

**Safety and efficacy of fecal microbiome transplantation (FMT) in the treatment of antibiotic dependent pouchitis (ADP)**

**A placebo-controlled proof of concept study with open label extension**

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# Safety and efficacy of fecal microbiome transplantation (FMT) in the treatment of antibiotic dependent pouchitis (ADP)

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## 1. PROTOCOL SUMMARY

<b>Title:</b>	Safety and efficacy of fecal microbiome transplant (FMT) in the treatment of antibiotic dependent pouchitis (ADP) in patients with IPAA after colectomy for therapy refractory ulcerative colitis
<b>Research Hypothesis:</b>	Fecal transplant is a safe and effective treatment for patients with antibiotic dependent pouchitis.
<b>Study design:</b>	Randomized, double blind study with open label extension
<b>Study population:</b>	Patients with ADP and IPAA after colectomy for ulcerative colitis
<b>Sample size:</b>	20 study patients
<b>Treatment:</b>	Fecal microbiome transplantation
<b>Treatment duration:</b>	Endoscopic FMT followed by oral FMT or placebo endoscopic and placebo oral FMT for 14 days in the placebo-controlled arm. In case of no clinical remission open label endoscopic FMT followed by oral FMT for 14 days.
<b>Main criteria for inclusion:</b>	<ul style="list-style-type: none"> <li>Signed informed consent.</li> <li>Man or woman between 18 and 70 years of age.</li> <li>IPAA after colectomy for ulcerative colitis</li> <li>Active pouchitis, defined as a mPDAI <math>\geq 5</math> and a history of <math>\geq 4</math> antibiotic therapies for pouchitis in the last 12 months</li> </ul> <p>or</p> <ul style="list-style-type: none"> <li>Need for ongoing antibiotic therapy (&gt; 4 weeks) to maintain clinical remission and a history of at least 2 attempts in the last 24 months to stop antibiotic therapy resulting in pouchitis episodes.</li> <li><u>Concomitant oral 5 ASA or local therapies containing 5-ASA (enemas, suppositories) or steroid enemas are permitted but not required but have to be stable for 4 (oral) or 2 weeks (topical) before inclusion in the study.</u></li> </ul>
<b>Main criteria for exclusion:</b>	<ul style="list-style-type: none"> <li>Treatment with biologics (e.g. infliximab, adalimumab, golimumab, vedolizumab)</li> <li>Treatment with immunomodulators (azathioprine, 6-MP, methotrexate), steroids or any investigational drugs</li> <li>Use of cholestyramine</li> <li>Crohn's disease of the pouch</li> <li>Known cytomegalovirus infection of the pouch</li> </ul>

	<ul style="list-style-type: none"> <li>• Clostridium difficile infection</li> <li>• Isolated moderate/severe cuffitis</li> <li>• Clinical significant strictures of the pouch inlet or outlet</li> <li>• Concurrent intestinal obstruction</li> <li>• History of familial adenomatous polyposis</li> <li>• History of uncontrolled lactose intolerance</li> <li>• History of confirmed (serological test and/or histology) celiac disease</li> <li>• Pregnancy, breast feeding, or planning to become pregnant during the trial</li> <li>• Non - steroidal inflammatory medications (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month. Daily use of aspirin up to 81 mg a day for cardiovascular prophylaxis is acceptable.</li> <li>• Dysphagia (oropharyngeal, esophageal, functional, neuromuscular)</li> <li>• History of recurrent aspiration episodes</li> <li>• Proven gastroparesis</li> <li>• Allergy to the following generally regarded as safe ingredients (GRAS): glycerol, acid resistant HPMC, gellan gum, cocoa butter, titanium dioxide</li> <li>• Adverse event attributable to previous FMT</li> <li>• Allergy/intolerance to pump inhibitor therapy</li> <li>• Any condition for which the investigator thinks the FMT treatment may pose a health risk (e.g. severely immunocompromised)</li> <li>• Participation in another clinical trial within the last 30 days, simultaneous participation in another clinical trial, or previous participation in this trial</li> <li>• During the trial period until one week after the trial end: Non-use of appropriate contraceptives in females of childbearing potential (e.g. condoms, intrauterine device {IUD}, hormonal contraception, or other means considered adequate by the responsible investigator) or in males with a child-fathering potential (condoms, or other means considered adequate by the responsible investigator during treatment</li> <li>• Well-founded doubt about the patient's cooperation</li> </ul>
<b>Concomitant medication:</b>	<ul style="list-style-type: none"> <li>• All antibiotic therapies for pouchitis have to be stopped 1 day before FMT.</li> </ul> <p>Following drug groups are permitted as concomitant medication:</p> <ul style="list-style-type: none"> <li>• Orally administered mesalazine containing drugs should be stable for the last 4 weeks</li> <li>• Local therapies containing 5-ASA (enemas, suppositories) or steroid enemas should be at a stable dose 2 weeks before inclusion in the study.</li> </ul> <p>Following drug groups are <b><u>not</u></b> permitted as concomitant medication:</p>

	<ul style="list-style-type: none"> <li>• Antibiotic therapy for treatment of pouchitis</li> <li>• Cytostatics/immunosuppressants, cyclosporine, tacrolimus, mycophenolate mofetil.</li> <li>• Anti-TNF therapies or anti-adhesion therapy.</li> <li>• Non - steroidal anti-inflammatories (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month. Daily use of aspirin up to 81 mg a day for cardiovascular prophylaxis is acceptable</li> </ul>
<b>Criteria for Evaluation:</b>	<p>Safety and Tolerability of FMT will be evaluated determining the number of study patients, who discontinued therapy, determining the adverse events grouped by body system and evaluation of changes in laboratory values.</p> <p>The outcome will be determined by the modified pouchitis disease activity index (mPDAI) and the need for antibiotic therapy to control symptoms. The mPDAI is a composite index consisting of 2 disease variables (clinical criteria of pouchitis: number of bowel movements, blood in stool, urgency and fever with a maximum score of 6 and endoscopy criteria of pouch inflammation with a maximum score of 6. The maximum combined score is 12).</p> <ul style="list-style-type: none"> <li>• Complete remission is defined as a mPDAI score = 0 and no need for antibiotics.</li> <li>• Clinical remission is defined as a clinical mPDAI score <math>\leq 4</math> and no need for antibiotics.</li> <li>• Symptomatic improvement is defined as a decrease from baseline in the mPDAI clinical subscore of 2 points and no need for antibiotics.</li> <li>• Symptomatic worsening of the disease is defined by an increase in the mPDAI clinical subscore of 2 points or the need for antibiotics.</li> </ul> <p>The severity of the pouch inflammation will be evaluated by two pouchoscopies (week 0 and week 4). Endoscopic improvement is defined as a decrease from baseline in the mPDAI endoscopic subscore of 2 points.</p>
<b>Primary variables</b>	Safety and tolerability of endoscopically and orally applied FMT
<b>Secondary variables:</b>	<ul style="list-style-type: none"> <li>• <u>Clinical remission (mPDAI score <math>\leq 4</math> points ) and no need for antibiotic therapy at week 4.</u></li> <li>• Clinical remission (clinical mPDAI score <math>\leq 4</math>) and no need for antibiotics at week 8*.</li> <li>• Clinical remission (clinical mPDAI score <math>\leq 4</math>) and no need for antibiotics at week 16*.</li> <li>• Endoscopic improvement of active pouchitis (decrease from baseline in mPDAI endoscopic subscore <math>\geq 2</math> points) at week 4.</li> </ul>



	<ul style="list-style-type: none"> <li>• Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) and no need for antibiotic therapy at week 4.</li> <li>• Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) and no need for antibiotic therapy at week 8*.</li> <li>• Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) and no need for antibiotic therapy at week 16*.</li> </ul> <p>*Only patients who do not continue in open label extension</p> <ul style="list-style-type: none"> <li>• <u>Exploratory analyses (OL=Open label):</u></li> <li>• Endoscopic improvement of active pouchitis (decrease from baseline in mPDAI endoscopic subscore <math>\geq 2</math> points) at in open label extension week 8.</li> <li>• Clinical remission (mPDAI score <math>\leq 4</math> points and a decrease in the baseline mPDAI <math>&gt; 2</math> points) and no need for antibiotic therapy at week 10 OL.</li> <li>• Clinical remission (clinical mPDAI score <math>\leq 4</math>) and no need for antibiotics at week 14 OL.</li> <li>• Clinical remission (clinical mPDAI score <math>\leq 4</math>) and no need for antibiotics at week 22 OL.</li> <li>• Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) and no need for antibiotic therapy at week 10 OL.</li> <li>• Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) and no need for antibiotic therapy at week 14 OL.</li> <li>• Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) and no need for antibiotic therapy at week 22 OL.</li> <li>• Symptomatic worsening of active pouchitis (decrease from baseline in mPDAI clinical subscore <math>\geq 2</math> points) from FMT week 0 – week 16</li> <li>• Quantitative changes in mPDAI from baseline values</li> <li>• Quantitative changes of calprotectin from baseline</li> <li>• Alterations in stool microbiome after FMT</li> <li>• Engraftment of donor FMT</li> </ul>
<b>Statistical analysis:</b>	Remission and response as defined by mPDAI scores will be analysed by intention-to-treat design. Wilcoxon rank sum tests will be carried out to assess differences in the pre- and post-treatment mPDAI total scores and subscores. P values $< 0.05$ will be considered to be statistically significant.
<b>Duration of study</b>	2 weeks of FMT/ placebo therapy followed by evaluation of clinical and endoscopic outcome at week 4, and clinical evaluation at week 8 and 16. In case the study patients does not achieve clinical remission at week 4 or

	<p>experiences a flare of disease on day 15-28 after start of the study he/she will be offered the possibility to participate in open label extension with an additional endoscopic FMT followed by 2 weeks of oral FMT and clinical follow up at week 10, 14 and 22.</p> <p>The anticipated time of the overall study is 18 months</p>
<b>Number of centres:</b>	Single center study

## 2. BACKGROUND:

Ulcerative colitis (UC) is a chronic inflammatory gastrointestinal disorder, which is limited to the colon and characterized by the involvement of the mucosa only (in contrast to the transmural inflammation seen in CD). UC affects approx. 500.000 Americans, most of them being primarily young adults (20 - 40 years) but the disease may present also at a very early age (5-10 years) or in later in life (>60 years)<sup>1-3</sup>. The inflammatory process in UC is primarily localized to the rectum (proctitis) or can extend proximally in a contiguous manner involving the mucosa up to the splenic flexure (left sided colitis) or involving the entire colon (extensive colitis). The key clinical feature is bloody diarrhea. Approximately 20-35% of patients with UC eventually have to undergo colectomy, which is most often performed in conjunction with ileal pouch-anal anastomosis (IPAA) due to a refractory course of UC or histological proven dysplasia<sup>4-6</sup>. The ileal pouch serves as a reservoir for the stool and improves functional outcomes following IPAA. Pouchitis is the most common long-term complication after IPAA affecting up to 50% of patients following operation for UC<sup>7-10</sup>. The clinical symptoms include diarrhea, crampy abdominal pain, fever, bloody bowel movements, dehydration as well as extraintestinal manifestations such as joint pain.

