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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

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7 3 Efficacy and safety of faecal microbiota transplantation in
8 patients with psoriatic arthritis:
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10 protocol for a 6-month, double-blind, randomised placebo-
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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.¹⁻⁵ 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,⁶ 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.⁷⁻¹²

10 PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum
11 and a varied disease course.¹³ 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.¹⁴ 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.¹⁵ Without disease modifying intervention, 40-60% of PsA patients will
16 develop erosive and deforming joint damage within a few years of disease onset.¹⁶ Methotrexate
17 (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug
18 (csDMARD) for initial therapy.¹⁷ However, a substantial number of patients does not benefit from
19 such treatment.¹⁸ Currently, other treatment options may include biological agents such as
20 tumour necrosis factor (TNF- α) inhibitors aiming to block some of the downstream molecular
21 pathways driving the disease.¹⁹ However, these drugs do not target the cause of PsA, which is
22 believed to be multifactorial comprising genetic, immunological and environmental factors.²⁰ The
23 interplay between these complex aetiological factors has yet to be fully understood.^{21,22}

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- α , interleukin (IL)-1, interferon- γ , IL-6, IL-12, IL-15, IL-18
28 and the IL-17/IL-23 axis.²³⁻²⁷ However, although microbial agents including dormant bacteria,
29 bacterial products, mycobacteria and viral antigens have been implicated as potential
30 initiators,^{28,29} 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.³⁰ 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.³¹ 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,³² a decrease in regulatory T cell activity,³³ and/or an altered mucosal permeability
40 thus compromising the capacity of the intestine to provide adequate containment of luminal
41 microorganisms and molecules.^{34,35} In support of these theories, several studies have documented
42 subclinical gut inflammation in PsA patients.³⁶⁻⁴¹ 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.⁴²

3 4 **Rationale**

5 If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen
6 in PsA,⁸ 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.^{44,45} Particularly, this procedure has demonstrated more than 90% clinical
9 resolution of recurrent or refractory *Clostridium difficile* infections.⁴⁶⁻⁵⁰ Also, multiple FMTs seem
10 to be able to induce remission in patients with inflammatory bowel disease (IBD).⁵¹ Due to these
11 results, FMT is now being tested as a potential novel treatment for other gastrointestinal and
12 extra-intestinal diseases.⁵² 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.⁵³ 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.^{1,3,5,6,20,32,43,54-60} 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),⁶¹ one in juvenile
29 idiopathic arthritis using exclusive enteral nutrition (n = 7),⁶² and one in rheumatoid arthritis
30 patients using probiotics in a placebo-controlled setting (n = 60).⁶³ 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.

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

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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.
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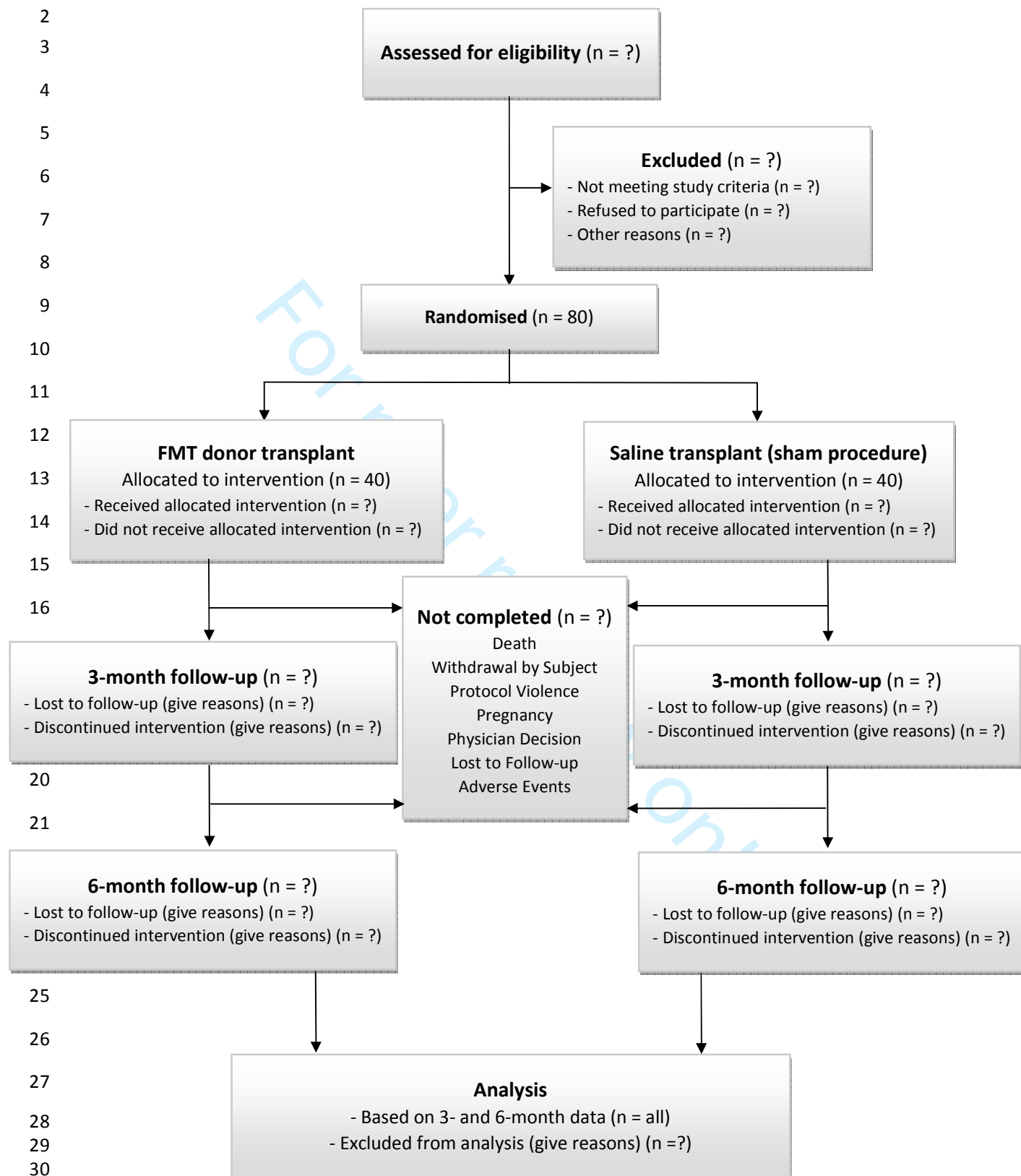


