Klingberg E, Carlsten H, Hilme E et al. Calprotectin in ankylosing spondylitis--frequently elevated in feces, but normal in serum. *Scand J Gastroenterol*. 2012;47:435-444.
- 6 86. Kelly CR, Kahn S, Kashyap P et al. Update on Fecal Microbiota Transplantation 2015: Indications, Methodologies, Mechanisms, and Outlook. *Gastroenterology*. 2015;149:223-237.
- 8 87. Wang S, Xu M, Wang W et al. Systematic Review: Adverse Events of Fecal Microbiota Transplantation. *PLoS One*. 2016;11:e0161174.
- 10 88. Rossen NG, MacDonald JK, de Vries EM et al. Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. *World J Gastroenterol*. 2015;21:5359-5371.
- 12 89. Girotra M, Garg S, Anand R et al. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection in the Elderly: Long-Term Outcomes and Microbiota Changes. *Dig Dis Sci*. 2016;61:3007-3015.
- 15 90. Gweon TG, Kim J, Lim CH et al. Fecal Microbiota Transplantation Using Upper Gastrointestinal Tract for the Treatment of Refractory or Severe Complicated Clostridium difficile Infection in Elderly Patients in Poor Medical Condition: The First Study in an Asian Country. *Gastroenterol Res Pract*. 2016;2016:2687605.
- 19 91. Agrawal M, Aroniadis OC, Brandt LJ et al. The Long-term Efficacy and Safety of Fecal Microbiota Transplant for Recurrent, Severe, and Complicated Clostridium difficile Infection in 146 Elderly Individuals. *J Clin Gastroenterol*. 2016;50:403-407.
- 22 92. Di BS, Gouliouris T, Petrosillo N. Fecal microbiota transplantation (FMT) for Clostridium difficile infection: focus on immunocompromised patients. *J Infect Chemother*. 2015;21:230-237.
- 24 93. Webb BJ, Brunner A, Ford CD et al. Fecal microbiota transplantation for recurrent Clostridium difficile infection in hematopoietic stem cell transplant recipients. *Transpl Infect Dis*. 2016.
- 26 94. Harris PA, Taylor R, Thielke R et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377-381.
- 29 95. Altman DG, Dore CJ. Randomisation and baseline comparisons in clinical trials. *Lancet*. 1990;335:149-153.
- 31 96. Normand SL, Sykora K, Li P et al. Readers guide to critical appraisal of cohort studies: 3. Analytical strategies to reduce confounding. *BMJ*. 2005;330:1021-1023.
- 33 97. White IR, Horton NJ, Carpenter J et al. Strategy for intention to treat analysis in randomised trials with missing outcome data. *BMJ*. 2011;342:d40.
- 35 98. Liu R, Hong J, Xu X et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med*. 2017;23:859-868.
- 37 99. Pedersen HK, Gudmundsdottir V, Nielsen HB et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*. 2016;535:376-381.
- 39 100. Thaiss CA, Zmora N, Levy M et al. The microbiome and innate immunity. *Nature*. 2016;535:65-74.

