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"Out of the box" new therapeutic strategies for Crohn´s disease: moving beyond biologics

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Introduction

Crohn´s disease (CD) is a chronic disabling systemic condition with a high impact in quality of life and growing incidence in industrialized countries (1)

Although CD can affect any part of the gastrointestinal tract, it typically involves the ileocecal area and is characterized by transmural inflammation which often leads to local complications like strictures, fistulae and abscesses (2,3). In addition, it may present associated extraintestinal manifestations like arthralgia, skin manifestations or ocular

inflammation in a third of patients. (4)

The systemic and recurrent nature of CD leads to the need of potent immunosuppressive medications, frequent hospitalizations and surgery to manage complications. In addition, this results in significantly compromised quality of life as well as a huge economic burden for society with high associated direct and indirect related costs. (5)

The biggest challenge for developing new effective and safe therapies is the current lack of a deep understanding of the pathogenesis of CD (6,7). There is growing evidence suggesting that CD is primarily an immune deficiency condition affecting mainly the innate immune response to the gut microbiome. (8,9)
Several alterations of mechanisms involving the recognition and clearance of intracellular organisms and an impaired function of key immune defense cells in the intestinal mucosa (like neutrophils, macrophages or unconventional T cells) have been extensively described in CD patients. (10-12) On the other hand, other factors can contribute to chronic intestinal inflammation, such as: alterations in the mucosal layer (13), alterations in intestinal permeability (14), dysfunction in the production of defensins by Paneth cells (15) or gut microbiome dysbiosis (16,17). This may lead to recurrent translocation of bacteria and fungi from the lumen that are not efficiently resolved by the immune system, perpetuating an exaggerated inflammatory response in genetically predisposed individuals.

The current treatment of CD includes glucocorticoids (conventional and other forms like budesonide), antibiotics (typically ciprofloxacine and metronidazole), immunosuppressants (azathioprine/6-mercaptopurine or methotrexate) and anti-TNF agents (infliximab and adalimumab more widely used). In recent years, the anti-integrin antibody vedolizumab and the antibody against IL-12/23 ustekinumab have also been approved for CD. (18,19)

However, despite the growing therapeutic armamentarium a substantial proportion of patients can´t maintain clinical remission or achieve deep endoscopic remission with complete mucosal healing, which leads to clinical relapses (20-22). Moreover, heterogeneity is a key feature of IBD since disease location and behavior (phenotypes) and therapy

response varies widely from patient to patient (23).

Thus, novel therapeutic strategies targeting inflammation in different ways are urgently needed. Ideally, the objective is to find effective therapies, with a good safety profile and reasonable cost that can improve current management. The implementation of diet modifications (exclusive enteral nutrition or specific diets), microbiome targeting therapies (FMT, phage therapy, helminths), T regulatory cell (Treg) engineering and other methods to tackle systemic inflammation (like hyperbaric oxygen, cannabis or vagal nerve stimulation) have shown promising initial results, although larger trials are still needed. (24-32)

In this review, we aim to summarize the current status of these "out of the box" novel therapeutical approaches for CD, discuss their potential use as complementary or primary treatments to biologics and small molecules and identify the gaps and promises for implementation in near future.

1.Exclusive enteral nutrition (EEN)

EEN is a liquid dietary therapy providing a full supply of required calories, excluding solid food. EEN can be administered as elemental, semi-elemental or polymeric diets. Elemental formulas consist of free single amino acids, are generally less palatable and require the use of a nasogastric tube for administration (24,25). Non-elemental diets can be administered orally and consist of semi-elemental diets containing short peptides (oligo-peptides of 4-5 amino acids), and polymeric diets consisting of whole proteins, typically from milk, meat, egg or soy-(24,26). EEN may be recommended following a relapse of CD, to induce remission. It is administered for a 6-8 weeks period either orally or by nasogastric or gastric tubes (26).

The role of EEN is not fully understood, but three mechanisms are believed to be essential to improve CD. First, nutritional deficiencies can be repleted. This avoids inflammation-induced hypercatabolism activity, malabsorption, and lead to weight gain and reduced hospital length of stay (27). Notably, EEN has also shown to reduce or alleviate the need for steroids, thus avoiding their negative nutritional side effects such as fat gain and osteomalacia (27).

Second, EEN may reduce the dysbiosis associated with CD, as diet is major factor known to alter the gut microbiome composition (24). Third, EEN can have anti-inflammatory effects by exclusion of dietary triggers of inflammation and reducing gut permeability through enhancing tight junction expression. This may attenuate an excessive the inflammatory immune response (27).

A Cochrane meta-analysis of 27 studies using EEN as induction therapy in adult CD showed high efficacy of EEN with no differences in remission rates between elemental and non-elemental (semi-elemental and polymeric) diets, of 64% vs. 62%, respectively (RR 1.02, 95% CI 0.88-1.18) but evidence was of low quality. There were also no difference in adverse events occurrence, such as nausea, vomiting and diarrhea, between these two groups (28).

A multicenter, randomized multicenter trial included 62 patients with active CD, and were treated with EEN either with 1) high oleate and low linoleate content or 2) with high linoleate and low oleate content versus corticosteroids for 4 weeks. Clinical remission was achieved in 2/3 of patients receiving EEN at the end of the study (29). In a prospective study incluidng 41 adult CD patients with complicated disease (either fistula/abscess or stricture formation), 12 weeks of EEN induced clinical remission in 80.5% and fistula closure in 75%. Moreover, up to 80% of patients had partial or total stricture healing response and 76% of patients had their intra-abdominal abscess resolved (patients with abscess formation received antibiotics with or without percutaneous drainage). In addition, 47% of patients with mucosal ulcers at inclusion achieved mucosal healing (30).

EEN has shown similar -or even superior- clinical remission rates than corticosteroids in paediatric CD patients (27). In adults, the results have varied, showing a remission rate ranging from 8 to 100% in a previous review of 11 studies (31). In fact, corticosteroid treatment was superior to EEN in inducing clinical remission in adult patients with CD, as reported in a Cochrane meta-analysis of 10 studies. Steroid-induced remission occurred in 73% of patients compared with 45% achieving remission after EEN (RR 0.65, 95% CI 0.52-0.82), although evidence was of low quality (28). In line with this results, a controlled trial including comparing 107 active CD patients receiving EEN vs corticosteroids for 6 weeks,

demonstrated 53% vs. 79% clinical remission rates, respectively (32).

Up to 80% of CD patients will require surgery within 20 years of diagnosis (33). EEN appears beneficial in the preoperative setting of CD patients to improve the nutritional status and thus reduce the surgical complication rate (34). EEN may also decrease the intestinal inflammatory activity and the need for corticosteroids (34). Corticosteroid treatment is associated with increased risk of postoperative infection and anastomotic leakage (35). Notably, EEN may also enhance recovery after surgery (34).