Figure 1. Flow diagram of the randomised, placebo-controlled trial

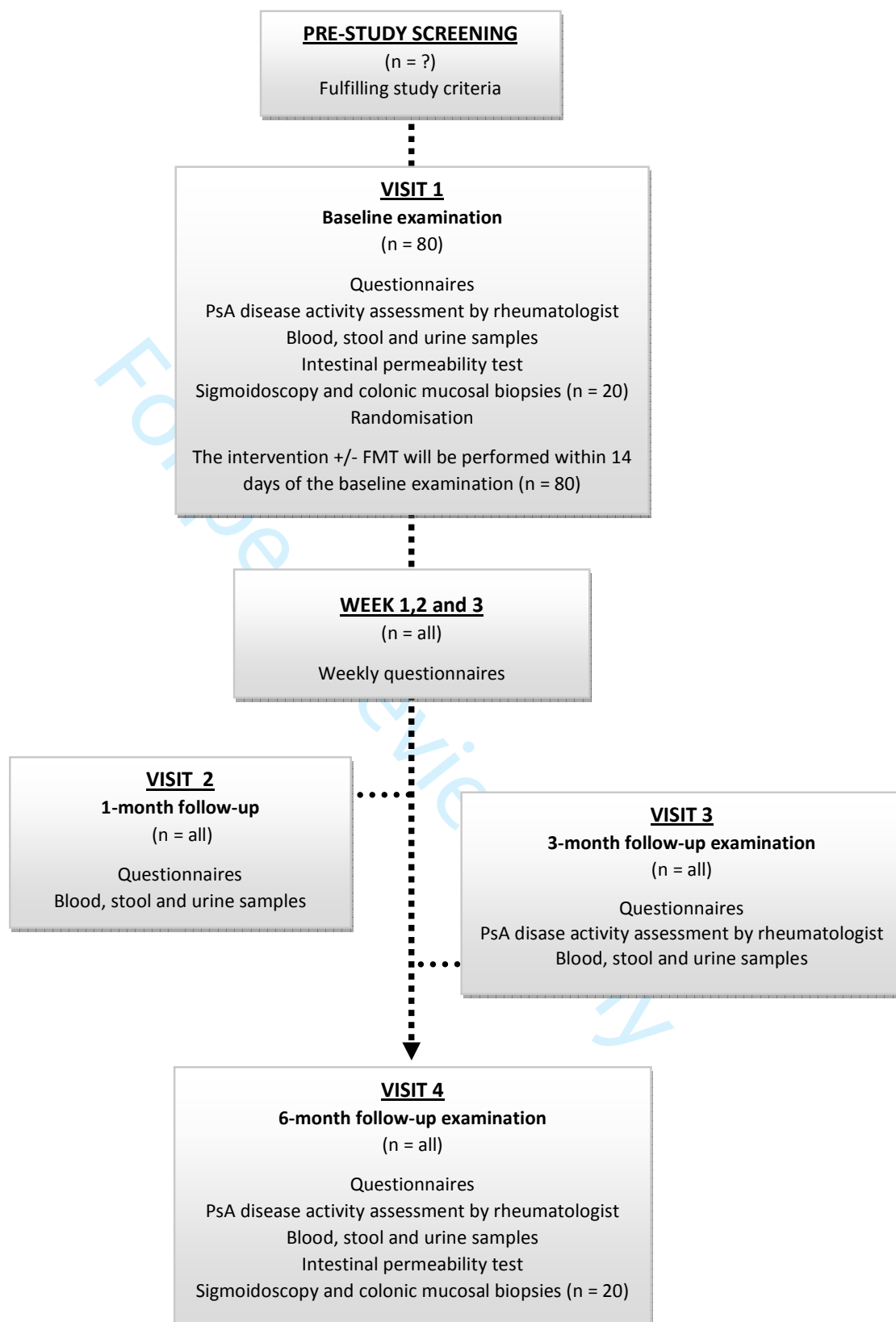


Figure 2. Participation timeline and general characteristics of each visit.

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).⁶⁴
- 13 • Presence of active peripheral arthritis defined as ≥ 3 swollen joints.
- 14 • Subcutaneously administered MTX treatment (≥ 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/m², 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)^{65,66}

[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) Questionnaire⁶⁷ [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

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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)]
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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)]
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13 8 Proportion of patients in each group achieving the American College of Rheumatology (ACR)⁶⁸
14 9 Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
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16 10 I. ACR20 response criteria⁶⁹

17 11 II. ACR50 response criteria⁷⁰

18 12 III. ACR70 response criteria⁷⁰
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23 14 Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)⁶⁸
24 15 [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
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28 17 Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis
29 18 Index⁶⁸ in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7
30 19 days), 6 months (+/- 14 days)]
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32 20

33 21 Change from baseline in the Psoriasis Area Severity Index (PASI)⁷¹ in the subset of patients who
34 22 have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
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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)]
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42 27 Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]
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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,⁷² changes in faecal bacteria composition and
51 34 metabolism, changes in intestinal permeability,⁷³ changes in plasma orosomucoid, changes in
52 35 plasma and faecal calprotectin,⁷⁴ 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_{1c} levels,
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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

6 4 **Safety**

8 5 The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild
9 6 diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the
10 7 procedure.⁷⁵ A recent systematic review on the adverse events of FMT identified 50 relevant
11 8 studies with a total of 1,089 patients. In this review, the incidences of serious adverse events
12 9 (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The
13 10 SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%),
14 11 death (0.3%), auto-immune diseases and FMT procedure related injury.⁷⁶ Although most of the
15 12 patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection
16 13 and/or IBD), these findings warrant caution when performing FMT; especially when introducing
17 14 the procedure in a new patient population. In addition, the potential long term side effects
18 15 following FMT remains largely unknown.⁷⁷ Still, when strict donor screening is conducted and the
19 16 procedure is performed by experienced practitioners, FMT is in general considered safe, and even
20 17 elderly patients with a poor medical condition and multiple co morbidities as well as
21 18 immunosuppressed patients have been proven to tolerate the FMT procedure well.⁷⁸⁻⁸²

22 19 In the present study, we will carefully monitor and evaluate safety by means of open
23 20 assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be
24 21 monitored until resolution, stabilisation or until it has been shown that the study intervention is
25 22 not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events,
26 23 version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events.
27 24 Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool
28 25 type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a
29 26 week for the first month following the randomised intervention. Routine blood screening for MTX
30 27 toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by
31 28 the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject
32 29 incidence rates of all treatment-emergent AE will be tabulated by system organ class and
33 30 preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant
34 31 treatment-emergent adverse events, will also be provided. For the long-term extension portion of
35 32 this study, exposure adjusted event rates will be summarised.
36 33

37 34 **Sample size and power considerations**

38 35 For a comparison of two independent binomial proportions using the Pearson's chi-squared
39 36 statistic with a Chi-square approximation (a two-sided significance level of 0.05), a sample size of
40 37 40 PsA patients per group has a power of 90% (0.895) if we assume that the proportions of
41 38 treatment failures are 35% (FMT-active group) and 70% (FMT-sham group), respectively.
42 39 Consequently, the inclusion of 80 PsA patients allocated (1:1) to two treatment arms is believed to
43 40 be sufficient to reveal any difference of clinical importance between treatment groups (i.e., an
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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 REDCap⁸³ 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.