- Pathogenesis of Pouchitis

The pathogenesis of pouchitis remains is only incompletely elucidated. The currently most favored underlying pathomechanisms of pouchitis are dysbiosis of the bacterial flora of the pouch and dysregulation of the mucosal inflammatory responses in genetically susceptible patients. Also bile acid toxicity, ischemia and infections may contribute in some patients to the clinical and endoscopic picture of pouchitis.

- Genetic susceptibility and pouchitis

The role of an aberrant regulation of the mucosal immune system is highlighted by the fact that pouchitis rarely occurs in patients with familial adenomatosis coli (FAP), who underwent a colectomy with IPAA<sup>11</sup>. There are also other known genetic or genetically influenced risk factors for the development of pouchitis, such as the presence of antineutrophil cytoplasmatic antibody with perinuclear staining pattern (pANCA), a history of primary sclerosing cholangitis (PSC) as well as an association of known IBD risk genes such as CARD15 with pouchitis pouchitis<sup>12</sup>.

- Bacterial Flora and pouchitis

The role of the bacterial flora in the pathogenesis of pouchitis has been strongly suggested by clinical trials demonstrating a high clinical efficacy of antibiotics (e.g. ciprofloxacin, metronidazole) to treat and prevent pouchitis. There seem to be a bacterial dysbiosis present in patients with pouchitis<sup>13-16</sup>. Studies comparing the bacterial flora of pouches of patients with FAP and UC found sulfate reducing bacteria in the majority of UC patients but none in FAP patients<sup>17</sup>. Also strict anaerobes (*Clostridium perfringens*, *Bifidobacteria* and *Bacteroides*) predominate over facultative anaerobes (*Enterococci*, *Coliforms* and *Lactobacilli*) in stool and mucosal biopsies of UC patients<sup>18</sup>. Using a 16S ribosomal RNA technique to determine qualitativ changes in the mucosa of the pouch, Komaduri et al. report the persistence of *Fusobacter* and *Enteric* species and the absence of specific bacteria such as *Streptococcus* species in association with pouchitis<sup>16</sup>. More recently an increase in *Ruminococcus gnavus*, *Bacteriodes vulgatus* and *Clostridium perfringens* with a concomitant reduction of *Lachnospiraceae* *Blautia* and *Roseburia* before colectomy was found to be a risk factor for developing pouchitis in the first year after IPAA.<sup>19</sup>

- Current therapeutic approaches for pouchitis

Different antibiotic therapies including ciprofloxacin, metronidazole, rifaximin have been shown to successfully treat pouchitis in subgroups of patients<sup>20-24</sup>. The probiotic VSL#3, which is a highly concentrated bacterial cocktail of 8 different bacterial species, prevents acute and chronic pouchitis, but is only effective in a subgroup of patients<sup>25-29</sup>. Agents known to be efficacious in left-sided ulcerative colitis including budesonide enemas<sup>30</sup>, mesalamine enemas<sup>31</sup> and suppositories<sup>32</sup>, butyrate and glutamine suppositories<sup>33</sup> have all been used with some success. Allopurinol, inulin and bismuth carboner foam enemas were also investigated without significant clinical efficacy<sup>34-36</sup>. More recently a single center study described a therapeutic efficacy of AST-120, a spherical carbon adsorbent in acute pouchitis<sup>37</sup>. A placebo-controlled trial has to confirm these findings in the future.

### **3. RATIONALE FOR THIS STUDY**

Active pouchitis can be treated successfully with antibiotics (metronidazole, ciprofloxacin), but relapse of pouchitis is common. The probiotic VSL#3 is effective to treat recurrent pouchitis, but is effective only in a subgroup of patients and is associated with high costs for the patients since considerable amounts of VSL#3 are necessary to achieve clinical effectiveness. Frequent antibiotic therapies, often in rotating therapeutic schedules are usually required to treat patient with pouchitis. The overuse of antibiotics predisposes to the development of bacterial resistance and is often associated with numerous side effects (nausea, vomiting, neurotoxicity, kidney toxicity, tendon rupture). There may also be the risk of secondary gastrointestinal infections, such as *Clostridium difficile* especially with fluoroquinolones<sup>38, 39</sup>. Patients may develop a refractory course of pouchitis after long-term antibiotic use, which may be due to bacterial selection<sup>40</sup>. Additionally patients with IPAA have an increased risk for the development of intra-abdominal infections and complications such as an ileus, which often require antibiotic therapy. Therefore the development of bacterial resistance from overuse of antibiotics for pouchitis might jeopardize the treatment of these complications.

#### *Fecal Microbial transplant (FMT)*

FMT has been shown to be an effective therapy for patients with dysbiosis in the setting of *C. diff* colitis.<sup>41, 42</sup> In patients with active UC 3 placebo controlled FMT studies have been reported.<sup>43-45</sup> One showed a beneficial effect mainly based on the efficacy of a single donor, whereas the other study revealed no effect of FMT. The most robust evidence for FMT comes from the so far largest placebo controlled study, which was conducted at 3 Australian centers and included 81 patients.<sup>45</sup> This study revealed a significant therapeutic efficacy of repeated intense FMT (5 multidonor FMT enemas/ week for 8 weeks) vs placebo (steroid free remission at week 8 27% FMT group vs 8% placebo group).

So far only two small case series and 2 case reports in patients with pouchitis (one with concomitant *C. difficile* infection) are published.<sup>46-49</sup> Two case reports describe success after a single endoscopic application of FMT, but the treatment success appeared weeks after the stool transplant, one patient also needed again antibiotic therapy.<sup>47, 48</sup> Landi et al published a case series of 8 patients with chronic refractory pouchitis treated with a single application of FMT via nasogastric tube.<sup>50</sup> FMT was not effective in achieving clinical remission, but significant changes in the pouch bacterial flora towards a supposedly healthier composition was observed in some FMT recipients and the quinolone sensitivity of 2 patients was re-established, who then were successfully maintained again on ciprofloxacin therapy. The engraftment success varied, which suggest that a single FMT in patients with refractory pouchitis might be not sufficient. Similar to the results of repeated FMT applications in patients with UC by

Paramsothy et al, a recently published case series by Stallmach et al. supports the need for repeated FMT in 5 patients with antibiotic dependent or refractory pouchitis.<sup>45, 49</sup> A single endoscopically performed FMT was only effective in 1/5 patients, but lasting remission (3/5) or improvement (1/5) was achieved with repeated FMT in the remaining patients. The efficacy of FMT in inflammatory bowel diseases (IBD) most likely depends on the frequency of donor microbiome applications. Several studies in IBD patients employed a single FMT, others performed daily or weekly repeated FMT. A recent meta-analysis of FMT in IBD revealed a trend to better success rates with a higher number of FMT, mostly in the form of enemas.<sup>51</sup> Thus a single FMT in patients with *C. difficile* colitis may be sufficient, but a prolonged therapy with daily FMT may be more beneficial in patients with chronic intestinal inflammation. Another important aspect of FMT is the microbial composition, the diversity and the microbiome metabolic profile of the donor feces. The beneficial effect of FMT derived from a unique donor (Donor B) in the Canadian UC FMT trial point towards donor specific microbiota quality such as diversity or specific bacterial products such as butyrate, which all may be key drivers of FMT therapeutic success.<sup>43, 44, 52</sup>

### Oral FMT

Just recently oral FMT in an encapsulated form has become available in the US (FMT capsule G3).<sup>53, 54</sup> This capsule was developed by OpenBiome, a non-profit stool bank. The company formulated a microbial emulsion matrix encapsulating rigorously screened and processed microbiota preparations ([www.openbiome.org](http://www.openbiome.org)). Initial trials indicate a high but slightly inferior efficacy in treating patients with *C. difficile* colitis with a single oral encapsulated FMT.<sup>53-56</sup> Data about the efficacy and safety of daily repeated oral FMT over longer time periods are currently not known, but weekly applications of 6 capsules in patients with Crohn's disease appear to be safe and well tolerated (OpenBiome, unpublished data). At this stage the encapsulated FMT offers an exciting possibility to explore the efficacy and safety of daily oral applications of fecal microbiota. In contrast to daily FMT enemas, as it has been performed in some studies, the oral FMT approach is also the patient preferred therapeutic modality, which increases compliance and the overall chance of therapeutic success.<sup>57</sup>

## **4. JUSTIFICATION AND OBJECTIVE OF THE CLINICAL TRIAL**

FMT for ADP is a promising approach, given the documented role of bacteria in the pathogenesis of this condition. However, whereas a single FMT in patients with *C. difficile* colitis seems to be sufficient to achieve a high therapeutic success rate, an intensified therapy with daily or repeated FMT to firmly establish the donor microbiome in the recipient appears to be the therapy of choice in patients with chronic intestinal inflammation (as outlined above). However, repeated endoscopic FMT is costly and not feasible. An alternative therapeutic modality is the repeated application of FMT via enemas. But this approach is suboptimal due a) patient preferences (oral > rectal), b) the occurrence of severe rectal pain during the application in patients with concomitant cuffitis and c) problems with retention of the enema content.<sup>58-60</sup> Thus the optimal approach is a combination of endoscopic FMT and consecutive therapy with oral FMT to help establish the donor microbiome in the host. With the recent availability of capsulated FMT this approach became feasible. The objective of this proof of concept trial is to evaluate the safety of endoscopic followed by oral FMT in patients with ADP and to estimate the effect size to be achieved from FMT therapy in patients with ADP for subsequent evaluation in a large definitive trial. A secondary objective is to study the microbial engraftment of donor FMT in the recipients.

## Specific hypothesis

We hypothesize that FMT is a novel therapeutic option for patients ADP. This form of pouchitis is predestined to benefit from FMT, since bacterial dysbiosis, which can only be controlled with antibiotics, appears to be the major driver of the clinical symptoms. We choose two distinct outcomes, a clinical and translational aim, to investigate the effect of FMT in patients with ADP.