A meta-analysis of 831 mostly adults CD patients reported that preoperative EEN vs. no EEN significantly reduced the rate of complications after surgery, of 21.9% vs. 73.2% respectively (36). A retrospective study of 120 adult CD patients receiving at least 4 weeks of EEN before undergoing laparoscopic bowel resection, showed an improvement in preoperative levels of albumin, haemoglobin and C-reactive protein (CRP) and a significant reduction in postoperative complications (37).

However, the most recent meta-analysis including seven studies on EEN as preoperative optimization in adult CD, concluded that current data are insufficient to demonstrate significant effects. Four randomized controlled trials are ongoing, and their results may clarify whether EEN improves perioperative outcomes or not (38).

Although treatment with biologic agents, such as anti-TNF, has become essential in the management of moderate to severe CD, 30% of patients do not respond and another 20-40% lose response over time. The addition of EEN may be beneficial in this setting due to its additive anti-inflammatory effects and improved biological response following nutritional repletion. In addition, EEN has no risks of severe adverse effects (27). A meta-analysis including 342 adult CD patients compared the clinical remission rates from EEN (≥ 600 kcal/day) and infliximab versus infliximab alone, showing a significant difference, 69.4% vs. 45.4% respectively. Of note, included patients were allowed to receive an oral diet to supplement their caloric requirement (39).

Another, more recent meta-analysis including 857 CD patients aimed to assess if EEN combined with anti-TNF therapy (infliximab or adalimumab) was effective in maintenance of remission. The addition of complementary EEN showed higher clinical remission or response rate than the use of anti-TNF alone, 70.5% vs. 53.8%, respectively (OR 2.23 95% CI 1.60-3.10), suggesting a favourable effect of the combination. However, several limitations were reported, regarding retrospective study design and only studies from Japan were included (40). In line with this, in a randomized controlled trial including 20 CD patients, the combination of EEN and infliximab achieved long-term (56 weeks) clinical remission or response in 78.6% versus 50.0% in the infliximab alone group (41). In contrast, another prospective study in 72 CD patients receiving EEN ($\geq$ 900 kcal/day) + anti-TNF versus anti-TNF alone, reported no significant difference in 2-year cumulative clinical remission rate (60.9% vs 56.7%, respectively) (42). Selected studies of EEN treatment are shown in Table 1.

EEN may induce remission in about 60% of active adult CD patients and seems promising also in complicated disease. However, EEN appears to have less impact on disease activity than corticosteroids in adults and should be considered when corticosteroid therapy is not tolerated or contraindicated. EEN combined with anti-TNF treatment may be favourable in maintaining remission and should be considered especially in patients with complicated or disabling disease factors. The major limitation using EEN in adults is low compliance, but it is a low-risk treatment with few side effects that can potentially improve outcomes in selected patients.

2.Diet

Diet and dysbiosis have been postulated to be central elements in the development and perpetuation of CD (7). Questions around diet are probably the most common among CD patients and many patients wish to influence disease course by modifying their diet. In addition, some CD patients experience irritable syndrome (IBS)-like symptoms independently of disease activity and benefit from symptom relief on certain diets (43). Different diets have demonstrated various success in alleviating gastrointestinal symptoms

and inflammation in CD.

As incidence rates for CD are increasing world-wide, the so-called Western diet is suspected to play a major role in disease pathogenesis. Western diet is a term covering a modern diet, including a high fat, high sugar, low fiber diet, as well as refined flours, red meat and processed food. At the same time, the intake of fruits, vegetables, nuts, whole grains, fish and poultry is low. Emulsifiers, important additives to better palatability in processed foods, such as maltodextrin (MDX), carboxymethyl cellulose (CMC) (E466/E469), carrageenans (E407), as well as other food additives have been associated with intestinal microbiome alteration and inflammation (44,45).

Diet is an environmental factor that can be altered or modified individually. Numerous diets, many of them not CD specific, i.e. low FODMAP diet, gluten-free diet, lactose-free diet, specific carbohydrate diet, modified specific carbohydrate diet, balanced fat diet, dietary fiber diet, Mediterranean diet, semi vegetarian diet, paleolithic diet, anti-immune protocol diet, anti-inflammatory diet, IgG4 exclusion diet, as well as diets avoiding food additives and microparticles, exist. In addition, there is exclusive enteral nutrition (EEN), partial enteral nutrition (PEN), the Crohn's Disease Exclusion Diet (CDED) and anti-inflammatory diet for IBD. The most important of these will be discussed in this review.

In the British Society of Gastroenterology consensus guidelines on the management of IBD in adults, practical dietary advice is described (46), but guidelines regarding dietary intervention from the large gastroenterology societies are generally missing. The last ESPEN (European Society for Clinical Nutrition and Metabolism) guidelines do not recommend any specific diet to induce or maintain remission in IBD patients. Counselling by a clinical dietist to avoid malnutrition is, however, advocated (47). Recently, IOIBD (International Organization for the Study of Inflammatory Bowel Diseases), published a paper to provide expert opinion on dietary guidance to control and prevent relapse of IBD (48). Simultaneously, several comprehensive reviews covering aspects of IBD and the role of diet intervention have been published (49-53).

Gluten-free diet and lactose-free diet

Animal studies have demonstrated that gluten ingestion may promote intestinal inflammation and increase intestinal permeability. Howevever, there have been no prospective studies evaluating the role of a gluten-free diet in the induction and maintenance of CD and UC. Several cross-sectional reports suggest that a gluten-free diet may improve symptoms in IBD patients (54-57), but due to a lack of high-quality prospective clinical studies, current data do not support the universal use of a gluten-free diet in IBD (58).

Many IBD patients, even in clinical remission, report intolerance to lactose. In a meta-analysis of 17 articles, Szilagyi et al concluded that lactose maldigestion in IBD is dependent on ethnic makeup of the population and usually not disease, although an increased risk on sub-analysis was found in CD with small bowel involvement. There was a suggestion that dairy foods may protect against IBD and that nutritional consequences of dairy restrictions might impact adversely on bone and colonic complications (59).

Low FODMAP diet

FODMAPs (Fermentable oligosaccharides, disaccharides, monosaccharides and polyols) are short chain carbohydrates that are poorly absorbed in the small intestine and are prone to absorb water and ferment in the colon. Although a low FODMAP diet is known to relieve gastrointestinal symptoms in subgroups of patients with IBS (60), there is a paucity of studies and no significant evidence that supports an anti-inflammatory effect in CD. In a RCT to investigate the effects of a low FODMAP diet on persistent gut symptoms, the intestinal microbiome and circulating markers of inflammation in patients with quiescent IBD, the low FODMAP diet reduced fecal abundance of microbes believed to regulate the immune response, compared with the control diet, but had no significant effect on markers of inflammation (61).