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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 _{1c} (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).

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1 DISCUSSION

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

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

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

41 Next, only patients with active peripheral PsA will be included. One reason for this is
42 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.⁹² 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,⁹⁵⁻⁹⁸ 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.⁹⁹⁻¹⁰¹ 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.¹⁰² 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,¹⁰³ 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.¹⁰¹ 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.

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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,¹⁰⁴ 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.¹⁰⁵ 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.^{51,106} 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,^{95,96,107,108} 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 al⁹³ 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 al¹⁰⁹
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,¹¹⁰ and animal models have shown
28 that sex¹¹¹ and age¹¹² 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.¹¹³
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.

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

CONCLUSION

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

AUTHORS' CONTRIBUTION

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

REGISTRATION

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

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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.
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17
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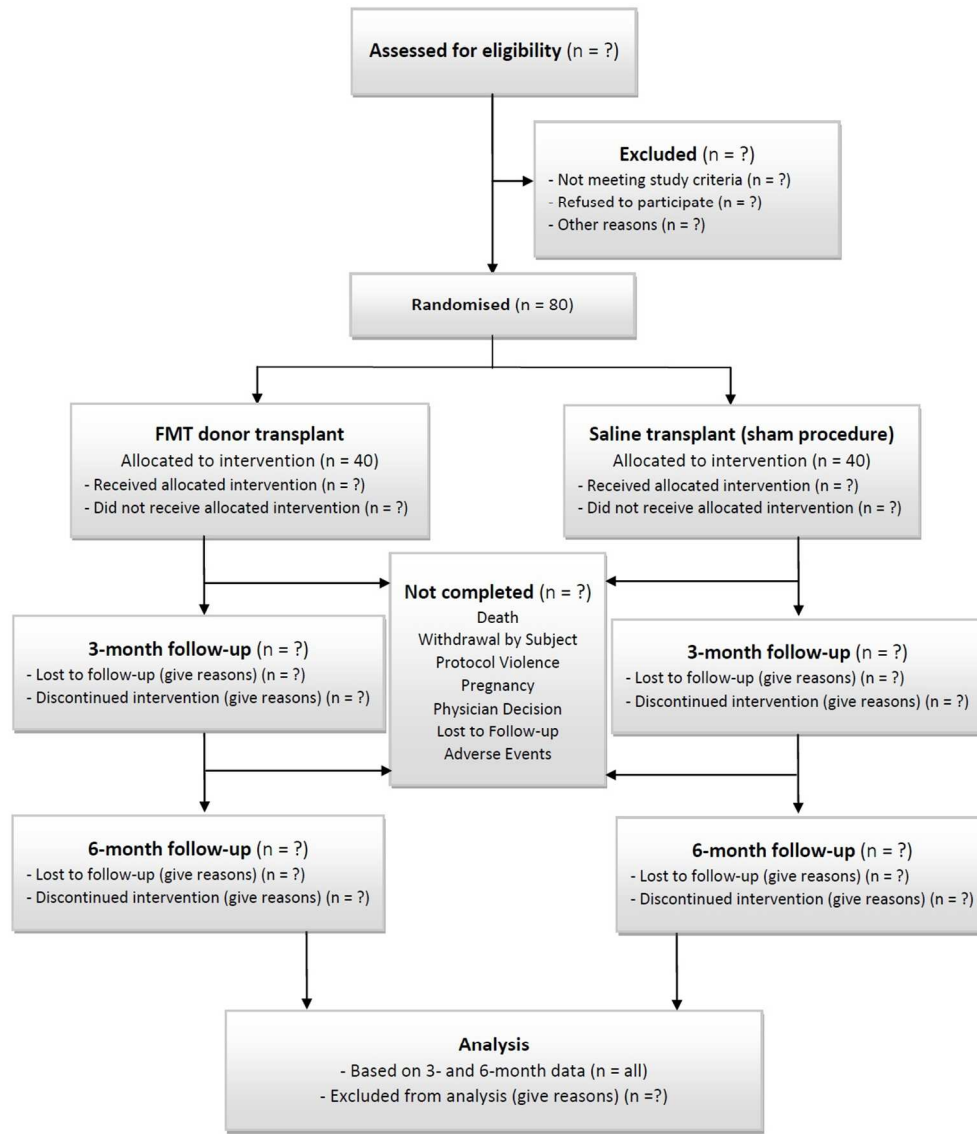


Figure. 1. Flow diagram of the randomised, placebo-controlled trial

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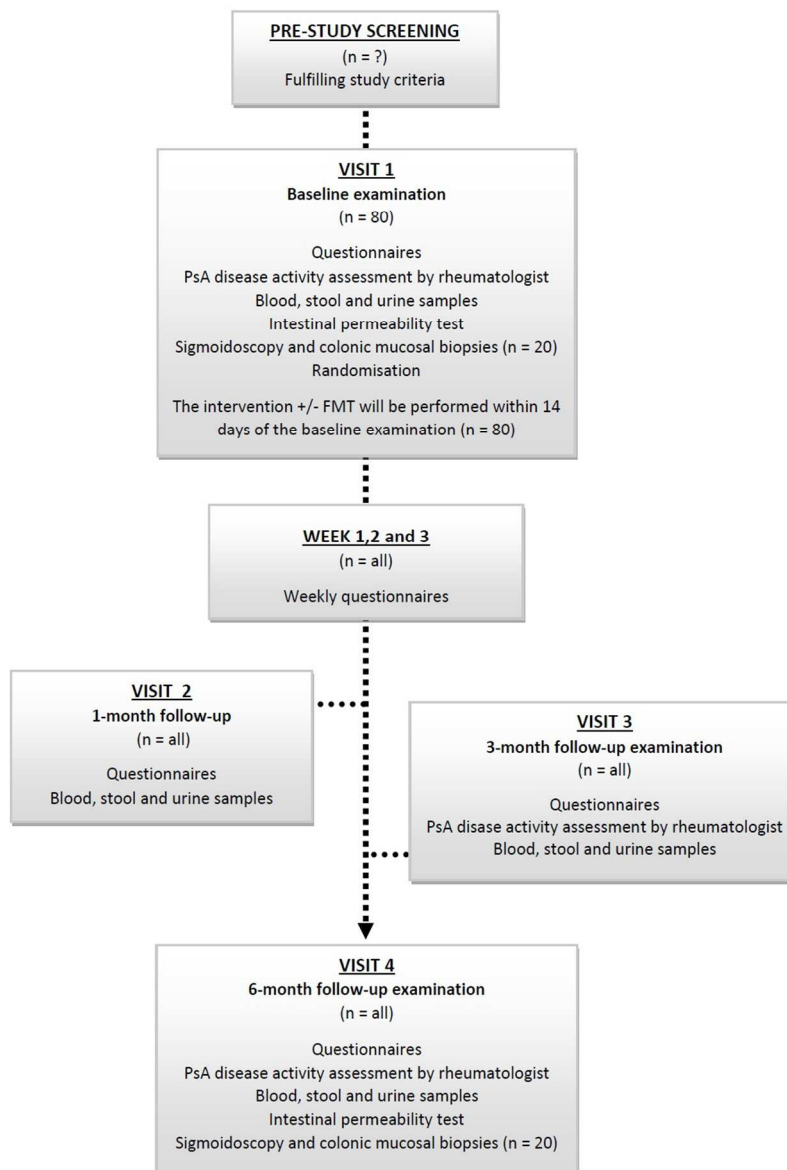