- 1  
2 1 101. McLean MH, Dieguez D, Jr., Miller LM et al. Does the microbiota play a role in the pathogenesis of  
3 2 autoimmune diseases? *Gut*. 2015;64:332-341.  
4  
5 3 102. Longman RS, Yang Y, Diehl GE et al. Microbiota: host interactions in mucosal homeostasis and  
6 4 systemic autoimmunity. *Cold Spring Harb Symp Quant Biol*. 2013;78:193-201.  
7  
8 5 103. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin*  
9 6 *Gastroenterol*. 2015;31:69-75.  
10  
11 7 104. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune  
12 8 system. *Science*. 2012;336:1268-1273.  
13  
14 9 105. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health  
15 10 and disease. *Nat Rev Immunol*. 2009;9:313-323.  
16  
17 11 106. Van de Wiele T, Van Praet JT, Marzorati M et al. How the microbiota shapes rheumatic diseases.  
18 12 *Nat Rev Rheumatol*. 2016;12:398-411.  
19  
20 13 107. Butto LF, Haller D. Dysbiosis in intestinal inflammation: Cause or consequence. *Int J Med Microbiol*.  
21 14 2016.  
22  
23 15 108. Moayyedi P, Surette MG, Kim PT et al. Fecal Microbiota Transplantation Induces Remission in  
24 16 Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology*.  
25 17 2015;149:102-109.  
26  
27 18 109. Rossen NG, Fuentes S, van der Spek MJ et al. Findings From a Randomized Controlled Trial of Fecal  
28 19 Transplantation for Patients With Ulcerative Colitis. *Gastroenterology*. 2015;149:110-118.  
29  
30 20 110. Smolen JS, Schols M, Braun J et al. Treating axial spondyloarthritis and peripheral spondyloarthritis,  
31 21 especially psoriatic arthritis, to target: 2017 update of recommendations by an international task  
32 22 force. *Ann Rheum Dis*. 2017.  
33  
34 23 111. Zhernakova A, Kurilshikov A, Bonder MJ et al. Population-based metagenomics analysis reveals  
35 24 markers for gut microbiome composition and diversity. *Science*. 2016;352:565-569.  
36  
37 25 112. Falony G, Joossens M, Vieira-Silva S et al. Population-level analysis of gut microbiome variation.  
38 26 *Science*. 2016;352:560-564.  
39  
40 27 113. Forslund K, Hildebrand F, Nielsen T et al. Disentangling type 2 diabetes and metformin treatment  
41 28 signatures in the human gut microbiota. *Nature*. 2015;528:262-266.  
42  
43 29 114. Zhang X, Zhang D, Jia H et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis  
44 30 and partly normalized after treatment. *Nat Med*. 2015;21:895-905.  
45  
46 31 115. Woodworth MH, Neish EM, Miller NS et al. Laboratory Testing of Donors and Stool for Fecal  
47 32 Microbiota Transplantation for Recurrent *C. difficile* Infection. *J Clin Microbiol*. 2017.  
48  
49 33 116. Costello SP, Tucker EC, La BJ et al. Establishing a Fecal Microbiota Transplant Service for the  
50 34 Treatment of *Clostridium difficile* Infection. *Clin Infect Dis*. 2016;62:908-914.  
51  
52 35 117. Cammarota G, Ianiro G, Tilg H et al. European consensus conference on faecal microbiota  
53 36 transplantation in clinical practice. *Gut*. 2017;66:569-580.  
54  
55 37 118. Chu ND, Smith MB, Perrotta AR et al. Profiling Living Bacteria Informs Preparation of Fecal  
56 38 Microbiota Transplantations. *PLoS One*. 2017;12:e0170922.  
57  
58  
59  
60