Adhering to a low FODMAP diet is complicated because of lengthy inventories, as well as a period of strict restriction of food high in FODMAPs, followed by a period of reintroduction, and finally a maintenance period, which should ideally last for a long time (61). Concerns regarding the low FODMAP diet have been raised, especially because of impact on the gut

microbiota and due to its restrictive nature with risk of an inadequate diet. A systematic review and meta-analysis, searching a number of databases from their establishment to December 2021, that will evaluate the efficacy and safety of the low FODMAP diet in the treatment of quiescent IBD patients with IBS-like symptoms has been planned (62).

Specific Carbohydrate Diet (SCD)

The Specific Carbohydrate Diet (SCD) is the most widely studied whole food intervention in CD and UC. SCD was initially created in 1924 to treat celiac disease (63). It was later used for the treatment of IBD (64). SCD eliminates all grains, most starches, sugar (except honey), dairy products (except butter, 24-hour fermented yogurt and hard cheeses, which are essentially lactose free), and most store-bought, processed or prepackaged foods. The majority of studies have been performed in pediatric cohorts.

Kakodkar et al performed a survey study of 50 adult and pediatric IBD patients in remission following the SCD (see Table 1). Among these, 36 had CD, 9 UC and 5 indeterminate colitis, and mean age was 36 years. Thirty-three subjects (66 %) noted complete symptom resolution, which did not occur until a mean of 9.9 months after starting the SCD (65). In another survey study also including adult and pediatric patients 47 % had CD, 43 % had UC, and 10 % had indeterminate colitis. Thirty-three percent reported symptomatic remission at 2 months after initiation of the SCD, and 42 % at both 6 and 12 months (66).

In adult CD, a symptomatic remission and fecal calprotectin (FC) response in 46.5 % and 34.6 % of patients, respectively, was demonstrated after a six-week SCD intervention of the DINE-CD Study (https://www.crohnscolitisfoundation.org/blog/dine-cd-study-results-something-to-chew), matching the response seen in a Mediterranean diet intervention arm (67). In a case report, Arjomand et al, described sustained clinical, biochemical, radiologic, endoscopic and histologic remission in a medical non-responsive complicated male CD patient on a 42-month long SCD. Prednisolone was prescribed in the induction phase, tapered after 26 weeks, and not reintroduced (68).

Modified Specific Carbohydrate Diet (mSCD)

The Modified Specific Carbohydrate Diet (mSCD), derived from SCD, attempts to add a few healthy whole foods to offer the patients a more varied diet and to potentially feed a more diverse microbiome. The PRODUCE study, which stands for Personalized Research on Diet in UC and CD (https://www.nimbal.org/education/produce-study), will compare the effectiveness of a strict SCD versus a mSCD in reducing symptoms and inflammation in patients with IBD. Participants will be asked to alternate diets during a period of 34 weeks and will receive results about the impact of the different diets on their symptoms and inflammation. All data will be combined to learn about the effects of diet in IBD overall.

Balanced fat diet and dietary fiber diet

In a meta-analysis of 83 RCTs, including 41 751 participants, investigating long-term effects of omega-3, omega-6 and total PUFA on IBD and inflammatory markers, the authors concluded that supplementation with PUFAs has little or no effect on prevention, treatment or modification of long-term inflammatory status (69).

Patinets suffering from IBD tend to consume a lower intake of dietary fiber than healthy subjects, especially in periods of clinical activity, and fiber intakes are inadequate compared with respective national fiber guidelines (70). In a review of RCTs 3/10 UC studies reported fiber supplementation to benefit disease outcomes, whereas 0/12 CD studies and 1/1 pouchitis study reported a benefit on disease activity. A number of studies have, however, reported favorable intragroup effects on physiological outcomes including fecal butyrate, FC, inflammatory cytokines, microbiota, and gastrointestinal symptoms. According to the authors, there was no evidence that fiber intake should be restricted in patients with IBD, except in obvious gastrointestinal obstruction (71).

Mediterranean diet (MD)

The Mediterranean diet (MD), first defined as such in the 1960s, describes a high consumption of vegetables, fruits and nuts, legumes and unprocessed cereals, and a low consumption of meat and meat products, as well as a low consumption of dairy products (with the exception of long-preservable cheeses), and a moderate intake of fish, where available. Alcohol consumption, usually as wine, is moderate and olive oil is liberally used

(72). In general, IBD patients have low compliance with strict MD in countries outside the Mediterranean. Despite its documented benefits for health and modulating inflammation, MD is still rarely used as treatment in IBD in many countries (73).

In a prospective, interventional study aiming at evaluating the impact of a MD on disease activity, obesity, obesity-related complications, and QoL 142 IBD patients, 84 UC and 58 CD, were included. After 6 months of the diet, fewer UC and CD patients with stable therapy had active disease (23.7 % in UC vs. 6.8 %;17.6 % CD vs. 3.0 %) and elevated inflammatory biomarkers. MD improved QoL in both UC and CD, but neither serum lipid profile nor liver function were modified by the diet (74). In another study, Lewis et al compared the effectiveness of the SCD to the MD as treatment for CD. The percentage of participants who achieved symptomatic remission at week 6 was not superior with the SCD (SCD, 46.5 %; MD, 43.5 %). Fecal calprotectin response was achieved in 34.8 % with the SCD and in 30.8 % with the MD. C-reactive protein response was achieved in 2 of 37 participants (5.4 %) with the SCD and in 1 of 28 participants (3.6 %) with the MD (p=0.68). The authors concluded that the SCD was not superior to the MD. Given these results, the greater ease of following the MD and other health benefits associated with the MD, the MD may be preferred to the SCD for most patients with CD with mild to moderate symptoms (67).

Partial enteral nutrition (PEN)

In partial enteral nutrition (PEN), whole food diet is supplemented with a liquid formula-based diet delivered to the gastrointestinal tract orally or through a nasogastric feeding tube. In a systematic review with meta-analysis of PEN for the maintenance of remission in CD, Yang et al included eight studies with 429 patients. The rate of clinical relapse at 0.5 to 2 years was significantly lower in patients receiving PEN (420-1800 kcal/d) than in those not receiving nutrition therapy and patients receiving PEN exhibited a higher frequency of clinical remission maintenance at 0.5 to 1 year (67 %) than those not receiving nutrition therapy. The authors concluded that PEN may be more effective than the absence of EN therapy for the maintenance of remission in CD with a good safety profile (75).

Crohn's Disease Exclusion Diet (CDED)

Until recently, EEN has been the only dietary intervention effective in CD and with documentation only for pediatric cohorts, whereas PEN with free diet has been ineffective for inducing remission (26). Therefore, the Crohn's disease exclusion diet (CDED), a three-phase elimination diet, which avoids products known to have a pro-inflammatory effect on the intestinal mucosa in combination with up to 50 % of dietary calories from PEN, was developed to induce remission in pediatric and young adult CD patients (76). CDED involves exclusion of dietary components that impair innate immunity, increase intestinal permeability, cause microbial dysbiosis, or allow bacteria to adhere and translocate through the intestinal epithelium in animal models. These components are animal and saturated fats, gluten, and emulsifiers. Half of the diet is provided as EEN, thereby allowing the double effect of avoiding nutritional deficiencies and improving dysbiosis (77). In phase 1 food restrictions are strict, in phase 2 the food list is expanded and phase 3 is a long-term maintenance phase.