Figure 2. Participation timeline and general characteristics of each visit

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8 Maja Skov Kragstnæs
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12 5000 Odense C
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**De Videnskabetiske Komitéer
for Region Syddanmark**

komite@rsyd.dk

25. juni 2015

Projekt-ID: S-20150080
HLP/bss

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17 **Forskningsprojekt:**
18 **Fæces-mikrobiom-transplantation hos patienter med perifer psoriasisgigt:**
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
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	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
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
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	
Randomisation:			
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
Results			
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
Discussion			
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	-
Other information			
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.

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
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7 3 Efficacy and safety of faecal microbiota transplantation in
8 patients with psoriatic arthritis:
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10 protocol for a 6-month, double-blind, randomised placebo-
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12 controlled trial
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17 8 The FLORA trial
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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.¹⁻⁵ 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,⁶ 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.⁷⁻¹²

10 PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum
11 and a varied disease course.¹³ 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.¹⁴ 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.¹⁵ Without disease modifying intervention, 40-60% of PsA patients will
16 develop erosive and deforming joint damage within a few years of disease onset.¹⁶ Methotrexate
17 (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug
18 (csDMARD) for initial therapy.¹⁷ However, the evidence for MTX in PsA is poor, and a substantial
19 number of patients does not benefit from such treatment.¹⁸ Currently, other treatment options
20 may include biological agents such as tumour necrosis factor (TNF- α) inhibitors aiming to block
21 some of the downstream molecular pathways driving the disease.¹⁹ Still, these drugs do not target
22 the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and
23 environmental factors.²⁰ The interplay between these complex aetiological factors has yet to be
24 fully understood.^{21,22}

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- α , interleukin (IL)-1, interferon- γ , IL-6, IL-12, IL-15, IL-18
29 and the IL-17/IL-23 axis.²³⁻²⁷ However, although microbial agents including dormant bacteria,
30 mycobacteria, bacterial products and viral antigens have been implicated as potential
31 initiators,^{28,29} 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.³⁰ 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.³¹ 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,³² a decrease in regulatory T cell activity,³³ and/or an altered
41 mucosal permeability thus compromising the capacity of the intestine to provide adequate
42 containment of luminal microorganisms and molecules.^{34,35} In support of these theories, several
43 studies have documented subclinical gut inflammation in PsA patients.³⁶⁻⁴¹ 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.⁴²

4 5 **Rationale**

6 If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen
7 in PsA,⁸ 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.^{44,45} Particularly, this procedure has demonstrated more than 90% clinical
10 resolution of recurrent or refractory *Clostridium difficile* infections.⁴⁶⁻⁵⁰ Also, multiple FMTs seem
11 to be able to induce remission in patients with inflammatory bowel disease (IBD).⁵¹ Due to these
12 results, FMT is now being tested as a potential novel treatment for other gastrointestinal and
13 extra-intestinal diseases.⁵² 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.⁵³ 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.^{1,3,5,6,20,32,43,54-60} 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),⁶¹ and one study in entheses-related
30 arthritis (n = 8) reported no beneficial effects of probiotic therapy,⁶² 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.⁶³ Five placebo-controlled trials of probiotic therapy in
33 rheumatoid arthritis patients⁶⁴⁻⁶⁸ (sample size between 26 and 60 patients) reported mixed
34 results.⁶⁹ However, two of these studies demonstrated positive clinical effects of probiotic therapy
35 which included improvement in HAQ-DI pain scale,⁶⁵ improvement in the Disease Activity Score of
36 28 joints (DAS-28), and improvement on the C-reactive protein concentrations.⁶⁶ 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).⁷⁰
- 28 • Presence of active peripheral arthritis defined as ≥ 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.

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11 Stool donors

12 The stool donor corps will consist of four anonymous (to the recipient) donors who must be
 13 healthy as assessed by a screening questionnaire, and be active members of the Danish blood
 14 donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m², and an average alcohol
 15 intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of
 16 donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior
 17 to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie
 18 diets), and must not be in a stressful life period. Before joining the stool donor corps, each
 19 potential donor will go through a screening process including stool analyses for faecal calprotectin
 20 and enteric pathogens (*Aeromonas*, *Campylobacter*, *C. difficile*, diarrhoeagenic *Escherichia coli*,
 21 *Salmonella*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, and multidrug-resistant bacteria, parasites
 22 including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium*
 23 (DNA) and *Giardia* (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human
 24 adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-
 25 reactive protein (CRP) (acceptable level: < 6.0 mg/L), white blood cell count (acceptable range:
 26 3.50-8.80 10⁹/L), haemoglobin (acceptable range: 8.3-10.5 mmol/L), albumin (acceptable range:
 27 36-50 g/L), alanine aminotransferase (ALAT) (acceptable range: 10-70 U/L), glomerular filtration
 28 rate (eGFR) (acceptable level: > 59 mL/min), and coeliac disease, and blood test for infectious
 29 agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM),
 30 hepatitis A, B, C and E, tuberculosis (QuantiFERON[®] TB-Gold test), syphilis, human
 31 immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies),
 32 and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing
 33 the screening tests, the donor will donate stool for the next month after which, the donor will
 34 have to pass the screening programme once more before the stool can be released for
 35 transplantation.

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37 Interventions

38 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,^{71,72} 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.⁷³

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.^{74,75}

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)^{76,77}
3 [Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14
4 days)]
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8 5 Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire⁷⁸ [Time Frame: 1
9 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]
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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)⁷⁹
21 Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
22 15
23 16 I. ACR20 response criteria⁸⁰
24 17 II. ACR50 response criteria⁸¹
25 18 III. ACR70 response criteria⁸¹
26 19
27 20 Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)⁷⁹
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 Index⁶⁸ in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7
33 days), 6 months (+/- 14 days)]
34 24
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36 26
37 27 Change from baseline in the Psoriasis Area Severity Index (PASI)⁸² 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
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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)]
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2 1 Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14
3 2 days)]
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5 4 *Tertiary (exploratory secondary) outcomes:* Proportion of patients in each group achieving changes
6 5 in plasma CRP, changes in tender point count,⁸³ 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,⁸⁴ 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 HbA₁C 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.⁸⁵ 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.⁸⁶ 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.⁸⁷ 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.⁸⁸⁻⁹²

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.