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1 119. Satokari R, Mattila E, Kainulainen V et al. Simple faecal preparation and efficacy of frozen inoculum  
2 in faecal microbiota transplantation for recurrent *Clostridium difficile* infection--an observational  
3 cohort study. *Aliment Pharmacol Ther.* 2015;41:46-53.
- 4 120. Li J, Jia H, Cai X et al. An integrated catalog of reference genes in the human gut microbiome. *Nat*  
5 *Biotechnol.* 2014;32:834-841.
- 6 121. Arumugam M, Raes J, Pelletier E et al. Enterotypes of the human gut microbiome. *Nature.*  
7 2011;473:174-180.
- 8 122. Kazerouni A, Wein LM. Exploring the Efficacy of Pooled Stools in Fecal Microbiota Transplantation  
9 for Microbiota-Associated Chronic Diseases. *PLoS One.* 2017;12:e0163956.
- 10 123. Vandeputte D, Falony G, Vieira-Silva S et al. Stool consistency is strongly associated with gut  
11 microbiota richness and composition, enterotypes and bacterial growth rates. *Gut.* 2016;65:57-62.
- 12 124. Ley RE. The gene-microbe link. *Nature.* 2015;518:57.
- 13 125. Li SS, Zhu A, Benes V et al. Durable coexistence of donor and recipient strains after fecal microbiota  
14 transplantation. *Science.* 2016;352:586-589.
- 15 126. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut  
16 microbiome. *Nat Rev Microbiol.* 2011;9:279-290.
- 17 127. Markle JG, Frank DN, Mortin-Toth S et al. Sex differences in the gut microbiome drive hormone-  
18 dependent regulation of autoimmunity. *Science.* 2013;339:1084-1088.
- 19 128. Xiao L, Estelle J, Kiilerich P et al. A reference gene catalogue of the pig gut microbiome. *Nat*  
20 *Microbiol.* 2016;16161.
- 21 129. Markle JG, Frank DN, Adeli K et al. Microbiome manipulation modifies sex-specific risk for  
22 autoimmunity. *Gut Microbes.* 2014;5:485-493.
- 23 130. Mills S, Shanahan F, Stanton C et al. Movers and shakers: influence of bacteriophages in shaping  
24 the mammalian gut microbiota. *Gut Microbes.* 2013;4:4-16.
- 25 131. Pfeiffer JK, Virgin HW. Viral immunity. Transkingdom control of viral infection and immunity in the  
26 mammalian intestine. *Science.* 2016;351.
- 27 132. Underhill DM, Pearlman E. Immune Interactions with Pathogenic and Commensal Fungi: A Two-  
28 Way Street. *Immunity.* 2015;43:845-858.
- 29 133. Castelino M, Eyre S, Moat J et al. The skin microbiome in psoriatic arthritis: methodology  
30 development and pilot data. *Lancet.* 2015;385 Suppl 1:S27.
- 31 134. Scher JU, Joshua V, Artacho A et al. The lung microbiota in early rheumatoid arthritis and  
32 autoimmunity. *Microbiome.* 2016;4:60.
- 33  
34  
35  
36



1  
2 1 FIGURE LEGENDS  
3

4 2

5 3 **Figure 1.** Flow diagram of the randomised, placebo-controlled trial.  
6  
7

8 4

9 5 **Figure 2.** Participation timeline and characteristics of each visit.  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