In a pediatric CD cohort, Levine et al demonstrated that the novel CDED coupled with PEN was better tolerated than EEN and induced sustained remission in a significantly higher proportion of patients (48). In an adult CD cohort with 32 patients, Szczubelek et al aimed to evaluate the effectiveness of the CDED in inducing remission. Clinical remission was obtained in 76.7 % patients after 6 weeks and in 82.1 % after 12 weeks of therapy. Fecal calprotectin levels were significantly lower in the second follow-up compared with baseline. The authors concluded that CDED is an effective therapy for inducing remission in the adult CD population (78). In another adult CD cohort, Yanai et al performed an open-label, pilot randomised, where eligible patients were biologic naïve adults with mild-to-moderate disease, randomly assigned to CDED plus PEN or CDED alone for 24 weeks. Forty patients were included in the study. At week 6, 13 of 19 (68 %) patients in the CDED plus PEN group and 12 of 21 (57 %) patients in the CDED group had achieved clinical remission (p=0.46). Among the 25 patients in remission at week 6, 80 % were in sustained remission at week 24 (12 patients in the CDED plus PEN group and eight in the CDED alone group). Fourteen of 40 (35 %) patients were in endoscopic remission at week 24 (eight patients in the CDED plus PEN group and six in the CDED alone group). The authors concluded that CDED with or without PEN was effective for induction and maintenance of remission in adults with mild-

to-moderate bio naïve CD (79).

CD-TREAT

CD treatment-with-eating diet (CD-TREAT) is an individualized solid food-based diet, with a similar nutrients and food ingredients composition as EEN, devised by Svolos et al (80). The effects of CD-TREAT on the gut microbiome, inflammation, and clinical response were evaluated in a rat model, in healthy adults, and in children with relapsing CD. For healthy adults, CD-TREAT was easier to comply with and more acceptable than EEN and induced similar effects on the gut microbiome. In the rat model, CD-TREAT and EEN produced similar changes in bacterial load, SCFA acids, microbiome, and ileitis severity vs. standard chow. In children receiving CD-TREAT, 80 % had a clinical response and 60 % entered remission, with significant decreases in FC. The authors concluded that CD-TREAT replicates EEN changes in the microbiome, decreases gut inflammation, is well tolerated, and is potentially effective in patients with active CD (80). A summary of the main studies of diet in CD are summarized in Table 1

Conclusion

Numerous diets, some general and others more specific for CD, have been suggested as treatment of active disease, or to keep the patient in sustained remission. Until recently, EEN was the only dietary intervention effective in CD and with documentation only for pediatric patients. Results from recent studies presented in this review suggest that other diets, such as the CDED (with or without PEN), CD-TREAT and the SCD hold promise for, not only clinical, but also inflammatory response in adult CD patients by excluding certain food components.

Longer, strict exclusion diets are not recommended and should be avoided. Low FODMAP diet and diets low on dietary fibers have an impact on gut microbiota and may lead to a bacterial dysbiosis, which again may lead to inflammation and a depletion of SCFA produced by bacteria. Low levels of SCFA deprive enterocytes of an important energy source, which may also lead to inflammation, and even exacerbation of disease. High levels of dietary fiber may be refrained from in active CD, especially in stricturing disease, but not in remission.

The aim should be to implement an effective diet with documented impact on inflammation, keeping in mind compliance and promoting if possible a while choosing non-restrictive and short duration diets, although, for instance the CDED has a long-term maintenance phase 3. Moreover, the risk of social stigmatization when on a diet should not be neglected.

It is important to bear in mind that there is potential for malnutrition and weight loss when following exclusion diets. Nutritional status, including macro- and micronutrient status should be assessed, ideally by a clinical dietist, especially when malnutrition is suspected. Dietary interventions should be performed in a coordinated care setting, under professional supervision, with support from a multi-disciplinary experience care team. Self-directed exclusion diets should be discouraged, and it is crucial that the patient is carefully characterized when planning a diet intervention. It is most important to define the target of treatment, such as disease state (preclinical, active disease, maintenance of remission), and if the diet intervention is primary or adjunctive/supplementary to other treatment.

In the study performed by Lewis et al, the authors concluded that the SCD was not superior to the MD to achieve symptomatic remission or biochemical response. Given these results, the greater ease of following the MD and the other health benefits associated with the MD, MD may be preferred to the SCD for most patients with active CD (67). Due to the lack of substantial data supporting the use of other diets in adult CD, ESPEN and BSG recommend a standard healthy diet, rich in fruit and vegetables, low in processed food and sugar, fat and red meat (similar to the MD), exactly as recommended for healthy and other persons who do not suffer from CD.

3.T regs therapeutic manipulation

Several animal and human studies have shown the key role of T cells in the maintenance of the inflammatory response in CD (81-83). A balance between T effector cells and regulatory T cells (Tregs) is key to maintain homeostasis in the gut barrier. This equilibrium is lost in CD, with a predominance of pro-inflammatory Th1 and Th17 responses over T reg suppressive

function (84,85). In addition, recent studies demonstrated how microbiota-derived signals induce the expression of RORγt in Treg cells that contribute to control intestinal inflammation regulating Th1 and Th17 responses (86,87). Thus, attempts to restore or boost Treg function are under study to fight chronic intestinal inflammation.

T regulatory cells are a specialized population of CD4(+) T cells that control excessive immune responses against self- and foreign antigens via excretion of anti-inflammatory cytokines like IL-10, transforming growth factor-beta (TGF-beta) and IL-35 (88). Based on their developmental origin, T regs can be divided into thymic T regs (tTregs) and peripheral T regs (pTregs) (88). Both types express the transcription factor Forkhead box P3 (Foxp3) that is key for their suppressive and immune tolerance promoting functions (89). Recently, a third type of type 1 T regs (Tr1) that are FoxP3 negative, that develop in the periphery and secrete high levels of IL-10 and TGF-beta1, have gained interest as a possible cell-base therapy for IBD (90).