Aim 1: Evaluation of safety, tolerability and clinical effectiveness (measured as clinical response or remission and discontinuation of antibiotic therapy) of FMT in patients with ADP.

Aim 2: Evaluation of the impact of FMT on the fecal bacterial microbiome in patients with ADP, which will provide functional data about possible mechanisms of this therapy.

- Safety profile of fecal transplant

Fecal transplant has an excellent safety profile in patients with *C. difficile* colitis. Also FMT for *C. difficile* colitis seems to be safe in immunocompromised patients.

In patients with IBD often mild flu symptoms after transplant have been observed.<sup>51</sup> However, these are only transitory and no organ failure or organ toxicity has been reported.

- Choice of Study Drug Dosage

The endoscopic FMT is an established method in patients with *C. difficile* colitis. The advantage of endoscopic rather than enema applied FMT is the possibility to install the FMT proximal to the pouch, which facilitates mucosal contact of the FMT not only in the pouch but also in the distal terminal ileum. However a single application most likely leads to an incomplete engraftment of the donor microbiome and repeated FMT is likely to have superior results as shown in several uncontrolled observations as well as in a large placebo controlled trial in patients with ulcerative colitis and a case series of 5 ADP patients.<sup>45, 49, 51, 61</sup> Repeated endoscopic FMT is not a feasible option. Oral FMT has been recently established and seems to have slightly inferior efficacy in patients with *C. difficile* colitis compared to endoscopic FMT.<sup>53, 55, 56</sup> Daily FMT using enema is another alternative, but in our opinion is suboptimal due to patient preferences, often pain during application of the enemas due to concurrent cuffitis and problems with retention in the setting of enema therapy.<sup>58-60</sup> Oral FMT offers the possibility to a superior engraftment and maintenance of the donor microbiome over longer time periods.<sup>58</sup> However, no data about such an approach are yet available. The only available data for patients with pouchitis and FMT are based on single FMT or repeated endoscopic FMT as outlined above.

## 5. TRIAL ENDPOINTS

- Primary Endpoint:

Safety and Tolerability of FMT

- Number of study patients, who discontinued therapy

- Adverse events grouped by body system
- Changes in laboratory values
- Secondary Endpoints
- Clinical remission (mPDAI score  $\leq 4$  points) and no need for antibiotic therapy at week 4.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 8\*.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 16\*.
- Endoscopic improvement of active pouchitis (decrease from baseline in mPDAI endoscopic subscore  $\geq 2$  points) at week 4.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 4.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 8\*.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 16\*.

\*Only patients who do not continue in open label extension

- Exploratory analyses (OL=Open label):
- Endoscopic improvement of active pouchitis (decrease from baseline in mPDAI endoscopic subscore  $\geq 2$  points) at in open label extension week 8.
- Clinical remission (mPDAI score  $\leq 4$  points and a decrease in the baseline mPDAI  $> 2$  points) and no need for antibiotic therapy at week 10 OL.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 14 OL.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 22 OL.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 10 OL.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 14 OL.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 22 OL.
- Symptomatic worsening of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) from FMT week 0 – week 16
- Quantitative changes in mPDAI from baseline values
- Quantitative changes of calprotectin from baseline
- Alterations in stool microbiome after FMT
- Engraftment of donor FMT
- Alterations in stool microbiome before and after FMT in study patients with antibiotic dependent pouchitis

Since the bacterial dysbiosis in the pouch plays an important role in the pathogenesis of pouchitis a secondary endpoint is the evaluation of the fecal microfloral pattern by 16S rDNA collected before and 2,4,8 and 16 weeks after FMT.

- Evaluation of calprotectin as a marker of pouch inflammation before and after FMT

Repeated pouchoscopies to evaluate the degree of pouch inflammation are not feasible. Also due to safety concerns, biopsies of the pouch will not be taken before FMT. Calprotectin, a member of the S100 family of calcium-binding proteins is abundant in the stool and its level correlates with the endoscopic degree of intestinal inflammation in patients with ulcerative colitis and pouchitis.<sup>62, 63</sup> Calprotectin can be readily quantified in feces using enzyme-linked immunosorbent assay (ELISA) or an immunoassay. We will collect stool samples for the measurement of calprotectin at the screening visit and on weeks 2, 4, 8 and 12 after FMT.

## 6. STUDY DESIGN

- Study Population

The study will include 20 patients who have undergone total proctocolectomy and ileal pouch-anal anastomosis (IPAA) for ulcerative colitis (UC). The study patient qualifies for the study if he/she has episode of active pouchitis, defined as a mPDAI  $\geq 5$  and a history of  $> 3$  antibiotic therapies for pouchitis in the last 12 months or if he/she is in need for chronic antibiotic therapy to maintain clinical remission and has history of at least 2 attempts in the last 24 months to stop continuous antibiotic therapy resulting in pouchitis episodes.

- Treatment period.

2 weeks of FMT/ placebo therapy followed by evaluation of clinical and endoscopic outcome at week 4, and clinical evaluation at week 8 and 16. In case the study patient does not achieve clinical remission at week 4 or experiences a flare of disease on day 15-28 after start of the study he/she will be offered the possibility to participate in open label extension with an additional endoscopic FMT followed by 2 weeks of oral FMT and clinical follow up at week 4, 8 and 16.

- Inclusion Criteria

- Signed informed consent.
- Man or woman between 18 and 70 years of age.
- IPAA after colectomy for ulcerative colitis
- The subject has been diagnosed with one of the following 2 pouchitis subtypes:
  - Active pouchitis, defined as a mPDAI  $\geq 5$  and a history of  $\geq 4$  antibiotic therapies for pouchitis in the last 12 months

or

- Need for ongoing antibiotic therapy ( $> 4$  weeks) to maintain clinical remission and a history of at least 2 attempts in the last 24 months to stop antibiotic therapy resulting in pouchitis episodes. Concomitant oral 5-ASA or local therapies containing 5-ASA (enemas, suppositories) or steroid enemas are permitted but not required but have to be stable for 4 (oral) or 2 weeks (topical) before inclusion in the study.



- Exclusion criteria
  - Treatment with biologics (e.g. infliximab, adalimumab, golimumab, vedolizumab)
  - Treatment with immunomodulators (azathioprine, 6-MP, methotrexate), steroids or any investigational drugs
  - Use of cholestyramine
  - Crohn's disease of the pouch
  - Known cytomegalovirus infection of the pouch
  - Clostridium difficile infection
  - Isolated moderate or severe cuffitis
  - Clinical significant strictures of the pouch inlet or outlet
  - Concurrent intestinal obstruction
  - History of familial adenomatous polyposis
  - History of uncontrolled lactose intolerance
  - History of confirmed (serological test and/or histology) celiac disease
  - Pregnancy, breast feeding, or planning to become pregnant during the trial
  - Non - steroidal inflammatory medications (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month
  - Dysphagia (oropharyngeal, esophageal, functional, neuromuscular)
  - History of recurrent aspiration episodes
  - Proven gastroparesis
  - Allergy to the following generally regarded as safe ingredients (GRAS): glycerol, acid resistant HPMC, gellan gum, cocoa butter, titanium dioxide
  - Adverse event attributable to previous FMT
  - Allergy/intolerance to pump inhibitor therapy
  - Any condition for which the investigator thinks the FMT treatment may pose a health risk (e.g. severely immunocompromised)
  - Participation in another clinical trial within the last 30 days, simultaneous participation in another clinical trial, or previous participation in this trial
  - During the trial period until one week after the trial end: Non-use of appropriate contraceptives in females of childbearing potential (e.g. condoms, intrauterine device {IUD}, hormonal contraception, or other means considered adequate by the responsible investigator) or in males with a child-fathering potential (condoms, or other means considered adequate by the responsible investigator during treatment)
  - Well-founded doubt about the patient's cooperation

- Concomitant Medications

All antibiotic therapies have to be stopped 1 day before FMT.

Following drug groups are permitted as concomitant medication:

- Orally administered mesalazine containing drugs should be stable for the last 4 weeks
- Local therapies containing 5-ASA (enemas, suppositories) or steroid enemas should be at a stable dose 2 weeks before inclusion in the study.

Following drug groups are **not** permitted as concomitant medication:

- Antibiotic therapy for treatment of pouchitis
- Cytostatics/immunosuppressants, cyclosporine, tacrolimus, mycophenolate mofetil.
- Anti-TNF therapies or anti-adhesion therapy.
- Non - steroidal anti-inflammatories (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month

## 7. OVERVIEW OF TRIAL SEQUENCE

*See also study flow chart in appendix as well as study visit charts in appendix.*

The trial will begin with the **screening visit (days -14 to 0)**. The investigator will ask patients who are interested in participating in the study after a careful explanation, to sign an informed consent form prior to study specific examinations. The study patient will receive a copy of the consent form, which outlines the sequence of events in brief and summarizes the advantages and disadvantages of participation in the study.

After the patient has given his/her written consent, the screening examination will be performed to evaluate whether the patient is probably eligible for the study.

At the screening visit, a complete history will be taken including concomitant medication and demographic data will be collected. A physical examination will be performed and vital signs will be documented. Blood samples (max. 20 ml) will be taken from the study patient (all study patients: hematology, serum chemistry, creatinine, female study patients: urine samples for pregnancy test). Also the study patient will be provided with a stool collection kit to provide a stool sample, which should be collected at this visit or before the week 0 visit. This stool kit will also contain a sample kit for *C. difficile* toxin. The study patient will also be provided with a diary, which he/she will be asked to complete the diary every day in the evening during the trial. It will be also evaluated if the patient is able to swallow the oral FMT test capsule.

At least 10 days before the planned endoscopic FMT at week 0 all patients will be started on antibiotic therapy as standard of care for treatment of pouchitis. Patients on continuous antibiotic therapy will continue their regimen. Since all patients must fulfil the criteria for antibiotic pouchitis, the antibiotic with the best efficacy to treat the pouchitis episodes in the past will be used. In patients on chronic antibiotic therapy to prevent pouchitis recurrence the antibiotic regimen will be continued. 24 hours before the planned FMT the antibiotic will be discontinued.

### *Placebo-controlled trial period*

At week 0 visit the study patient will have a physical exam and a urine pregnancy test. The patient's AE and symptom diary should be reviewed and the clinically modified PDAI calculated. The patient's concomitant medications will be reviewed. If the study patient fulfils all inclusion criteria and does not have an exclusion criterion the study patient will be randomized to either the placebo or the FMT group and a unique study ID number will be assigned, which will appear on all medication packs.