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2 **Sample size and power considerations**

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

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

21 Beyond the primary endpoint, a total sample size of 80 (with a balanced design)
22 corresponds to a sufficient statistical power (82%) to detect a standardised mean difference of
23 0.65 SD units (i.e. Cohen's effect size) in any of the Patient-Reported Outcome Measures.

25 **Randomisation, allocation concealment and blinding**

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

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

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9 5 Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central
10 REDCap⁹³ 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.
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21 13 **Statistical methods**

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

45 33 Our strategy for ITT analysis with incomplete observations will be based on the
46 recommendations from White et al⁹⁶:
47

- 48 34 1: Attempt to follow up all randomised participants, even if they withdraw from allocated
49 treatment.
- 50 35 2: Perform a main analysis of all observed data (data as observed).
- 51 36 3: Perform sensitivity analyses to explore the effect of departures from the assumption made in
52 the main analysis (Baseline Observation Carried Forward [BOCF] imputations, repeated measures
53 mixed models, and multiple imputations).
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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]).

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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.

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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.

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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.

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33 The faecal metagenomics bioinformatics analyses will be performed using
34 comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics
35 species,^{71,72} 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.⁷³ 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-

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1 Hochberg false discovery rate control procedure. To control for confounders, we will use blocked
2 Spearman tests as implemented in COIN.^{97,98}
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

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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 _{1c} (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		

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

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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.^{22,99,100} In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.¹⁰¹⁻¹⁰⁴ 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.¹⁰⁵ Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic diseases are causal related,⁵⁵ and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.^{54,106} 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.^{51,107,108} 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,⁴⁷ 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.¹⁰⁹ 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

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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.¹⁰⁷ 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,¹¹⁰⁻¹¹³ 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.¹¹⁴⁻¹¹⁶ 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.¹¹⁷ 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,¹¹⁸ 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.¹¹⁶ 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

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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,¹¹⁹ 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.¹²⁰ 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.^{51,121} 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,^{110,111,122,123} 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 al¹⁰⁸ 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 al¹²⁴
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,¹²⁵ and animal models have shown
35 that sex¹²⁶ and age¹²⁷ 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.¹²⁸
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

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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.

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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.¹²⁹⁻¹³¹ Other complicating factors may include the composition of other microbiological
10 niches such as the oral, lung, genitourinary, and skin microbiota.^{132,133} 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.

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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**

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20 T. Ellingsen, M.S. Kragssnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for
21 the study. T. Ellingsen and M.S. Kragssnaes are the principal investigators and wrote the first study
22 protocol draft. T. Ellingsen and M.S. Kragssnaes 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. Kragssnaes, 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**

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

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9 **COMPETING INTEREST STATEMENT**

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

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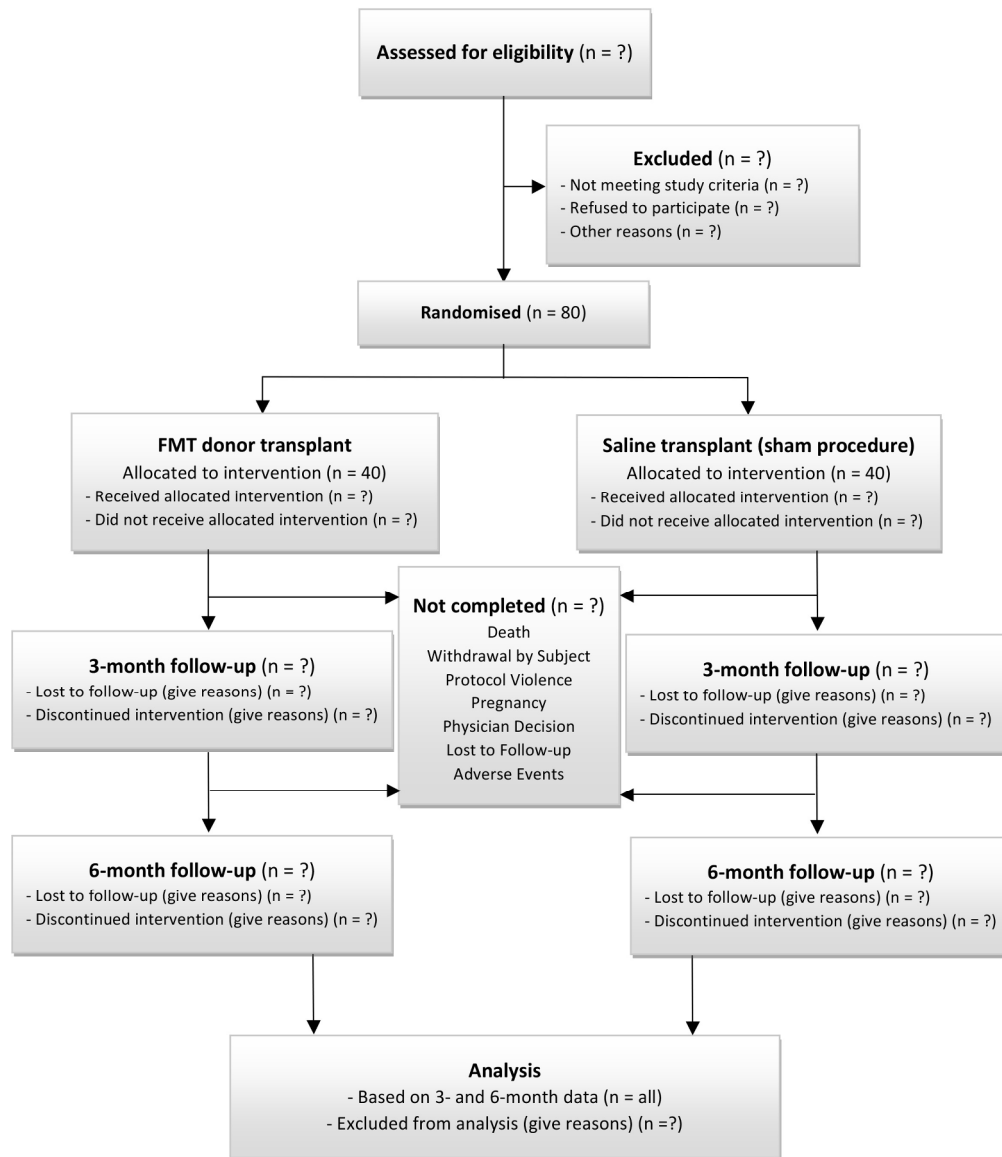


Figure 1. Flow diagram of the randomised, placebo-controlled trial.