T regs have shown a key role in controlling inflammation and in the maintenance of local immune homeostasis in the gut in several animal models of colitis (91-93). Interestingly, Tregs from peripheral blood from IBD patients retain their normal suppression properties, but their numbers are decreased during activity. In addition, T regs are increased in the inflamed intestinal mucosa compared with noninflamed mucosa, but lower compared to patients with diverticulitis, suggesting a possible alteration in the compensatory Treg trafficking to control inflammation in IBD (94). Fantini et al showed that IBD mucosal lamina propria T cells are resistant to Treg suppression potentially contributing to inflammation and this phenomenon could be reverted using Smad7, an inhibitory molecule of TGF-beta. (95)

There is increasing interest in the use of adoptively transferred Tregs as a cell-based therapy for IBD. In theory, this approach could achieve an *ad hoc* anti-inflammatory and immune-regulating effect, without the side effects of standard drug induced systemic immunosuppression. In fact, T regs have been tested in several autoimmune diseases like diabetes type 1, graft versus host disease after hematopoietic stem cell transplantation, and in tolerance to solid organ transplants with promising preliminary results. (96-100)

In 2012, Desmeraux et al published the results of the first attempt to implement a therapy with Tregs (Tr1) in refractory CD showing promising results regarding efficacy and safety. (101). In this phase 1/2a 12-week open-label study, ovalbumin-specific Treg cells were isolated from patients' peripheral blood mononuclear cells, exposed to ovalbumin, and administrated in a single intravenous infusion. A diet supplemented with meringue cakes was used to activate T regs locally. Of the 20 CD patients included, 40% showed clinical improvement (CDAI reduction of 100 points) at weeks 5 and 8 with no major safety issues suggesting its feasibility. However, biochemical improvement measured by CRP and fecal calprotectin was only modest. See Table 1.

A major leap in the field was achieved after a later study showed that functional Tregs could be expanded from the blood of CD patients to potential target doses in 22-24 days. A specific subpopulation of CD45RA+ Tregs was stable, expressed gut homing integrins (like α4β7, CD62L and CCR7) and was able to home to human small bowel in a xenotransplant mouse model (102). Importantly, the authors showed that these Tregs do not transform into Th17 T cells invitro -which could potentially be deleterious in CD- and could suppress the activation of T cells isolated from CD intestinal mucosa (102). A recent study by the same group demonstrated that incubation of *ex vivo* expanded Treg cells with rapamycin and an agonists of the retinoic acid receptor-α induced the expression of α4β7 integrins, improving their migration to the gut in a humanized mouse model of colitis (103). Based on these findings, a Phase I/IIa randomized clinical trial using *ex vivo* Treg expansion for CD is underway (NCT03185000). In addition, A Phase I open label study using autologous expanded Tregs in UC is also ongoing (104).

Although preliminary evidence of Treg therapies is promising and seems well tolerated, several uncertainty areas need to be addressed before implantation in the clinic. Specifically, the protocol for the expansion of T regs and the right subtype, finding the most effective dose without major side effects and the interval and timing for the infusion have to be better defined. Another important aspect to explore is to understand how current medications used refractory CD patients -like immunosuppressants, anti TNF or the new

biologics and small molecules- may modify the effect and safety profile of T reg cell therapies.

4.Hyperbaric oxygen

Hyperbaric oxygen therapy (HBOT) involves breathing 100% oxygen for 60-90 minutes at a greater pressure than one atmospheric absolute (ATA), typically 2.0-2.5 ATA in a pressurized chamber. Treatment can be given daily, in periods for up to 6-8 weeks for several chronical conditions (105). HBOT can be used to treat ischemic and hypoxic conditions such as carbon monoxide poisoning, brain hypoxia, gangrene and decompression sickness (106,107).

Beneficial effects of HBOT in CD can be attributed to wound-healing- and anti-infection effects resulting from hyperoxygenation of plasma and tissues, neovascularization and anti-oxidant effects. In addition, HBOT may reduce the production of pro-inflammatory cytokines and chemokines, which are also essential factors in the intestinal inflammatory process in CD (106,107).

A previous systematic review included 12 preclinical studies of HBOT use in rat colitis models showed a reduction in blood concentrations of inflammatory markers such as TNF-α and IL-1β (105). In addition, myeloperoxidase activity was decreased reflecting accumulation of neutrophils and markers of oxidation were favourably altered such as decreased malondialdehyde levels and increased glutathione peroxidase levels in several studies (105). HBOT also reduced nitric oxide and nitric oxidase synthase levels in three studies analyzed.

In an early study of HBOT in an indomethacin-induced enteropathy rat model, HBOT by 2.3 ATA 100% oxygen, 1-2 treatments of 60 minutes daily for 2 or 5 days significantly reduced TNF-α and IL-1β levels, intestinal ulcerations, as well as myeloperoxidase and nitric oxide synthase activities (108). These findings support anti-inflammatory and antioxidant effects from HBOT.

In 1989, the first clinical case showing beneficial effects from HBOT in CD was reported (109). A later systematic review including 13 studies in treatment refractory CD, reported

improvement in 78% (31/40) of patients altogether. Most participants had perianal disease, and all were refractory to multiple medical treatments, including conventional IBD treatment, antibiotics or even an elemental diet (105).

In a case series, 29 treatment refractory complicated CD patients (perianal-, enterocutaneous fistulas, pyoderma gangrenosum) received HBOT, 2.4 ATA for 2 hours, 10-86 sessions (median 20). Clinical response (closure of fistulas, complete healing of pyoderma gangrenosum) was observed in a high percentage of patients (22/29) (110).

Another case series included 20 CD patients with perianal fistulas, all had failed conventional treatment and 15 had failed biological treatment. Patients received HBOT, 243-253 kPa for 80 minutes, 40 sessions over 8 weeks. At week 16, the perianal disease activity index was reduced, as was the modified van Assche index (MRI). Clinical response (reduction of $\geq$ 50% of draining fistulas) was reported in 12/20 and clinical remission (absence of draining fistulas) in 4/20 participants (111). Selected HBOT studies are shown in Table 1.
More recently, a systematic review and meta-analysis including 10 studies with 353 CD patients treated with HBOT showed a clinical response rate as high as 81.9% (107).

Based on preclinical studies, HBOT appears to have beneficial anti-inflammatory, antioxidant and healing properties. Clinical response rates in CD have been around 80% and, notably, HBOT may be effective also in complicated, treatment refractory cases, suggesting it as an adjunct treatment in more severe cases. The availability of pressurized chambers could be a limiting factor for the implantation of HBOT, but HBOT is safe and has few and mild side effects which may increase its implantation as an adjuvant therapy in complicated patients. Current studies are small and non-controlled and prospective, larger studies are mandatory to identify the best potential candidates for HBOT.

5.Fecal microbiota transplantation

The aim of treatment with fecal microbiota transplantation (FMT) is to restore dysbiosis of the gut microbiota, potentially not only bacterial, but possibly also viral and fungal, by transferring stool from a healthy donor to a patient. The effect of FMT in medically severe, recurrent and refractory *Clostridium difficile* infection is effective and well documented (112,113) and FMT is emerging as a feasible treatment to induce remission in active UC based on several RCTs (114-118).