The study patient then undergoes a pouchoscopy for evaluation of the pouch inflammation and to rule out complications in the pouch structure (e.g. strictures, isolated cuffitis, which would lead to exclusion of the trial). If all inclusion criteria are met during an endoscopically guided placebo or real FMT will be performed. Additionally, the study patient will be started on omeprazole 20 mg (1 tablet/daily) as

long as he/she undergoes oral FMT or placebo therapy. The study patient will receive 2 diaries, which need to be filled out on a daily basis, the stool diary and the adverse event diary. The stool diary should be daily completed either until Visit 8 or if the patient participates in the open label extension until visit 12OL. The adverse event diary has to be daily completed until visit 6 or visit 10OL.

At visits 2 and 4 (phone visits) the coordinator will ask the study patient if adverse events occurred and will review the adverse event diary with the study patient. The coordinator will also evaluate the clinical components of the mPDAI of the last 3 days before the phone call (stool frequency, rectal bleeding, fecal urgency/ abdominal cramping, fever) as well as the concomitant medications.

Visit 3 (site visit) the coordinator will review the adverse event diary with the study patient, and will assess the mPDAI and concomitant medication. The patient will also bring a stool sample for the stool bank, which was collected earliest on day 1 after the end of the oral or placebo FMT period.

At phone visit 4 the coordinator will ask the study patient if adverse events occurred and review the adverse event diary. The coordinator will evaluate the clinical components of the mPDAI of the last 3 days before the phone call (stool frequency, rectal bleeding, fecal urgency/ abdominal cramping, fever) as well as the concomitant medications. If the patient is in remission according to the diary ( $mPDAI \leq 4$ ) and is on no concomitant medications, a pouchoscopy will be planned for site visit 5. If the study patient is not in remission ( $mPDAI > 4$ ) or had an early relapse after FMT the open label extension will be offered and the patient can be started on oral antibiotics again. For safety analyses a site visit 5 should be planned in the following 3-4 days. If the study patient chooses not to participate, he should be followed according the same schedule as patients who are in remission.

*Early termination visit due to flare on day 1-28 after FMT or due to adverse event or patient unwillingness to continue in study necessitating early termination*

If at any time between day 1-28 after endoscopic FMT the patient experiences recurrence of his pouchitis symptoms, he/she will contact the coordinator. The coordinator will ask the study patient if adverse events occurred and review the adverse event diary. The coordinator will evaluate the clinical components of the mPDAI of the last 3 days before the phone call (stool frequency, rectal bleeding, fecal urgency/ abdominal cramping, fever) as well as the concomitant medications.

If the study patient has active disease ( $mPDAI > 4$ ), an early termination study visit will be scheduled. A physical exam will be completed and, if possible, stool will be collected before the start of antibiotic therapy as standard of care. Blood labs will also be collected. The patient will be started on the same antibiotic, which he was on before entering the study. At the study visit the coordinator will review possible adverse events, evaluate the clinical components of the mPDAI of the last 3 days before the visit (stool frequency, rectal bleeding, fecal urgency/ abdominal cramping, fever) as well as the concomitant medications. If the patient wants to proceed into the open label study, he has to undergo at least 10 days of standard of care antibiotic therapy, before he can undergo the open label therapy with FMT (endoscopic and oral FMT).

If the patient is not willing to continue in the open label part of the study, he/she will be followed in the regular arm with visits 6-9.

*Study visits for patients at remission at phone visit 4 and site visit 5*

Phone visit 6 will evaluate for adverse events and review the adverse event diary. At this point (4 weeks after stopping the oral FMT) the patient can stop filling out the daily adverse event diary. However, the patient will be reminded to bring the completed adverse event diary to site visit 7. Additionally, the mPDAI will be calculated and the concomitant medications will be assessed.

Site visit 7 will collect all pages of the adverse event diary, and review the adverse events, which occurred since visit 6. A physical exam and blood tests will be performed and the mPDAI will be calculated. The concomitant medications will be assessed. A stool sample will be collected from the patient.

Site visit 8 will review new adverse events, concomitant medications and the mPDAI score. A stool sample will be collected from the patient.

On the last phone visit 9 the coordinator will review serious adverse events only, which may have occurred since visit 8.

#### *Open label extension period*

After at least 10 days of antibiotic therapy a pouchoscopy and FMT will be performed at site visit 6OL. A physical exam will be completed, adverse events and concomitant medications reviewed. Female patients will provide a urine sample for a pregnancy test before FMT. Similar to the previous FMT the study patient will be started on omeprazole 20 mg (1 tablet/daily) as long as he/she undergoes oral FMT therapy.

In phone visit 7OL the coordinator will ask the study patient if adverse events occurred, review the adverse event diary, and will evaluate the clinical components of the mPDAI of the last 3 days before the phone call (stool frequency, rectal bleeding, fecal urgency/ abdominal cramping, fever) as well as the concomitant medications.

At site visit 8OL the study coordinator will ask the study patient if adverse events occurred, review the adverse event diary, and will evaluate the clinical components of the mPDAI of the last 3 days before the visit (stool frequency, rectal bleeding, fecal urgency/ abdominal cramping, fever) as well as review the concomitant medications. The coordinator will collect the stool sample from the patient, which should be collected 1-3 days after the end of the oral FMT therapy .

The 9OL site visit will review adverse events and the adverse event diary, collect safety blood labs, perform a physical exam and calculate the mPDAI. A stool sample for the stool bank will be collected from the patient. A pouchoscopy will also be performed.

Phone visit 10OL will evaluate for adverse events and review the adverse event diary. At this point 4 weeks after stopping the oral FMT the patient can stop filling out the daily adverse event diary. The patient will be reminded to bring the filled out adverse event diary to site visit 11OL. Additionally, mPDAI will be calculated and the concomitant medications will be assessed.

Site visit 11OL will collect all pages of the adverse event diary, and review the adverse events, which occurred since visit 10OL. A physical exam and blood tests will be performed and the mPDAI will be calculated. The concomitant medications will be reviewed. A stool sample for the stool bank will be collected from the patient.

Site visit 12OL will review new adverse events, concomitant medications and the mPDAI score. A stool sample for the stool bank will be collected from the patient.

On the last phone visit 13OL the coordinator will review serious adverse events only, which may have occurred since visit 12OL.

*Study patients experiencing a recurrence of symptoms in the open label extension period*

If the study patient is experiencing worsening of disease after the second FMT, antibiotic therapy will be re-initiated as a standard of care approach. The patient will nevertheless be followed up according to the same schedule as patients not experiencing a relapse.

## **8. SCREENING VISIT AND STUDY VISITS 1-4**

- Site screening visit

*Data to be recorded:*

General parameters:

Initials

Date of birth

Sex

Smoker (yes/no/ex-smoker)

Race (NIH categories)

Ethnicity (NIH categories)

*Case history*

Date of confirmation of the diagnosis

Classification of UC before IPAA (proctitis, left sided colitis, pancolitis)

Type of extraintestinal symptoms

Specific therapies for pouchitis within the last year

Type of medication

Specific therapies for pouchitis within the last 4 weeks

Type of medication

*Examinations*

Vital signs: blood pressure (mm Hg), heart rate (min<sup>-1</sup>), weight (kg), calculation of BMI, temperature

Height (cm)

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

*Blood sampling and measurement of laboratory parameters*

- Hematology
- Serum chemistry
- Urine pregnancy test
- Stool sample for C. difficile toxin

Formal aspects:

Informed consent procedure

Verification of inclusion / exclusion criteria (eligibility check), as far as possible

Dispense stool collection kit for stool sample (stool sample should be brought back at visit week 0)

Explain and distribute stool diary to study patient

Perform test if patient is able to swallow an empty FMT test capsule

- Visit 1: Site visit week 0

Examinations

Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg), temperature

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

*Blood sampling and measurement of laboratory parameters (must be performed in the 24 hours before FMT)*

- Urine pregnancy test

Dispense stool collection kit for stool sample (stool sample should be brought back at visit week 2)

Formal Aspects:

Calculation of the mPDAI

Verification of inclusion / exclusion criteria (eligibility check)

Randomization

Dispense oral FMT or placebo

Dispense omeprazole 20 mg (1 tablet) daily for 14 days during oral FMT

Dispense PDAI diary

Dispense adverse events diary

Pouchoscopy: Evaluation of endoscopic PDAI and perform “blinded” (real or placebo) FMT.

- Visit 2: Phone visit week 1 (7 (+1 day) days after endoscopic FMT /placebo and on oral FMT/placebo)

Formal aspects:

Adverse events (review adverse events diary)

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

- Visit 3: Site visit week 2 (15 +3 days after endoscopic FMT /placebo and on oral FMT/placebo)

Formal aspects:

Adverse events (review adverse events diary)

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

Reminder stool collection on day 15, 16 or 17 (day 1-3 after end of oral FMT) and bring in stool.

- Visit 4: Phone visit week 4 (2-3 days before planned week 4 site visit)

Formal aspects:

Adverse events (review adverse events diary)

Concomitant medication

Reminder to bring stool day 25, 26 or 27.

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

*If patient is in clinical remission (mPDAI  $\leq$ 4) and no need for antibiotics plan for pouchoscopy week 4*

*If patient is not in clinical remission ( $mPDAI > 4$ ) or need for antibiotics or comes in due to exacerbation of symptoms with possibility to continue with open label extension. Need to restart 10 day course of antibiotics as standard of care similar to initial antibiotic treatment before the first FMT*

- Visit 5: Site visit Week 4 (day 28 +/- 3 days after first transplant) or early termination visit due to flare (day 1-28 after first transplant)

#### Examinations

Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg), temperature

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

#### Measurement of laboratory parameters

Hematology  
Serum chemistry

Stool collection: Bring to visit.

#### Formal aspects:

Return of study medication (empty containers)

Compliance assessment (count of empty containers returned)

Concomitant medication

Adverse events (review adverse events diary)

Calculation of mPDAI score (before pouchoscopy)

Pouchoscopy (in patients in clinical remission and no need for antibiotics only): Evaluation of endoscopic PDAI.