198x236mm (300 x 300 DPI)

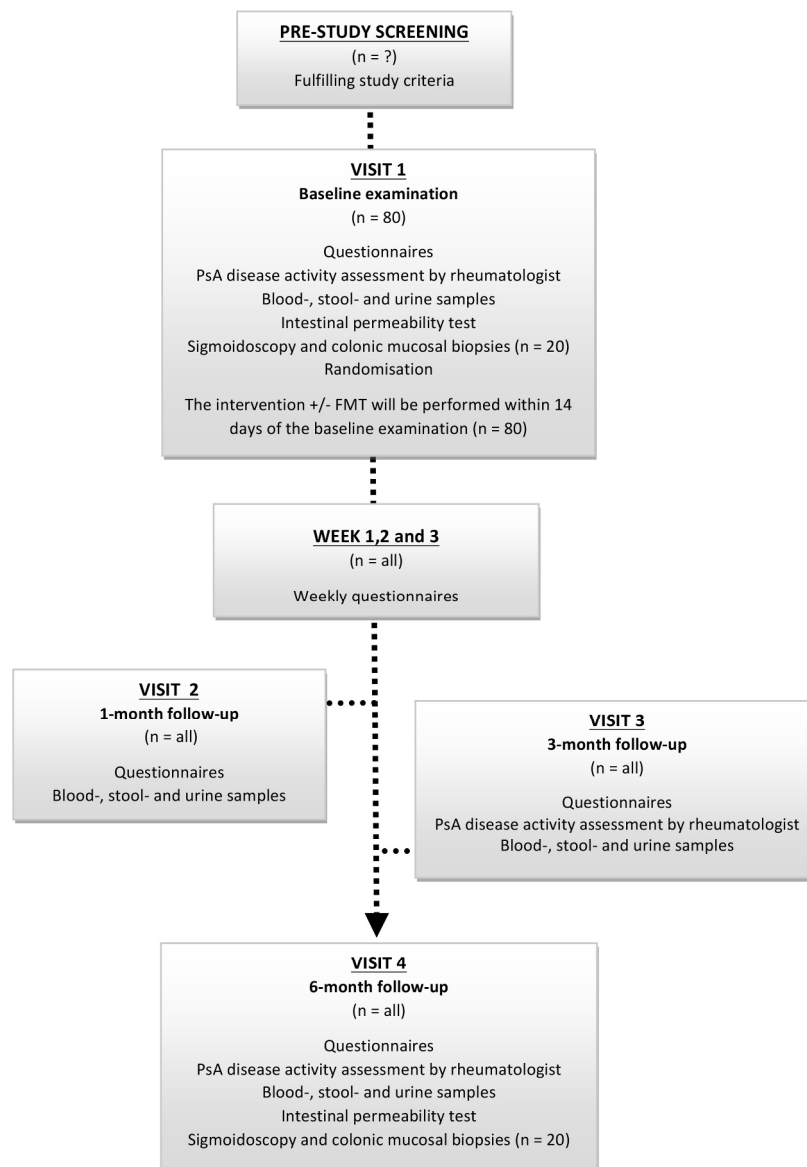


Figure 2. Participation timeline and characteristics of each visit.

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
Administrative information			
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			
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13			
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 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)
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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>
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4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>8</u>
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7	Methods: Assignment of interventions (for controlled trials)			
8	Allocation:			
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>
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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>
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20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>14</u>
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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>
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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>
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31	Methods: Data collection, management, and analysis			
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>
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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>
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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>
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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>
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>15-16</u>
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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>
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14	Methods: Monitoring			
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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>
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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>
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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>
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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>
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32	Ethics and dissemination			
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34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>18</u>
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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>
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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>
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>Not applicable</u>
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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>
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>23</u>
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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>
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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>
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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>
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>22</u>
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26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Not applicable</u>
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29	Appendices			
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31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>18</u>
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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>
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*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

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Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Psoriasis < DERMATOLOGY, Clinical trials < THERAPEUTICS, Faecal microbiota transplantation, Intestinal microbiota, Psoriatic arthritis

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20 10 *Kragsnaes MS^{1,2*}, Kjeldsen J³, Horn HC¹, Munk HL¹, Pedersen FM³, Holt HM⁴, Pedersen JK¹, Holm*
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41 28 Protocol manuscript version: 04.

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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.¹⁻⁵ 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,⁶ 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.⁷⁻¹²

10 PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum
11 and a varied disease course.¹³ 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.¹⁴ 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.¹⁵ Without disease modifying intervention, 40-60% of PsA patients will
16 develop erosive and deforming joint damage within a few years of disease onset.¹⁶ Methotrexate
17 (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug
18 (csDMARD) for initial therapy.¹⁷ However, the evidence for MTX in PsA is poor, and a substantial
19 number of patients does not benefit from such treatment.¹⁸ Currently, other treatment options
20 may include biological agents such as tumour necrosis factor (TNF- α) inhibitors aiming to block
21 some of the downstream molecular pathways driving the disease.¹⁹ Still, these drugs do not target
22 the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and
23 environmental factors.²⁰ The interplay between these complex aetiological factors has yet to be
24 fully understood.^{21,22}

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- α , interleukin (IL)-1, interferon- γ , IL-6, IL-12, IL-15, IL-18
29 and the IL-17/IL-23 axis.²³⁻²⁷ However, although microbial agents including dormant bacteria,
30 mycobacteria, bacterial products and viral antigens have been implicated as potential
31 initiators,^{28,29} 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.³⁰ 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.³¹ 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,³² a decrease in regulatory T cell activity,³³ and/or an altered
41 mucosal permeability thus compromising the capacity of the intestine to provide adequate
42 containment of luminal microorganisms and molecules.^{34,35} In support of these theories, several
43 studies have documented subclinical gut inflammation in PsA patients.³⁶⁻⁴¹ 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.⁴²

4 5 **Rationale**

6 If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen
7 in PsA,⁸ 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.^{44,45} Particularly, this procedure has demonstrated more than 90% clinical
10 resolution of recurrent or refractory *Clostridium difficile* infections.⁴⁶⁻⁵⁰ Also, multiple FMTs seem
11 to be able to induce remission in patients with inflammatory bowel disease (IBD).⁵¹ Due to these
12 results, FMT is now being tested as a potential novel treatment for other gastrointestinal and
13 extra-intestinal diseases.⁵² 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.⁵³ 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.^{1,3,5,6,20,32,43,54-60} 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),⁶¹ and one study in enthesitis-related
30 arthritis (n = 8) reported no beneficial effects of probiotic therapy,⁶² 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.⁶³ Five placebo-controlled trials of probiotic therapy in
33 rheumatoid arthritis patients⁶⁴⁻⁶⁸ (sample size between 26 and 60 patients) reported mixed
34 results.⁶⁹ However, two of these studies demonstrated positive clinical effects of probiotic therapy
35 which included improvement in HAQ-DI pain scale,⁶⁵ improvement in the Disease Activity Score of
36 28 joints (DAS-28), and improvement on the C-reactive protein concentrations.⁶⁶ 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).⁷⁰
- 28 • Presence of active peripheral arthritis defined as ≥ 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.