Regarding CD, RCTs are more scarce. Currently, 13 cohort studies and only two RCTs have been published (119). These studies suggest that FMT may be an effective therapy in CD.
In the first RCT, 21 patients with long-standing disease, mainly ileocolonic, received a single dose of fresh stool or sham FMT by colonoscopy after an induction therapy with corticosteroids. Steroid-free clinical remission rate at 10 and 24 weeks was 44.4 % and 33.3 % in the sham FMT group and 87.5 % and 50.0 % in the FMT group. The primary endpoint, implantation of donor microbiota at week 6 was not reached for any patient (see Table 1 – continuation) (120).
In the second RCT, 31 patients with mild to moderate colonic disease on stable medication received two doses of fresh stool on two consecutive weeks by either gastroscopy or colonoscopy. CD patients had a lower level of operational taxonomic units (OTUs) vs. donors with lower levels of *Bacteroides, Eubacterium, Faecalibacterium*, and *Roseburia*, and higher levels of *Clostridium, Cronobacter, Fusobacterium*, and *Streptococcus*. A significant increase in OTU and Shannon diversity index was demonstrated after two weeks. Clinical remission was achieved in 66.7 % (18/27). A significant percentage of patients experienced mild adverse events during or shortly after treatment both in the gastroscopy and colonoscopy-delivered groups, but no significant differences were seen (121)

There are many questions related to FMT that need to be resolved. Key issues are to identify donors with an ideal gut microbiota, such as a “super donor”, and match them with the right patient. Also, to determine if stool from a single donor or multiple donors should be used, and to what extent should donor stool be tested for transmittable diseases. To avoid the latter, other options are to use standardized bacterial mixtures such as for instance anaerobic cultivated human intestinal microbiota (ACHIM), which has been investigated as

treatment for IBS (122). The benefit of donor pre-treatment (for instance with antibiotics) as well as the type of fecal material to administer (fresh or frozen stools), the number and periodicity of treatments or the optimal administration route (lower or upper) needs to be standardized.

Additionally, certain safety issues must be considered. Clinicians must ensure that FMT recipients do not acquire any transmittable disease through serological and fecal testing of the donor pre-treatment. In fact, It is impossible to foresee all potential future long-term infectious complications with Hepatitis B, HIV and Creutzfeldt-Jacob disease as historic examples. Also, it is important to account for the possibility of sepsis in some severely immunocompromised patients due to ongoing CD treatment.

In conclusion, FMT seems to be a promising and relatively safe treatment option for CD. However, a better understanding of the match between donor and recipient microbiome (not only bacteria, but also viruses and fungi) and the standardization of FMT whether delivered from a “super donor” or from a well-defined bacterial cocktail as a commercial product needs further study.

6.Phage Therapy

The human body hosts microorganisms including viruses, bacteria, archaea, fungi and parasites. The viral part of the microbiome, the virome, consists mainly of bacteriophages (123). These are viruses that infect bacteria and have the potential to shape and modulate bacterial communities in the gut. Bacteriophages may be classified as virulent or temperate (non-virulent) (124).

Phages bind to its receptor on the host cell (bacteria and its genome is inserted into the cell before replication through either lytic, lysogenic, or alternatively chronic or pseudolysogenic cycles. While virulent phages use lysis of the host cell to release mature virions ready to infect other cells, temperate phages use lysogeny, a mechanism allowing genome replication without virion production or cell death/lysis (124). Bacteriphages, such as *microviridae*

(single-stranded DNA) and *caudovirales* (double-stranded DNA), are the main contributors to the intestinal virome (125) and the interplay of bacteria and phages is key to preserve homeostasis in the human gut (126).

As the phage genome is incorporated in the bacterium, phages can modulate bacterial host communities through several mechanisms. The introduction of genetic material by gene-transfer between bacteria can improve host virulence or improve resistance (123,127). In addition, host bacterial gene expression can be changed providing protection against other phages, or change the bacterial composition via lysis of competing bacteria (128). The interactions between phages and bacteria are complex and may also be influenced by the amount of nutrients available and the microbial density, and some authors have hypothesized an "arms race" dynamic between lytic phages and the bacterial host (124).

In IBD, the phage composition -*phageome*– appears to be altered, but findings are inconsistent (129,130). Bacteriophage richness was reported to be increased, whereas bacterial richness was reduced (129). Most studies have also showed elevated number but lower diversity of *Caudovirales* phages in UC and CD compared with healthy subjects (130). In contrast, the number of phages in ulcers were lower than in non-ulcerated mucosa in CD. A recent study demonstrated that the "healthy" core virome was shifted from a lytic toward a lysogenic cycle in IBD, suggesting that lytic phages help maintain a healthy gut (129).

Phage therapy refers to treatment by modulation of the phageome, and subsequently the bacteriome, in a disease assumed to be of bacterial origin. The first successful attempt with phage therapy was in 1921, where patients with dysentery were cured (123). Phage therapy appears promising for IBD as it could be used to alter the intestinal microbiota and selectively eliminate bacterial pathogens (123). However, engineering of the applied phage is essential, since these phages should not be immunogenic, leading to activation the immune system and inappropriate immune responses in the host.

Several pre-clinical study have demonstrated the possible benefits of phage therapy. In a preclinical study, applying a purified T4 phage preparation reduced the production of

reactive oxygen species in peripheral blood polymorphonuclear leukocytes stimulated with lipopolysaccharides or bacteria (*E. coli*) (133). In another study, T4 phage administration inhibited human T-cell activation and proliferation, and nuclear factor-kappa β activation in allogenic skin allografts (134). These findings support immune-modulating and possibly anti-inflammatory effects from phage therapy.

Galtier et al showed for the first time a potential clinical application of phage therapy to target Adherent Invasive Escherichia coli (AIEC) in CD (135). In a combined animal and human intestinal sample study, AIEC strain LF82 (predominantly in the ileum in CD), was the target of a cocktail of three virulent bacteriophages. The phage cocktail significantly reduced AIEC numbers in feces and intestinal sections in mice. These phages were also able to target LF82 bacteria in ileal biopsies from patients with CD. The findings support bacteriophages as a new treatment option in CD (135).

A more recent randomized controlled cross-over clinical study confirmed the efficacy of phage therapy targeting *E. coli*. This work included 43 adult healthy individuals reporting mild to moderate gastrointestinal symptoms who received a cocktail of 4 different phages targeting *E.coli* orally for 4 weeks, with a 2 week wash-out period. *E. coli* was reduced or not detectable in 71% of participants after treatment (15 of 21 cases had *E.coli* detected in stool before treatment), and IL-4, a pro-inflammatory marker, was significantly reduced. No other changes in diversity parameters were found suggesting that bacteriophages can selectively reduce target bacteria without altering gut microbial diversity (136) (See Table 1).

The gut microbiota has an essential pathogenic role in IBD. Phages have the potential to change the bacterial phenotype and modulate the immune response and possibly the inflammatory activity in IBD. However, the current data are scarce, the knowledge regarding the reference phageome of healthy individuals is limited, as well as the virome changes in IBD patients. Although phage therapy appears safe, phages may induce both pro- and/or anti-inflammatory responses. More research, including proper randomized controlled IBD trials are thus needed to establish the future role for such treatment in CD.