*If patient is in clinical remission ( $mPDAI \leq 4$ ) and no need for antibiotics proceed to pouchoscopy*

*If patient is not in clinical remission ( $mPDAI > 4$ ) or need for antibiotics or comes in due to exacerbation of symptoms with possibility to continue with open label extension. Need to restart 10 day course of antibiotics as standard of care similar to initial antibiotic treatment before the first FMT*



In case of **premature withdrawal** from the study (yes, no)

If yes, reason:

- Intolerance of medication
- Lack of efficacy (need for steroids e.g. increase of steroids or re-introduction of steroids after previously successful taper)
- Severe adverse event
- Lack of patient's cooperation
- Inclusion criterion not fulfilled or exclusion criterion fulfilled coming to knowledge after recruitment
- Other reason

## **9. FOLLOW-UP VISITS OF PATIENTS IN REMISSION AT WEEK 4 OR NOT PARTICIPATING IN THE OPEN LABEL EXTENSION**

- Visit 6: Phone visit week 6 (42 +/- 3 days after first transplant)

### Formal aspects:

Adverse events (review adverse events diary, last day of filling out the diary is 28 days after end of oral FMT, which is around week 6; patient needs to be advised to bring filled out diaries to visit 7)

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

- Visit 7: Site visit week 8 (56 +/- 5 days after first transplant)

### Examinations

Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg), temperature

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

Measurement of laboratory parameters

Hematology  
Serum chemistry

Stool collection: Bring to clinic.

Formal aspects:

Concomitant medication  
Adverse events (adverse events diary filled out until visit 6)  
Calculation of mPDAI score  
Reminder stool collection and bring in stool week 16.

Collect adverse event diary (filled out until week 6)

- Visit 8: Site visit week 16 (112 +/- 7 days after first transplant)

Formal aspects:

Adverse events  
Concomitant medication  
Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

Stool collection: Stool collection

- Visit 9: Phone visit week 24 (168 +/- 10 days after first transplant)

Formal aspects:

Review if serious adverse events occurred in previous 8 weeks

## **10. FOLLOW UP VISITS OF PATIENTS PARTICIPATING IN OPEN LABEL EXTENSION AFTER WEEK 4**

- Visit 6OL: Site visit week 6 (week 0 OL)

**This visit should be performed after the patient completed at least 10 days of antibiotics**

### Examinations

Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

### Formal aspects:

Concomitant medication

Calculation of mPDAI score (before pouchoscopy)

Dispense oral FMT capsules

Dispense omeprazole 20 mg (1 tablet) daily for 14 days during oral FMT

Dispense PDAI diary

Dispense adverse events diary for OL follow-up phase

### Blood sampling and measurement of laboratory parameters (be performed in the 24 hours before FMT)

- Urine pregnancy test

Pouchoscopy: Evaluation of endoscopic PDAI and perform FMT.

- Visit 7OL: Phone visit week 7 (week 1 OL; 7 days (+1 day) after second transplant)

Formal aspects:

Adverse events (review adverse events diary)

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

- Visit 8OL: Site visit week 8 (week 2 OL, 15 + 3 days after second transplant)

Formal aspects:

Adverse events (review adverse events diary)

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

Reminder stool collection on day 15, 16 or 17 (day 1-3 after end of oral FMT) and bring in stool.

Plan for pouchoscopy visit 9OL

- Visit 9OL: Site visit week 10 (week 4 OL, 28 +/- 3 days after second transplant)

Examinations

Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

Measurement of laboratory parameters

Hematology

Serum chemistry

Stool collection: Bring in.

Formal aspects:

Concomitant medication

Adverse events (review adverse events diary)  
Calculation of mPDAI score  
Pouchoscopy: Evaluation of endoscopic PDAI.

- Visit 10OL: Phone visit week 12 (week 6 OL; 42 +/- 3 days after second transplant)

Formal aspects:

Adverse events (review adverse events diary, which needs to be filled out 28 days after the last FMT, which is week 6 OL)

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

- Visit 11OL: Site visit week 14 (week 8 OL; 56 +/- 5 days after second transplant)

Examinations

Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)

Physical examination:

- |                                 |   |
|---------------------------------|---|
| - Head (including ENT and eyes) | - Gastrointestinal tract, liver, spleen |
| - Lymphatic system              | - Urogenital system                     |
| - Endocrine system              | - Nervous system                        |
| - Lungs and respiratory tract   | - Muscular and skeletal system          |
| - Peripheral vascular system    | - Skin and connective tissue            |
| - Cardiovascular system         | - Other                                 |

**each: actual status: Present finding / normal condition / not examined**

Measurement of laboratory parameters

Hematology  
Serum chemistry

Stool collection: Bring in.

Formal aspects:

Concomitant medication

Adverse events

Calculation of mPDAI score

Reminder for stool collection week 16

Collect adverse event diary (filled out until week 6OL)

- Visit 12OL: Site visit week 22 (week 16 OL; 112 +/- 5 days after second transplant)

Formal aspects:

Adverse events

Concomitant medication

Calculation of clinical mPDAI (stool frequency, rectal bleeding, fecal urgency/abdominal cramping, fever)

Stool collection: Stool collection

- Visit 13OL: Phone visits week 30 (week 24OL 168 +/- 10 days after second transplant)

Formal aspects:

Review if serious adverse events occurred in previous 8 weeks

## **11. POUCHOSCOPY AND LABORATORY PARAMETERS**

- Pouchoscopy (Screening, visit 5 or visit 6OL)

Pouchoscopy will be performed to evaluate the pouch, prepouch ileal segment and the cuff. The pouch and prepouch inflammation will be graded according mPDAI (see attachment).

- Laboratory parameters

All laboratory analyses will be carried out in at the local laboratory of the investigational site.

*Acute phase reactants (nonspecific inflammatory markers)*

C-reactive protein (CRP)

*Hematology:*

Blood count (erythrocytes, hemoglobin, hematocrit, leukocytes, platelets)

*Serum chemistry:*

Kidney function: creatinine

Liver function (alanine aminotransferase {ALT}, aspartate aminotransferase {AST}, alkaline phosphatase, bilirubin)

Urine pregnancy test screening visit and in the 24 hours before FMT

## **12. RECORDING OF COMPLIANCE**

Compliance will be assessed by checking the study medication (containers) returned at the follow-up visits and the final visit by the investigator.

All returned containers will be counted by the study coordinator and the number will be documented in the CRF.

## **13. ADVERSE EVENTS**

- Definitions

### Adverse events (AEs)

Adverse events (AEs) will be recorded at each regular scheduled study visit or study phone contact in the study patient record (source document) as well as on a specific AE form on the CRF.

An AE is any untoward medical occurrence in a study patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product, e.g.:

- any new diagnosis
- any symptom that requires medical clarification or leads to in-patient admission (surgery or accident)
- any suspected adverse drug reaction (ADR)
- any symptom that appears on the study patient's medical records
- any event related in time with the application of the study medication and affecting the health of the study patient (including laboratory value changes)

### Serious Adverse Events (SAE)

A serious adverse event (experience) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity

Non-serious adverse events are all AEs that do not fall into any of the above categories.

### Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction (ADR) is an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

### Expected Adverse Drug Reactions

Fecal transplant has an excellent safety profile in patients with *C. difficile* colitis. Also FMT for *C. difficile* colitis seems to be safe in immunocompromised patients.<sup>64, 65</sup>

In patients with IBD mild flu symptoms, abdominal pain and diarrhea after transplant have been observed.<sup>51</sup> However, these are only transitory and no organ failure or organ toxicity has been reported.

## **14. DOCUMENTATION AND REPORTING OF ADVERSE EVENTS**

The study patients will be instructed to contact the investigator, if any serious or unexpected AE occurs, so that appropriate action can be taken.

Moreover, the investigator must ask at each follow-up visit a generally worded question without searching for any special symptoms, e.g. "Has your state of health worsened since we last met?" If the answer to this question is "no", no further questions will be asked. If the answer to the question is "yes", the investigator will document the nature, time, severity, seriousness and duration as well as the causality of the AE. For each AE a specific AE documentation form will be provided, which should be completed by the PI as an initial report. The report must contain a detailed description of the symptoms observed and the concomitant treatment administered. Furthermore, the investigator must comment on a possible causative relationship between the AE and the trial medication. Each AE must be followed until it is resolved or can be explained satisfactory.

The following has to be documented for each AE:

- Nature of the event
- Time of onset: date, time
- Concomitant treatment: product (generic name), indication, dosage, dosage interval, presentation, mode of administration, administration regimen
- Duration of the AE
- Severity
- Seriousness
- Causality
- Outcome

### **Severity**

The severity is evaluated as follows:

1. Mild: - event/symptom does not interfere with normal daily activities
2. Moderate: - event/symptom interferes with normal daily activities



3. Severe: - event/symptom prevents normal daily activities

### **Causality**

The relationship between an AE and the study medication is classified according to the WHO classification:

#### **Certain**

A clinical event, including laboratory test abnormality, is occurring in a plausible time relationship to drug administration, and which concurrent disease or other drugs or chemicals cannot explain. The response of the study patient to withdrawal of the drug should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

#### **Probable/Likely**

A clinical event, including laboratory test abnormality, with a reasonable time sequence to the administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de challenge). Rechallenge information is not required to fulfil this definition.

#### **Possible**

A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

#### **Unlikely**

A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

#### **Conditional/Unclassified**

A clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data are essential for a proper assessment or the additional data are under examination.

#### **Not assessable / Unclassifiable**

A report suggesting an adverse reaction, which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

As an alternative to the above-mentioned WHO assessments, the following assessment can be made:

#### **Not related**

There is sufficient information available to show that the etiology is unrelated to the study medication.

#### **Measures at the onset of adverse events**

Measures at the onset of adverse events are classified and described as follows:

- 1) None, i.e. the study medication was not changed
- 2) The dose of the study medication was reduced
- 3) The study medication was withdrawn and/or
- 4) Other measures (clear text)

The **course** and **outcome** of the adverse event will be commented on as follows:

- Recovered without sequelae
  - Not yet recovered
  - Recovered with sequelae
  - Fatal
- 
- Documentation and Reporting of Serious Adverse Events

On enrolment in the study, the study patients will be instructed to contact the investigator if a serious or unexpected AE occurs, so that appropriate measures can be taken.

**Any SAE (including death, irrespective of the cause) occurring during or for up to 14 days after the end of the study will be immediately reviewed by the PI, i.e. within 24 hours.**

A specific SAE documentation form will be provided. In case of an SAE, this form will be completed and reviewed by the PI as an initial report, and if the SAE is judged to be possible related or related to study drug will be sent (via fax) to the IRB of the University of North Carolina. The report must contain a detailed description of the symptoms observed and the concomitant treatment administered. Furthermore, the investigator must comment on a possible causative relationship between the AE and the trial medication. Each SAE must be followed until it is resolved or can be explained satisfactorily.

The general procedure for the observation, collection and analysis of drug risks (regulatory affairs) in conformity with the appropriate national Drug Law shall apply without qualification.

In accordance with drug safety and national requirements, the study PI will inform the IRB of the study at the University of North Carolina and will make sure that the involved persons will obtain adequate information.