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- 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.

11 Stool donors

12 The stool donor corps will consist of four anonymous (to the recipient) donors who must be
 13 healthy as assessed by a screening questionnaire, and be active members of the Danish blood
 14 donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m², and an average alcohol
 15 intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of
 16 donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior
 17 to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie
 18 diets), and must not be in a stressful life period. Before joining the stool donor corps, each
 19 potential donor will go through a screening process including stool analyses for faecal calprotectin
 20 and enteric pathogens (*Aeromonas*, *Campylobacter*, *C. difficile*, diarrhoeagenic *Escherichia coli*,
 21 *Salmonella*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, and multidrug-resistant bacteria, parasites
 22 including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium*
 23 (DNA) and *Giardia* (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human
 24 adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-
 25 reactive protein (CRP) (acceptable level: < 6.0 mg/L), white blood cell count (acceptable range:
 26 3.50-8.80 10⁹/L), haemoglobin (acceptable range: 8.3-10.5 mmol/L), albumin (acceptable range:
 27 36-50 g/L), alanine aminotransferase (ALAT) (acceptable range: 10-70 U/L), glomerular filtration
 28 rate (eGFR) (acceptable level: > 59 mL/min), and coeliac disease, and blood test for infectious
 29 agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM),
 30 hepatitis A, B, C and E, tuberculosis (QuantiFERON[®] TB-Gold test), syphilis, human
 31 immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies),
 32 and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing
 33 the screening tests, the donor will donate stool for the next month after which, the donor will
 34 have to pass the screening programme once more before the stool can be released for
 35 transplantation.

37 Interventions

38 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.⁷¹ The faecal
14 metagenomics bioinformatics analyses will be performed using comprehensive pipelines including
15 the assembly of metagenomics linkage groups/metagenomics species,^{72,73} 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.⁷⁴

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.^{75,76}

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)^{77,78}

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

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4 2 Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire⁷⁹ [Time Frame: 1
5 3 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]
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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)]
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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
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16 11 Proportion of patients in each group achieving the American College of Rheumatology (ACR)⁸⁰
17 12 Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

18 13 I. ACR20 response criteria⁸¹

19 14 II. ACR50 response criteria⁸²

20 15 III. ACR70 response criteria⁸²
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27 17 Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)⁸⁰
28 18 [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
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32 20 Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis
33 21 Index⁶⁸ in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7
34 22 days), 6 months (+/- 14 days)]
35
36 23

37 24 Change from baseline in the Psoriasis Area Severity Index (PASI)⁸³ in the subset of patients who
38 25 have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
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42 27 Change from baseline in the number of digits affected with dactylitis in the subset of patients who
43 28 have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]
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47 30 Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]
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49 31

50 32 Number of adverse events in each group leading to discontinuation [Time Frame: 6 months (+/- 14
51 33 days)]
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54 35 Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14
55 36 days)]
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1 *Tertiary (exploratory secondary) outcomes:* Proportion of patients in each group achieving changes
2 in plasma CRP, changes in tender point count,⁸⁴ changes in faecal bacteria composition and
3 metabolism, changes in intestinal permeability, changes in plasma orosomucoid, changes in
4 plasma and faecal calprotectin,⁸⁵ 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 HbA₁C 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.⁸⁶ 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.⁸⁷ 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.⁸⁸ 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.⁸⁹⁻⁹³

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.
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40 **Sample size and power considerations**

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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 REDCap⁹⁴ 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;⁹⁵ 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.⁹⁶

30 Our strategy for ITT analysis with incomplete observations will be based on the
31 recommendations from White et al⁹⁷:

- 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,^{72,73} 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.⁷⁴ 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.^{98,99}

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 _{1c} (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.^{22,100,101} In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.¹⁰²⁻¹⁰⁵ 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.¹⁰⁶ Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic diseases are causal related,⁵⁵ and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.^{54,107} 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.^{51,108,109} 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,⁴⁷ 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.¹¹⁰ 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

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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.

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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.¹⁰⁸ 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,¹¹¹⁻¹¹⁴ bypassing the intestine
during MTX administration will ensure that no local non-disease related effects on the intestinal
microbiota will occur.

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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.¹¹⁵⁻¹¹⁷ 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.¹¹⁸ 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,¹¹⁹ the optimal transplant preparation
method in treating inflammatory diseases remains to be established.

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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.¹¹⁷ 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

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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,¹²⁰ 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.¹²¹ 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.^{51,122} 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,^{111,112,123,124} 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 al¹⁰⁹ 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 al¹²⁵
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,¹²⁶ and animal models have shown
36 35 that sex¹²⁷ and age¹²⁸ 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.¹²⁹
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.¹³⁰⁻¹³² Other complicating factors may include the composition of other microbiological
10 niches such as the oral, lung, genitourinary, and skin microbiota.^{133,134} 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).

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9

10 **COMPETING INTEREST STATEMENT**

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

13

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2 1 FIGURE LEGENDS
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5 3 **Figure 1.** Flow diagram of the randomised, placebo-controlled trial.
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9 5 **Figure 2.** Participation timeline and characteristics of each visit.
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For peer review only