7.Helminths

Helminth therapy is an attractive strategy for controlling chronic inflammation in CD, taking advantage of the anti-inflammatory responses helminths elicit in the host.

Over millenia, parasitic worms have colonized mammals, developing a vast array of protective mechanisms to help them survive minimizing damage to the host. This co-evolution of the immune system in the presence of helminth infections has shaped how the body self-regulates and controls immune responses to minimize harm (137-139).

The hygiene hypothesis suggests that reduced exposure to common infections due to improve living standards and better hygiene, particularly in early childhood, could lead to an increase in the incidence of allergic and immune-mediated diseases later in life (140-142). There is some evidence that this lack of exposure to "old" infectious agents, like helminths and other parasites, may have contributed to the increased incidence of CD in the developed world (143,144).

Helminths can down-regulate host immunity to protect themselves from the host and produce several immunomodulatory molecules that can block Th1/Th17-mediated anti-parasite responses and promote Th2 responses, limiting local inflammation (145-147).

Furthermore, helminths have shown to improve colitis by inducing IL-10 production by macrophages (148-150). Helminths can also down-regulate T cell proliferation (151), suppress cytokine production by epithelial cells (152) and induce the differentiation of T regs (153-154) in experimental studies. In addition, helminth infection can regulate positively the gut microbiome causing the expansion of protective bacterial communities (*Clostridiales*) that inhibit pro-inflammatory bacterial taxa (like *Bacteroidales*), which could be beneficial for some patients with CD (146). Thus, several clinical trials using attenuated helminths or helminth-derived compounds have been published to treat several immune-mediated conditions (155-156).

Almost two decades ago, Summers et al published the first results of the use of the porcine whipworm, *Trichuris suis* in IBD (157). The authors chose *T suis* as therapeutic agent because it is is genetically related to *Trichuris trichiura* (the human whipworm) but it is not a natural human parasite that can colonize without causing disease (157). The first open label trial demonstrated the safety of a single dose of 2500 live *T suis* eggs given orally and showed improvement in disease activity in CD patients (157). A subsequent study from the same group demonstrated that *T suis* ova ingested every three weeks achieved remission in 72% of CD patients in an open label at week 24, with no major side effects (158). Another randomized control trial demonstrated that a single dose of up to 7500 *T suis* ova was well tolerated and did not result in short- or long-term (6 months) side effects. (159)

The positive results in terms of safety and efficacy of *T suis* ova in a randomized controlled trial for UC (160), were followed by the publication by Schölmerich et al of a Phase II randomised, placebo controlled trial of *T suis* ova in mildly-to-moderately active, ileocolonic CD (161). The administration of 250–7500 *T suis* ova fortnightly over 12 weeks was not more effective than placebo for induction of clinical remission or response. However, the authors reported a dose-dependent immunological response measured by *T. suis* E/S antigen-specific total IgG and good safety profile (161). The study had a very high placebo response, and the authors argue that probably the study period was not long enough and that maintenance of remission could have been a better outcome measure. See Table 1.

In conclusion, there is a large evidence that helminth therapy can down-regulate the immune response and the implemented therapies seem to be safe in clinical trials in CD. However, although the preliminary results were promising, the largest well designed clinical trial failed to meet its endpoint in the induction of remission in CD. The question of whether higher doses and/or longer study periods could have improved the outcomes remains open and needs validation in further trials. An alternative approach could be to use *T. suis* ova to maintain remission after the induction of remission with conventional therapies or in the setting of post-operative recurrence prevention, to improve the immune-regulatory effects after the acute inflammation is controlled. This approach has not been yet tested to date.

Although most of the studies for CD test *T suis*, it is possible that other helminth species can be used. For example, *Necator americanus* hookworm was recently tested in a Phase 2 trial showing a good safety profile and an expansion of T regs suggesting an immunological effect in patients with multiple sclerosis. A small pilot study with *N. americanus* showed good tolerance and some clinical effect in CD, but a concerning increase in CDAI in 2 of 9 patients studied was reported. (162)

A very interesting alternative approach is to use helminth-derived molecules (instead of eggs or whole parasites) to control inflammation. Helminths secrete a variety of products including proteins, lipids and small molecular compounds commonly called excretory/secretory products (ES) (155,163). These ES are essential for the survival of the helminth allowing the parasite to evade immune surveillance. Thus, there has been increasing interest in characterizing and identifying these anti-inflammatory products that can be used as biologic therapeutic compounds.

In fact, several proteins and metabolites secreted by helminths have proved to have a potent immune-modulating effect and ameliorate colitis in animal models (164-166), as well as some synthetic hookwork-derived peptides (167). Of note, a promising anti-inflammatory protein secreted by *Schistosoma* (P28GST) in its recombinant form has showed efficacy and a good safety profile in a pilot Phase 2a trial in patients with mild CD (168).

A better understanding of the interactions between helminths and the host and the different mechanisms of downregulating the immune response will help identify potential new therapeutic targets. Finally, although there seems to be a general good safety profile in the published trials (160.161,169), the effects on helminths and their derived products in the long term and in combination with standard potent immune suppressive drugs deserve further investigation.

8.Cannabis

Cannabis refers to the plant family *Cannabis sativa*, that includes both hemp and marijuana, and has been used in both recreational and medical treatment purposes for many centuries. The *Cannabis sativa* family contains over 70 different cannabinoids, of which

tetrahydrocannabinol (THC) is the essential, most pharmacologically active ingredient with psychoactive properties like altered sensory perception and euphoria (170,171). *Cannabidiol (CBD)* is the other main natural cannabis compound, but it has no psychoactivity related adverse events. The medical use of cannabinoids has been debated due to legal constraints, risk of dependency as well as toxicity and adverse effects (170). Potential detrimental effects on brain development, cognitive functioning and cases of psychosis and paranoia have led to a more restricted use of cannabis in clinical studies (172). Nevertheless, a medical drug containing 1:1 formulation of THC and CBD administered as mouth spray has been used in patients with spasticity and pain in multiple sclerosis (MS), with low reported potential for dependency (173).

The endocannabinoid system is present throughout the human body, and consists of endogenous cannabinoids, their metabolizing enzymes, along with the cannabinoid receptors, CB1 and CB2 (172). Cannabis may exert beneficial effects trough stimulation of these receptors that are located in multiple organ systems, including the nervous system, GI tract and immune cells, particularly mast cells and plasma cells (172). Anti-inflammatory and pain-modulating effects have been reported in GI-related disease (174), as well as anti-emetic, anti-motility effects and a reduced secretory response (170,172). Reports of symptomatic relief of abdominal pain and cramping, diarrhea and joint pain in IBD patients, has increased the interest in cannabis use among patients (172).