The following instructions must be heeded:

In the case of an intolerable SAE, the study patient must, at the decision of the investigator, be withdrawn from the clinical trial, and symptomatic treatment must be administered. The measures taken must be recorded on the CRF.

In accordance with local legislation, the investigators will submit copies of the final SAE-report to the Regulatory Authorities concerned, if necessary.

## **15. ABNORMAL LABORATORY RESULTS**

All laboratory values outside of the normal range will be repeated as judged appropriate to ensure the validity of the abnormal result. The investigator will document all laboratory values on the relevant page of the CRF and will assess the etiology of the clinically relevant abnormal laboratory values.

Clinically relevant abnormal laboratory values are only documented on the AE form of the CRF, if the study patient is discontinued, hospitalized, or, in the investigator's opinion, it should be considered as an AE.

Abnormal laboratory results caused by ulcerative colitis should not be reported on the AE form.

## 16. WITHDRAWAL CRITERIA

- Criteria in individual cases

Any study patient can withdraw from the study at any time without personal disadvantages and without having to give a reason. The time of withdrawal, the results available up to that time, and, if known, the reason for withdrawal must be documented on the CRF.

The investigator can also discontinue the study after considering the risk-to-benefit ratio, if he/she no longer considers the further participation of the study patient justifiable. The date of and the primary reason for the withdrawal as well as the observations available at the time of withdrawal are to be documented on the CRF.

Reasons leading to the withdrawal of a study patient can include the following (primary reason must be determined):

- Development of a SAE that is thought to be likely secondary to study drug or study intervention.
- Development of an AE with  $\geq$  grade 3 toxicity, using the FDA toxicity grading scale for preventive vaccines (

If the PI or DSMB believes it is no longer in the subject's best interest to continue participation in the study. Examples include:

- A serious or unexpected adverse event
- Serious inter-current illness, or
- Progression of disease that requires alternative treatment.
- The subject no longer meets the inclusion criteria.
- Lack of compliance with study procedures or study treatment, as determined by the PI.

In all study patients who finish the study prematurely, a withdrawal examination should be carried out within 3 weeks after the last application of the study medication. The withdrawal examination must be conducted as a final examination and documented in the CRF. It is most important to calculate at least the final mPDAI score for each study patient. If possible, an endoscopy with biopsies according to the protocol should be performed and blood samples for hematology and serum chemistry should be taken.

The investigator will continue to observe study patients withdrawn from the study because of intolerable AEs until the findings have been clarified.

- Criteria for the termination of the whole study

If serious safety concerns arise, the coordinating investigator can terminate or interrupt the study by agreement with the sponsor and the DSMB. If new information on the risk-to-benefit ratio of the drug or on the treatment methods used in the study is obtained in the meantime, the coordinating investigator reserves the right to interrupt or terminate the project by agreement with the sponsor. Premature termination is also possible if the coordinating investigator, or the investigators and the sponsor if study patient recruitment is insufficient and cannot be expedited by appropriate measures.

The study should also be stopped or paused until thorough review of all safety data by the DSMB for the following:

- An SAE occurs in at least one subject in which the SAE is determined to be possibly, probably, or definitely related to test treatment.

- The same grade 3 or higher AE assessed as possibly, probably, or definitively related, occurring in two or more subjects, using the FDA toxicity grading scale for preventive vaccines.

## **17. DATA SAFETY MONITORING BOARD (DSMB) AND SAFETY OFFICER**

The DSMB will consist of at least external 3 members. Additionally one independent medical safety officer will monitor the study. On a monthly basis, the Data Management Center (DMC) will issue standard safety reports (enumerated by event and category, both by total and by type) as well as a cumulative report of all Adverse Events (AEs) to include Event Code, Dates of Onset/Resolution, Grade, and Outcome to the an independent safety officer. In addition, these standard safety reports will be presented to the DSMB at each conference. The DSMB will review these data and make appropriate safety decisions based on these events. Participants will be monitored for potential AEs, including signs or symptoms related or unrelated to the condition under study, any clinically significant laboratory abnormality, or any abnormality detected during physical examination.

## **18. RANDOMIZATION, DATA MANAGEMENT AND DATA MONITORING**

The randomization of the study patients and the processing and analysis of the data will be carried out by the Biostatistics core of the Center for Gastrointestinal Biology and Disease at UNC Chapel Hill.

Study data for this study will be collected and stored using electronic records. Data captured will be entered in real time at each clinical site using web forms developed to replicate paper case report forms. All data will be created, modified, maintained, archived, retrieved and distributed by a computer system. The use of electronic records will increase the speed of data collection and exchange. This will reduce the manpower necessary to perform double-data entry from paper forms and transcription error. In addition, electronic records permit economical storage of study data and ease of accessibility and analysis. Data management and data quality systems will be built into the system.

Data quality using electronic records will ensure that data are attributable, legible, contemporaneous and original.

The DMC at the CGIBD at the University of North Carolina will track the data collection, provide data security, control for confidentiality of study data, maintain computer backups to protect data until study closure and archive study data according to FDA requirements (21 CFR 11). Electronic signatures will be linked to each entry.

All computer systems and programs will be password protected, and all electronic communications of study and other confidential information will be encrypted. Personnel at the CGIBD have extensive training and experience using electronic data systems. Good computer security practice (restricting physical access to machines, prohibition of password sharing, and logging off computers after work hours or when away from the machine) will be required of all study personnel.

Standard Operating Procedures exist for users of the DMC. Only authorized persons are authorized for data entry and access. Data security systems require password protected identification codes for data entry and provide protection against data manipulation. The database is located on a server protected by firewalls. Access to the database server will not be allowed by users on computers outside of the firewall-

protected zone. Virus protection software is installed on each study machine. System access to computer systems will be audited. Redundant backups and off-site backup storage will allow for quick restoration of data in the unlikely event that a hardware failure, disaster, or security breach should occur. Servers and backups will be located in a secured location with access limited to authorized personnel.

Data cleaning will include range and edit checks, cross form edit checks, query generating and tracking and periodic data status reports. Any data errors or inconsistencies detected after data entry will be automatically tracked, communicated and resolved using a web-based application. An audit trail of all data changes over the life of the study will be maintained. All study raw data, forms, documents, software programs, software applications and computer data files will be indexed and archived routinely. Strict version control of documents and software applications will be instituted. Retention of study documentation after study completion will conform to FDA and NIH requirements.

Standardized study management reports will be generated monthly during the recruitment phase of the study. These reports will be used to track study progress including study patient enrollment, randomization, compliance, study patient status changes, and study events. The data will be reported for each Study Center individually and summarized for the study as a whole. Every six months, a standardized report will also be generated for the DSMB meeting. This report will include additional information on clinical events and adverse events that is coded by blinded treatment group. Other than the study statistician and statistical analyst, no study personnel will see this report.

## **19. MODIFIED POUCHITIS DISEASE ACTIVITY INDEX (PDAI)**

The Pouchitis Disease Activity Index (PDAI) was developed by Sandborn et al for diagnosing active pouchitis and quantifying the severity of pouchitis <sup>66</sup>. Shen et al. later on modified the index (mPDAI) and showed that similar outcomes can be measured after omitting the evaluation of histology, thus omitting the biopsy and histology costs.<sup>67</sup> The PDAI or the modified PDAI has been used in a number of clinical studies where it appears quite useful in discriminating between subjects with and without pouchitis and in quantifying the severity of pouchitis <sup>20, 22, 25, 27-29, 68-70</sup>

- **Subject Data Collected via Diary Card**

Subjects will be instructed to complete the diary at the same time each night before going to bed. The following information will be collected daily using a subject completed diary card:

- Current daily stool number via the question, “During the past 24 hours how many bowel movements did you have?” (Provide number)
- Rectal bleeding via the question, “During the past 24 hours, what percentage of your bowel movements contained blood?” a. 0% (none); b. <20% (rare); c. ≥20% (usual)
- Fecal urgency/abdominal cramps via the question, “During the past 24 hours what percentage of your bowel movements were preceded with abdominal cramps or an urgent need to go to the bathroom?” a. 0% (none); b. <50% (occasional); c. ≥50% (usual)
- Fever defined as temperature >37.8°C via the question, “During the past 24 hours have you experienced a fever higher than 37.8°C (100.0°F) (Yes/No)

Please note that responses to the rectal bleeding and fecal urgency questions are each associated with a word in parentheses. These words are the ratings that are used on the mPDAI. For example, if on a given day a subject responds that their percentage of bowel movements that had blood present was <20% and the percentage of bowel movements that were preceded by abdominal cramps was 0%, then their

rectal bleeding and fecal urgency/abdominal cramps mPDAI ratings would be “rare” and “none”, respectively.

Diary cards will be distributed to pouchitis subjects only at the screening visit and week 0 visit and should be reviewed with the study patient at visit week 0 and week 4, respectively, to ensure that all headers are completed and that entries for all questions are made for each day of the study period. Subjects should not be asked to provide answers retrospectively to unanswered diary questions but can be counselled on the importance of providing answers to all questions on each study day during the treatment period.

- Calculating the mPDAI

At the screening visit subjects will be questioned regarding their post-IPAA daily stool frequency. This number will represent a subject’s “normal” stool frequency after undergoing IPAA. Current stool frequency as well as current symptoms of stool blood, fecal urgency and cramps, and fever will be determined from the subjects’ daily diaries. To calculate the subjects’ baseline mPDAI scores, symptom data recorded in the subjects’ diaries from the three days prior to Visit week 0 will be used. To calculate their mPDAI score, the investigator will question the subject on their stool frequency, percentage of stools with blood, percentage of bowel movements associated with urgency and cramping, and fever, over the three days prior to their visit. Their responses to these questions will be used to calculate the clinical subscore of the mPDAI. To calculate the Month 1 mPDAI scores symptom data recorded in the subjects’ diaries from the three days prior to Visit week 4 will be used. If a subject fails to provide symptom responses for one of more days of the three days prior to Visit the three days prior and closest to the visit date for which symptom data was recorded will be used to calculate the clinical subscore of the mPDAI. Active pouchitis is defined as a total mPDAI score  $\geq 5$  points.

### Pouchoscopy

During the endoscopic examination of the pouch, the presence or absence of the following endoscopic findings will be noted: edema; granularity; friability; loss of vascular pattern; mucous exudate; and ulceration. No biopsies will be taken in the context of the study.

### **Stool frequency**

The mean stool frequency will be calculated for the three evaluation days. The subject’s “normal” stool frequency will be subtracted from the mean stool frequency and the result will be evaluated against the stool frequency categories on the mPDAI. The appropriate mPDAI stool frequency score will be assigned.