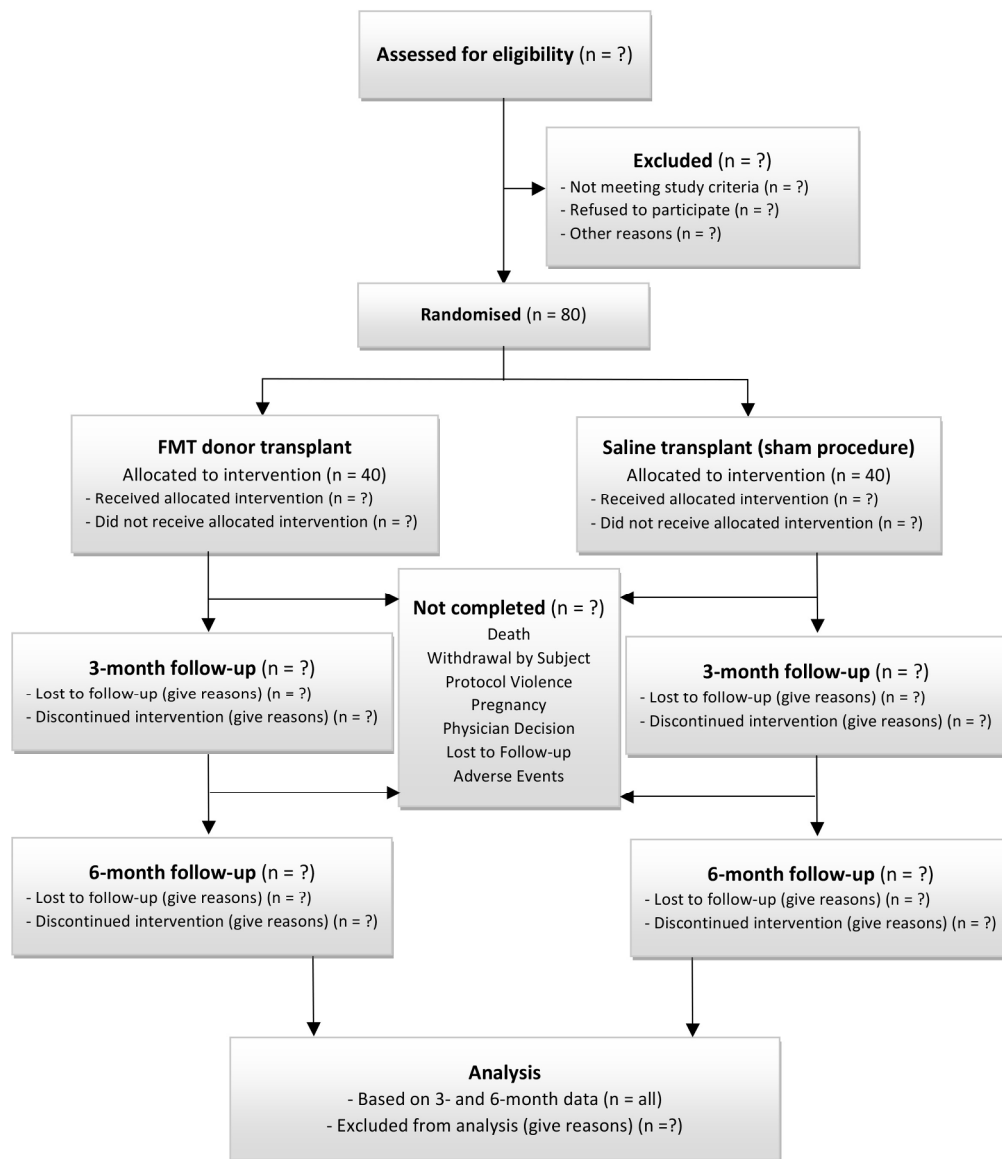


Figure 1. Flow diagram of the randomised, placebo-controlled trial.

198x236mm (300 x 300 DPI)

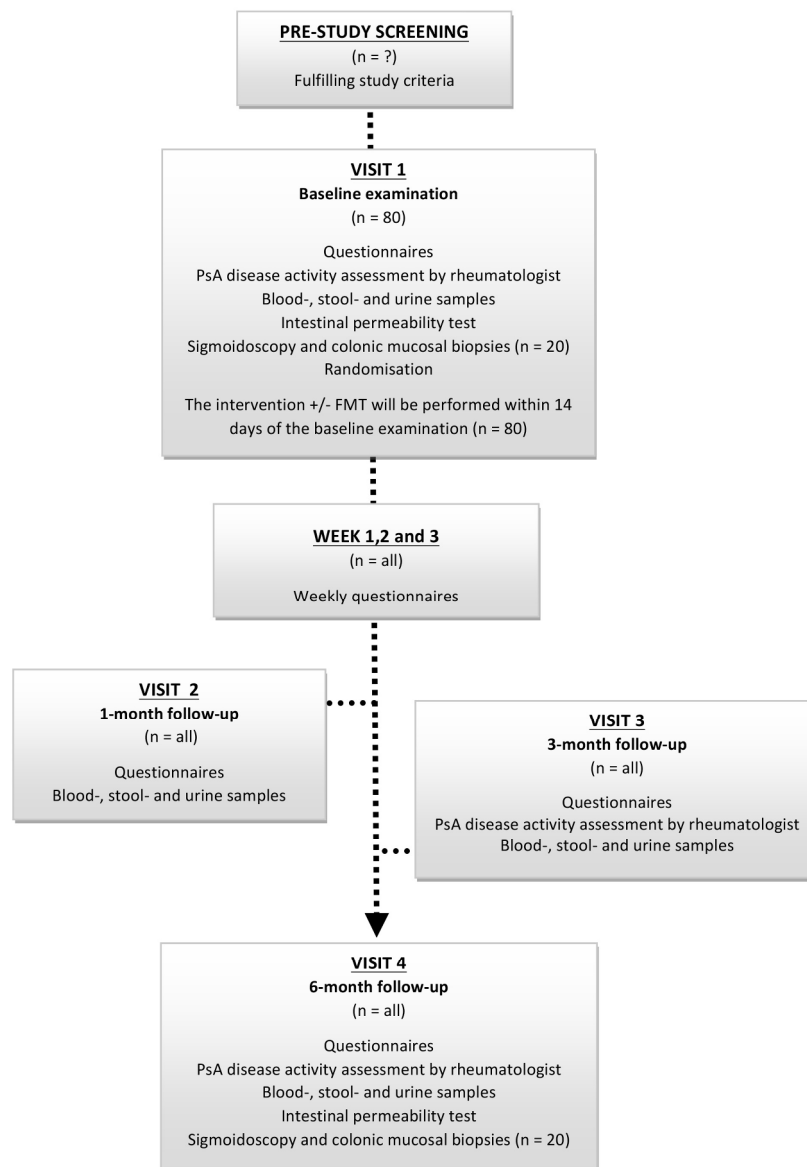


Figure 2. Participation timeline and characteristics of each visit.

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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
Administrative information			
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>
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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), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
11			<u>5</u>
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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 be collected. Reference to where list of study sites can be obtained
17			<u>8</u>
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19	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)
20			<u>8-9</u>
21			
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
23			<u>9-10</u>
24			
25		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)
26			<u>10</u>
27			
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
29			<u>Not applicable</u>
30			
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
32			<u>8 and 9</u>
33			
34	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
35			<u>11-12</u>
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37	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)
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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>
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4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>8</u>
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6	Methods: Assignment of interventions (for controlled trials)			
7	Allocation:			
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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>
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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>
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20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>14</u>
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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>
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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>
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31	Methods: Data collection, management, and analysis			
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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>
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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>
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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>
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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>
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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>
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14	Methods: Monitoring			
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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>
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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>
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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>
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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>
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32	Ethics and dissemination			
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34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>18</u>
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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>
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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>
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>Not applicable</u>
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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>
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>23</u>
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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>
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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>
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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>
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>22</u>
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26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Not applicable</u>
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29	Appendices			
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31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>Appendix</u>
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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>
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*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.