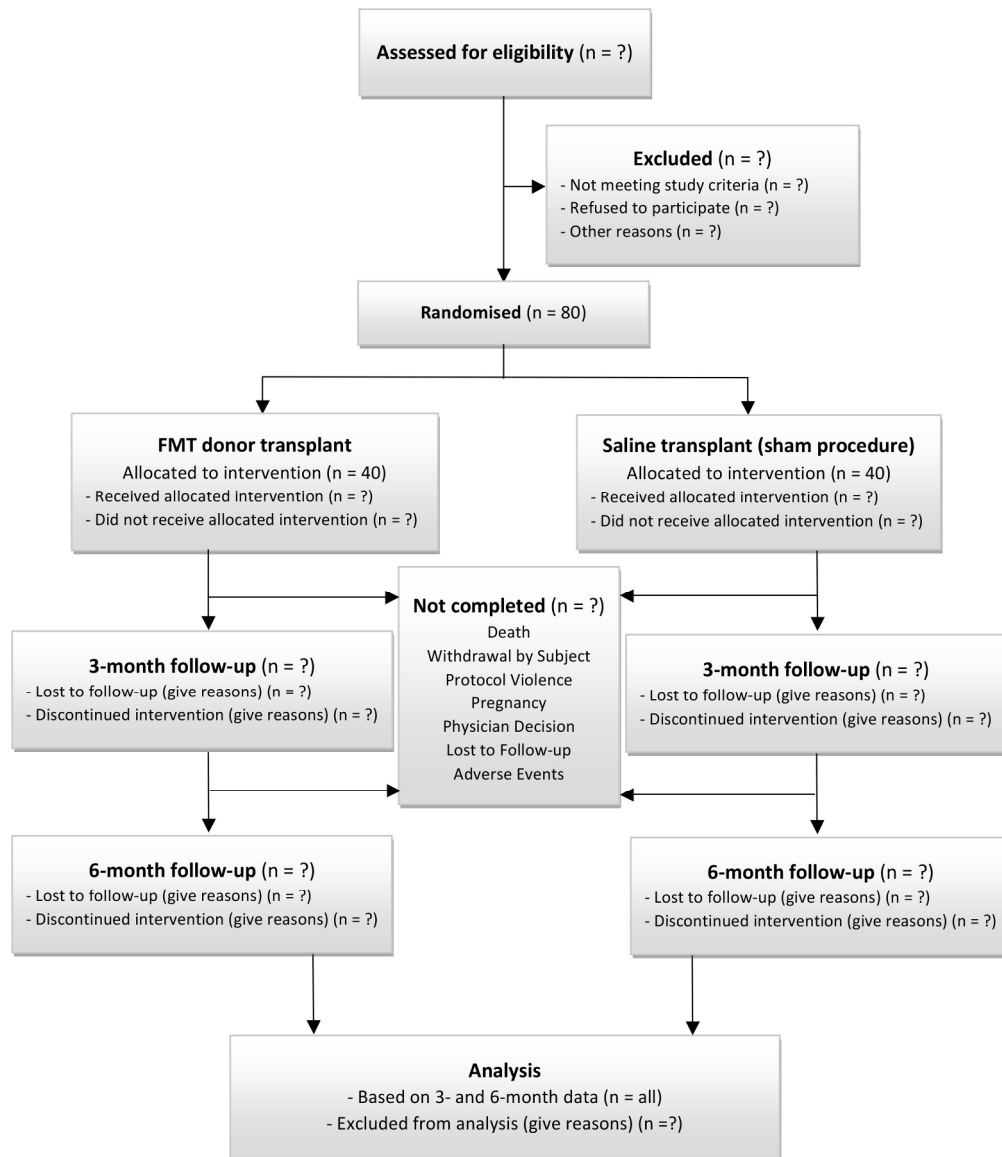


Figure 1. Flow diagram of the randomised, placebo-controlled trial.

198x236mm (300 x 300 DPI)