### **Rectal bleeding**

The rectal bleeding frequency will be calculated for the three evaluation days. The relative frequency for rectal bleeding will be calculated based on the total number of stools passed over the same three evaluation days ((total #stools with blood/total #stools)\*100). The relative frequency will be evaluated against the following categories:

0% (none) < 20% (rare) > 20% (present daily)

Please note that the words, “none”, “rare”, and “present daily” in parentheses, correspond to the rectal bleeding categories on the mPDAI. These categories will be used to assign a mPDAI score for rectal bleeding.

## **Fecal urgency/abdominal cramping**

The fecal urgency/abdominal cramping frequency will be calculated for the three evaluation days. The relative frequency for fecal urgency/abdominal cramping will be calculated based on the total number of stools for the same three evaluation days ((total #stools with cramping or urgency/total #stools)\*100). The relative frequency will be evaluated against the following categories:

0% (none) < 50% (occasional) > 50% (frequent)

As with rectal bleeding, the words, “none”, “occasional”, and “frequent” in parentheses, correspond to the fecal urgency/abdominal cramping categories on the mPDAI. These categories will be used to assign a mPDAI score for fecal urgency/abdominal cramping.

## **Fever**

If a subject indicates that they had a fever on none of the three evaluation days, the mPDAI score for “absent” will be recorded. If a subject indicates that they had a fever on one or more of the three evaluation days, the mPDAI score for “present” will be recorded.

## **20. STATISTICAL ANALYSIS**

The following endpoints will be assessed

- Primary Endpoint:

Safety and Tolerability of FMT

- Number of study patients, who discontinued therapy
- Adverse events grouped by body system
- Changes in laboratory values
- Secondary Endpoints      Clinical remission (mPDAI score  $\leq 4$  points and a decrease in the baseline mPDAI  $> 2$  points) and no need for antibiotic therapy at week 4.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 8\*.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 16\*.
- Endoscopic improvement of active pouchitis (decrease from baseline in mPDAI endoscopic subscore  $\geq 2$  points) at week 4.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 4.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 8\*.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 16\*.

\*Only patients who do not continue in open label extension

- Exploratory analyses (OL=Open label):
- Endoscopic improvement of active pouchitis (decrease from baseline in mPDAI endoscopic subscore  $\geq 2$  points) at in open label extension week 8.

- Clinical remission (mPDAI score  $\leq 4$  points and a decrease in the baseline mPDAI  $> 2$  points) and no need for antibiotic therapy at week 10 OL.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 14 OL.
- Clinical remission (clinical mPDAI score  $\leq 4$ ) and no need for antibiotics at week 22 OL.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 10 OL.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 14 OL.
- Response: Symptomatic improvement of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) and no need for antibiotic therapy at week 22 OL.
- Symptomatic worsening of active pouchitis (decrease from baseline in mPDAI clinical subscore  $\geq 2$  points) from FMT week 0 – week 16
- Quantitative changes in mPDAI from baseline values
- Quantitative changes of calprotectin from baseline
- Alterations in stool microbiome after FMT
- Engraftment of donor FMT

### Clinical analyses

The primary endpoint is the evaluation of safety and tolerability of FMT in patients with ADP. Secondary endpoints are endoscopic and clinical remission at week 4 in conjunction with no need for antibiotic therapy, clinical response and remission and no need for antibiotics at week 4, 8 and 16, endoscopic improvement of pouchitis and quantitative changes of calprotectin after the initial FMT. All subjects with missing data for determination of endpoint status will be considered as a non-responder in the intention-to-treat analysis. For dichotomous outcomes we will compute the percentage of subjects having the event along with a 95% confidence interval. For continuous outcomes we will compute the mean and a 95% confidence interval for the mean as well as descriptive statistics such as median, range and upper and lower quartiles. In addition to computing these measures for all 20 subjects, we will compute them for subgroups of interest (e.g. endoscopic active vs inactive ADP). These estimates can then be used in the planning of a larger, hypothesis-based study.

- Sample size calculation

This trial is designed as a proof-of-concept study of FMT therapy, not a definitive evaluation of FMT therapy in patients with ADP. The goal is to evaluate the safety and feasibility of such a trial, as well as to estimate the effect sizes in placebo and FMT therapy groups in order to accurately estimate the sample size needed for subsequent evaluation in a larger definitive trial. Nonetheless, we estimated the minimum effect size this pilot study can detect. We assume a placebo remission rate (defined as clinical remission and no need for antibiotics) of 10%. The low placebo response rate is based on the inclusion criterion, which selects only patients needing continuous or recurrent antibiotic therapy in the setting of ADP. With a follow-up of 16 weeks with the requirement of clinical remission without antibiotic therapy at end of follow-up, a placebo response rate beyond 10% is highly unlikely. The recent case series of Stallmach et al. suggests an antibiotic-free remission rate of 80% and a response rate of 100% in the setting of repeated FMT.<sup>49</sup> With 20 patients (1:1 randomization), and a placebo response rate of 10%, we will have 80% power to detect an effect difference of 67.5%, that is, an antibiotic-free remission rate



of 77.5% in the treatment group (2-sided,  $\alpha=0.05$ , Fisher-Irwin's exact conditional test). Thus, even with this small proof-of-concept design, we could observe a significant effect in the range of effect size reported in recent case series.

### Microbiome Analyses

From each patient 1 stool sample will be collected before transplant and twice after the transplant (week 3 and 4). Patients in remission will collect 2 more stool samples in week 8 and 16. Patients in the open label extension trial will collect stool samples after transplant in week 8, 10, 14 and 22. Assuming that 9 patients enter remission in the placebo controlled phase ( $9 \times 4 = 36$  samples) and 11 patients enter the open label extension ( $11 \times 7 = 77$  samples) a total of 116 samples will be analysed. All microbiome analyses will be conducted by OpenBiome, which will donate time and materials for the analyses. The gut microbiome of donors and patients will be analysed through deep sequencing of the 16S ribosomal RNA gene amplified from stool samples. Microbial engraftment will be operationalized through the donor similarity index (DSI) used to evaluate the degree of engraftment in FMT recipients over time (after 2, 4, 8 and 16 weeks).<sup>61</sup> We will measure DSI over time to elucidate the stability of engraftment and its relationship to clinical observations. In addition to 16S rRNA sequencing, we will save aliquots of stool for metabolomic characterization. In particular, given the potential role of butyrate in ameliorating inflammation, we're especially interested in measuring short chain fatty acid levels in stool before and after FMT. We plan to collect stool samples for characterization at baseline and at 2, 4, 8 and 16 week intervals after treatment.

## 21. STUDY VISITS CHART

### 1.1 STUDY VISITS CHART PLACEBO CONTROLLED TRIAL SCREENING – WEEK 4 (SITE VISIT)

Visit	Screening	1	2	3	4	5	Early termination <sup>g</sup>
Type of visit	Site	Site	Phone	Site	Phone	Site	Phone/site
Day	-14	0	7 +/-1	15 +3	26 +/-1	28 +/-3	
Week	-2	0	1	2	4	4	
Medical History	X						
mPDAI score		X	X <sup>d</sup>	X <sup>d</sup>	X <sup>g(l)</sup>	X	X
Concomitant Medication	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X
Physical Exam	X					X	X
Blood Tests	X					X	X
Pregnancy Test (urine)	X	X					
Test FMT capsule	X						
Dispense FMT capsules		X					
10-14 day antibiotic therapy pre-transplant	X				X <sup>c</sup>	X <sup>c</sup>	X <sup>c</sup>
Stool sample	X <sup>e</sup>			X <sup>f</sup>	X <sup>f</sup>	X <sup>g</sup>	X
Sigmoidoscopy (Pouchoscopy)		X				X <sup>a</sup>	
Endoscopic FMT		X					
Distribute/Review Subject Diaries <sup>b</sup>	X	X	X	X	X	X	X
Distribute/Review Subject adverse events diary <sup>b</sup>			X	X	X	X	X

Study visits Chart for patients continuing in observation phase

Visit	6	7	8	9	Early termination <sup>g</sup>
Type of visit	Phone	Site	Site	Phone	Phone/site
Day	42 +/-3	56 +/-5	112 +/-7	168 +/-10	
Week	6	8	16	24	
mPDAI score	X	X <sup>d</sup>	X <sup>d</sup>		X
Concomitant Medication	X	X	X		X
Adverse Events	X	X	X	X <sup>l</sup>	X
Physical Exam		X			X
Blood Tests <sup>a</sup>		X			X
Stool sample		X <sup>f</sup>	X <sup>f</sup>		
Sigmoidoscopy (Pouchoscopy)					
Distribute/Review Subject Diaries <sup>b</sup>	X	X			X
Distribute/Review Subject adverse events diary <sup>b</sup>	X	X <sup>j</sup>			<u>X</u>

**1.2 STUDY VISITS CHART OPEN LABEL EXTENSION FOR PATIENTS WITH RESPONSE ONLY OR NO RESPONSE OR EARLY TERMINATION DUE TO FLARE IN PLACEBO-CONTROLLED TRIAL SECTION**

Visit	6OL	7OL	8OL	9OL	10OL	11OL	12OL	13OL	Early termination <sup>g</sup>
Type of visit	Site	Phone	Site	Site	Phone	Site	Site	Phone	Phone/site
Day (post 2nd transplant)	38 (0)	46 (8) +/-1	53 (15) +3	66 (28) +/-3	80 (42) +/-3	94 (56) +/-5	150 (112) +/-7	206 (168) +/-10	
Week (post 2nd transplant)	6 (0)	7 (1)	8 (2)	10 (4)	12 (6)	14 (8)	22 (16)	30 (24)	
mPDAI score		X <sup>d</sup>	X <sup>d</sup>	X	X	X <sup>d</sup>	X <sup>d</sup>		X
Concomitant Medication	X	X	X	X	X	X	X		X
Adverse Events	X	X	X	X	X	X	X	X <sup>i</sup>	X
Physical Exam	X			X		X			X
Blood Tests				X		X			X
Pregnacy test (urine)	X								
Dispense FMT capsules + omeprazole	X <sup>g</sup>								
Stool sample			X <sup>f</sup>	X <sup>f</sup>		X <sup>f</sup>	X <sup>f</sup>		X
Sigmoidoscopy (Pouchoscopy)	X			X					
Endoscopic FMT	X <sup>h</sup>								
Distribute/Review Subject PDAI Diary <sup>b</sup>	X	X	X	X	X	X	X		<u>X</u>
Distribute/Review Subject adverse events diary <sup>b</sup>	X	X	X	X	X	X <sup>j</sup>			<u>X</u>

<sup>a</sup> Pouchoscopy visit 5 only in patients who are in remission (mPDAI<4) as assessed in visit 4 by phone or in patients with clinical active pouchitis, but not willing to participate in open label extension

<sup>b</sup> There will be two subject diaries provided to the patient: One for the evaluation of the mPDAI will be provided at Screening. The study patient will complete this diary each day, beginning with the Screening visit, and ending with the Week 16 visit. Stool frequency, rectal bleeding, fecal urgency or abdominal cramps, and/or fever will be recorded. A minimum of three days of diary entries will be required during the week prior to randomization, Phone visit week 1 and 2 and Visit Week 4 and 8. The last three days of diary entries will be used to calculate stool frequency and fecal urgency/abdominal pain scores and clinical mPDAI subscores.

The “adverse events diary” will assess for adverse events during the FMT phase (14 days) and daily for 28 days after the last day of oral FMT.

<sup>c</sup> Visit 4: Start of pretransplant antibiotic therapy for 10-14 days only in patients qualifying for open label extension

<sup>d</sup> Clinical mPDAI.

<sup>e</sup> Stool samples for microbiome, calprotectin and C. diff analyses.

<sup>f</sup> Stool samples for calprotectin and microbiome analyses.

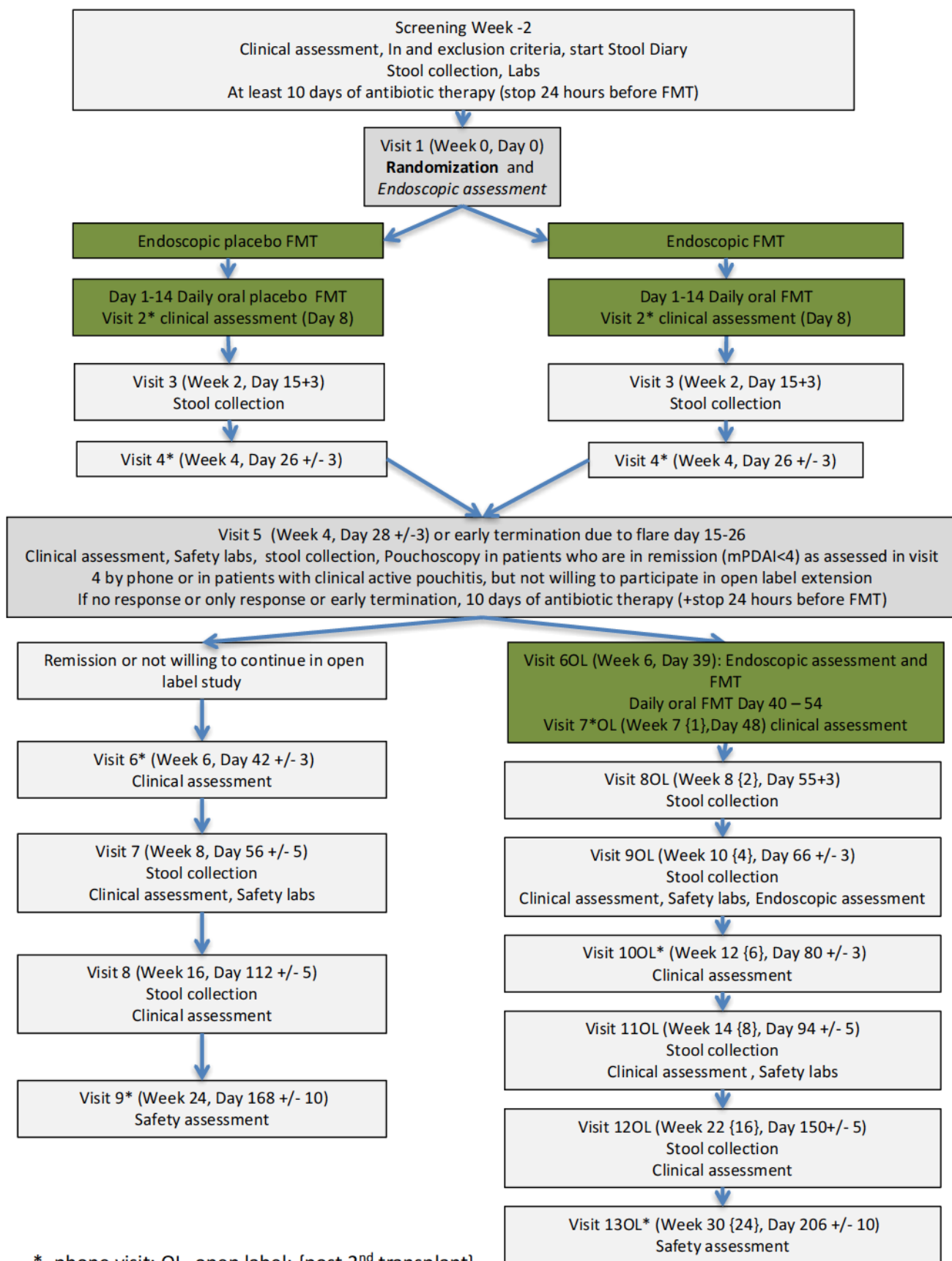
<sup>g</sup> between visit 1-5 or visit 6-8 or visit week 6OL-12OL

<sup>h</sup> Endoscopic open label FMT followed by oral FMT only if no clinical remission according to mPDAI at visit 4 and study patients wants to continue in open label FMT.

<sup>i</sup> Evaluation of serious adverse events occurring in the follow-up period

<sup>j</sup> Collect adverse event diary

## 22. APPENDIX B: STUDY FLOW CHART







### **23. APPENDIX C: MODIFIED POUCHITIS DISEASE ACTIVITY INDEX (MPDAI)**

According to Shen et al.; 2003 <sup>67</sup>

<b><u>Clinical Criteria</u></b>	<b>Score</b>
---------------------------------	--------------

<b>Stool Frequency</b>	
------------------------	--

Usual post-op stool frequency	0
-------------------------------	---

1-2 stools/day > post-op usual	1
--------------------------------	---

3 or more stools/day > post-op usual	2
--------------------------------------	---

<b>Rectal Bleeding</b>	
------------------------	--

None or rare	0
--------------	---

Present daily	1
---------------	---

<b>Fecal Urgency / Abdominal Cramps</b>	
---	--

None	0
------	---

Occasional	1
------------	---

Usual	2
-------	---

<b>Fever (temperature&gt; 100°F)</b>	
--------------------------------------	--

Absent	0
--------	---

Present	1
---------	---

<b><u>Endoscopic Criteria</u></b>	
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Edema	1
-------	---

Granularity	1
-------------	---

Friability	1
------------	---

Loss of vascular pattern	1
--------------------------	---

Mucus exudate	1
---------------	---

Ulceration	1
------------	---

## 24. STUDY PATIENT STOOL DIARY

[illegible]

## 25. ADVERSE EVENT DIARY

Front pages of diary

### **Safety and efficacy of fecal microbiome transplantation (FMT) in the treatment of antibiotic dependent pouchitis (ADP)**

#### **Record of Side Effects**

This diary is one way researchers will get information from you regarding any possible problems or side effects in this study.

- ❖ **What you are going to do is simple.** Just keep a record of any unpleasant thing that happens to you while you are in the study, before, during, and after we have completed the stool transplant. We even want you to record things that do not seem to be part of the stool therapy, at all.
- ❖ **When do you start? When do you end?** You will complete one entry per day during the time of oral treatment with the FMT capsule (14 days) as well as daily for 28 days after the oral treatment stopped.
- ❖ **What do you look for? What do you report?** Any symptom or problem whether or not it may be from the medicine, stool therapy. This could include: fever, abdominal pain, a big belly, lots of gas, diarrhea, nosebleeds, and anything else you know is not quite right.
- ❖ **What will you do?** In the first 42 days after the initial endoscopic transplant, you will report some of the specific things that have bothered you by checking the boxes in the diary (see below). You can also write any other problems that you may have had during that time. **Additionally, you will record your temperature once for each day for the 42 days after the initial endoscopic transplant, unless you feel hot. If you feel hot, please take your temperature again.** Please make sure to record the highest temperature taken that day if you take it more than once.

How will you record it? Like this...

EVENT	DATE OF ONSET	INTENSITY	ACTION TAKEN	MEDICATION	DATE RESOLVED
Fever	3/1/12	3	Missed 2 days of school	Tylenol-200mg	3/3/12
Sore throat	3/5/12	1	None	None	3/6/12

#### OTHER SYMPTOMS

Record each symptom at its **worst** level for each day.

For example, a sore throat that starts at 'Grade 1" but increases to 'Grade 2' should be recorded as 'Grade 2".

Examples of Grades:

**Grade 1 – Mild:** I noticed the symptom. It did not keep me from doing my normal activities.

**Grade 2 – Moderate:** I noticed the symptom and it kept me from doing some of my normal activities.

**Grade 3 – Severe:** I really noticed the symptom and it kept me from doing activities that I wanted or needed to do.

**Grade 4 – Very severe:** The symptom made me unable to perform basic self-care functions such as washing myself **OR** medical or surgical intervention was needed to prevent serious consequences.

**Daily diary page day 0 (endoscopic FMT-day 42 after endoscopic FMT {day 28 after stop of oral FMT})**

Day after transplant: \_\_\_\_\_

Subject ID: \_\_\_\_\_

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Check here is no side effects present:

☐

Highest temperature of the day: \_\_\_\_°F

Check if symptom present	Event	Date of Onset	Intensity	Action taken	Medications	Date Resolved
<input type="checkbox"/>	Fever					
<input type="checkbox"/>	Abdominal Pain					
<input type="checkbox"/>	Diarrhea					
<input type="checkbox"/>	Nausea/Vomiting					
<input type="checkbox"/>	Blood in Stool					
<input type="checkbox"/>	Other 1					
<input type="checkbox"/>	Other 2					
<input type="checkbox"/>	Other 3					
<input type="checkbox"/>	Other 4					

**Grade 1 – Mild:** I noticed the symptom. It did not keep me from doing my normal activities.

**Grade 2 – Moderate:** I noticed the symptom and it kept me from doing some of my normal activities.

**Grade 3 – Severe:** I really noticed the symptom and it kept me from doing activities that I wanted or needed to do.

**Grade 4 – Very severe:** The symptom made me unable to perform basic self-care functions such as washing myself **OR** medical or surgical intervention was needed to prevent serious consequences.

Completed by: \_\_\_\_\_

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