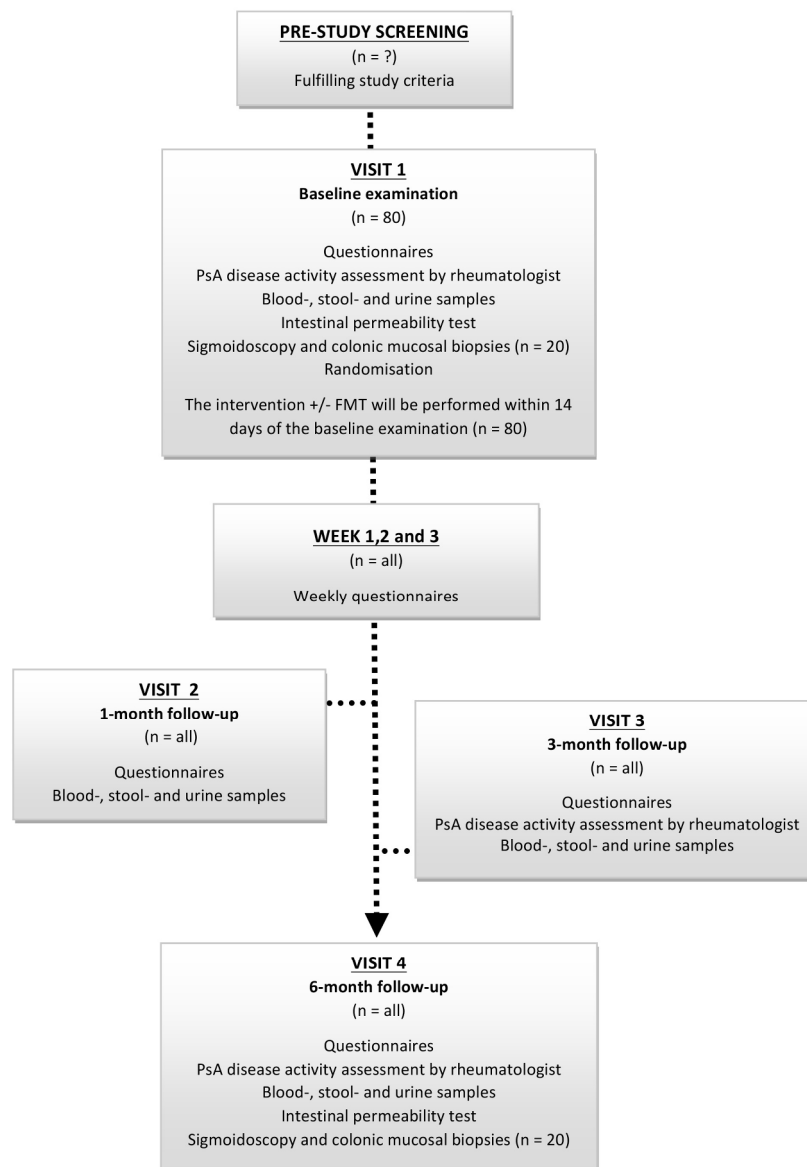


Figure 2. Participation timeline and characteristics of each visit.

160x237mm (300 x 300 DPI)



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

## SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	<u>1</u>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<u>2</u>
	2b	All items from the World Health Organization Trial Registration Data Set	<u>1-23</u>
Protocol version	3	Date and version identifier	<u>1</u>
Funding	4	Sources and types of financial, material, and other support	<u>23</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>1 and 22</u>
	5b	Name and contact information for the trial sponsor	<u>1</u>
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	<u>22</u>
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	<u>22</u>

## 1 Introduction

2			
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention
5			<u>3-4</u>
6		6b	Explanation for choice of comparators
7			<u>4</u>
8	Objectives	7	Specific objectives or hypotheses
9			<u>4-5</u>
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, or single group),
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
12			<u>5</u>

## 14 Methods: Participants, interventions, and outcomes

15			
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will
17			be collected. Reference to where list of study sites can be obtained
18			<u>8</u>
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)
21			<u>8-9</u>
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be
23			administered
24		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose
25			change in response to harms, participant request, or improving/worsening disease)
26			<u>10</u>
27		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence
28			(eg, drug tablet return, laboratory tests)
29			<u>Not applicable</u>
30		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
31			<u>8 and 9</u>
32	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood
33			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,
34			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen
35			efficacy and harm outcomes is strongly recommended
36			<u>11-12</u>
37	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for
38			participants. A schematic diagram is highly recommended (see Figure)
39			<u>7</u>

1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	<u>13-14</u>
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>8</u>
5				
6	<b>Methods: Assignment of interventions (for controlled trials)</b>			
7	<b>Allocation:</b>			
8				
9				
10	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	<u>14</u>
11				
12				
13				
14				
15				
16	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	<u>14</u>
17				
18				
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>14</u>
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>14</u>
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>14</u>
28				
29				
30				
31	<b>Methods: Data collection, management, and analysis</b>			
32				
33	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	<u>14</u>
34				
35				
36				
37				
38				
39		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	<u>16</u>
40				
41				
42				
43				
44				
45				
46				

1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	<u>14</u>
2				
3				
4				
5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	<u>15</u>
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>15-16</u>
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	<u>15</u>
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	<u>18</u>
17				
18				
19				
20				
21				
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	<u>18</u>
23				
24				
25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	<u>13</u>
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<u>18</u>
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>18</u>
35				
36				
37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	<u>18</u>
38				
39				
40				
41				
42				
43				
44				
45				
46				

1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	<u>14</u>
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>Not applicable</u>
5				
6				
7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	<u>14</u>
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>23</u>
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	<u>Not applicable</u>
14				
15				
16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	<u>18</u>
17				
18				
19				
20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	<u>18</u>
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>22</u>
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Not applicable</u>
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>Appendix</u>
32				
33				
34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<u>10-11</u>
35				
36				

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by/4.0/)" license.