A potential anti-inflammatory effect of the modulation of the endocannabinoid system has been demonstrated in experimental studies. Colitis was exaggerated in mice deficient in CB1-receptor compared with wild-type mice in two different animal models of colitis, suggesting that CB1-receptor protects against inflammation. In addition, treatment with a cannabinoid receptor agonist, R(-)-7-hydroxy-Δ6-tetra-hydrocannabinol-dimethylheptyl (HU210), protected against trinitrobenzene sulfonic acid (TNBS) induced colitis (175). Another animal experimental study showed that injections of the CB2-receptor agonists (JWH133, AM1241) protected against colitis development in a TNBS mouse model (176).

In a prospective, placebo-controlled clinical study, 21 CD patients unresponsive to conventional treatment received either 115 mg THC (cigarettes) twice daily for 8 weeks (n=11) or placebo (cigarettes) (n=10). Clinical response (CDAI decrease by > 100) was observed in 10/11 of patients receiving cannabis vs. 4/10 patients receiving placebo (p=0.028). however, there were no significant differences in complete remission rates (177). A later randomized controlled trial including 20 patients with moderate CD having resistant towards conventional treatment showed no effect of cannabis. Participants were randomized to receive 10 mg CBD or placebo two times daily for eight weeks, administered sublingually as oil drops. There were no observed differences in CDAI scores between the groups (178). (See Table 1 for selected studies).

A Cochrane meta-analysis including three studies on active CD (93 patients), evaluated the clinical remission rates following cannabis treatment (179). Cannabis was administered as cigarettes (115 mg THC) vs. placebo cigarettes in one study (n=21) (177), as cannabis oil (5% CBD) vs. placebo in one study (n=22) (178) and as cannabis oil (15% CBD and 4% THC) vs. placebo in one study (n=50) (171). The authors were unable to draw a definite conclusion based on the limited amount of data.

Cannabinoids, where the main forms are THC or CBD, exert biological effects through the endocannabinoid system. Cannabinoid agonists have shown effects on pain-modulation, gut motiliy, secretion and anti-emetic regulation, as well as anti-inflammatory effects in preclinical studies. Although symptomatic relief has been reported in IBD, there is a lack of data supporting an anti-inflammatory effect based on objective variables, such as endoscopy and inflammatory markers. The existing data are scarce, and current meta-analyses are unable to conclude on the effect of cannabis in CD. Serious concerns regarding mental-health issues, dependency and toxicity, suggest that cannabis use should be restricted and be based on individual case assessments.

9.Vagal nerve stimulation (VNS)

The use of Bioelectrical Medicine (BM) is a novel non-pharmacological method to treat inflammation using devices to modulate the electrical activity of the nervous system (180,181).

The stimulation of the vagus nerve (VN), the longest nerve of the organism that innervates the gastrointestinal tract, has anti-inflammatory effects via TNF reduction and cholinergic stimulation. Classically, neuromodulation with VNS has been used to treat neuropsychiatric conditions like refractory epilepsy and depression, but preliminary clinical studies have shown promising results in the treatment of immune-mediated diseases like rheumatoid arthritis (182,183) and CD (184,185).

The VN is composed of 80% afferent and 20% efferent fibers and is a key regulator of brain-gut interactions as a fundamental component of the parasympathetic autonomic nervous system (181). Several studies suggest that the production of pro-inflammatory cytokines may be attenuated by the VN through the cholinergic anti-inflammatory pathway (182). The VN can inhibit quickly and significantly the release of macrophage TNF and attenuate systemic inflammatory responses inhibiting the production of other key pro-inflammatory cytokines like IL-1β, and IL-6 via the release of acetylcholine (182,186). This immunomodulatory effects could be beneficial in CD. Interestingly, recent studies have shown a decreased vagal tone in CD correlated with TNF levels (187) and the stimulation of the VN can also dampen peripheral inflammation through the activation of the hypothalamic-pituitary adrenal axis and the release of glucocorticoids (188).

The anti-inflammatory effects of VNS have been widely demonstrated in several experimental models of colitis (189-191). The results of the first pilot trial of VNS in CD were published by Bonaz et al (193). Originally, 9 patients with moderately active CD (2 failure to azathioprine and 7 treatment-naïve) were implanted with a VNS device and electrode under general anesthesia. An electrode was wrapped around the left VN in the neck region and connected to a bipolar pulse generator subcutaneously implanted in the chest, and VNS was continuously performed for 12 months. At 6 months, the authors report the results of VNS in 7 patients (193). Two of them were removed from the study at 3 months for clinical worsening and 5 achieved clinical remission with a restored vagal tone assessed by heart rate variability. The procedure for VNS was feasible and well-tolerated in all patients (193).

After a longer follow up of 12 months of the same cohort, VNS was reported to be effective and well tolerated (194). Five out of the original 9 patients achieved clinical remission and 6 patients achieved endoscopical remission, with a restoration of vagal tone in 7 of them. A biological effect was also demonstrated by a reduction in CRP and fecal calprotectin in some patients (in 4 and 3 patients, respectively) and a decrease in several pro-inflammatory cytokines like TNF, IL-6, IL-12, and IL-23. (194). No major adverse events related to the device were reported except discomfort to the intensity/output current levels.

D´Haens et al published the results of a preliminary prospective trial of VNS in CD patients refractory to biologics in abstract form (195). A VNS device was implanted and used as mono- or adjunctive therapy in 16 CD patients. In this difficult to treat population, VNS achieved clinical response in almost half of the patients at 4 months (7/16 CDAI-70 responders) with and associated biomarker and endoscopic improvement measured by fecal calprotectin and SES-CD endoscopic scores. All serious adverse events in both cohorts were related to worsening of CD except for one device-related postoperative infection and one device deficiency at PE (195).

In conclusion, VNS is a promising option for CD with a good safety profile to date. However, several questions remained to be answered before this technique can be implemented in the clinic. First, the specific type of device to provide an effective and durable VNS needs to be defined. Also, the definition of the specific treatment parameters to be used (pulse width, amplitude and timing) for the different CD phenotypes and clinical situations needs to be clarified. The criteria to select the best potential candidates for VNS treatment needs to be addressed, as well. Larger placebo-controlled clinical trials are warranted to test the real clinical efficacy of VNS (alone or in combination with current treatments), as well as its use in different clinical scenarios (induction vs maintenance of remission) and to determine long-term safety.

The use of other non-invasive modalities of VNS (like transcutaneous cervical or auricular VNS) or focused ultrasound stimulation (rather than through an implanted device) are promising alternatives that may minimize side effects. The results of two pilot studies using non-invasive VNS applied to the cervical (183) or the auricular branch (196) of the VN in

rheumatoid arthritis patients are encouraging. In fact, transcutaneous auricular VNS is currently under clinical investigation in a double-blind placebo-controlled study in pediatric patients with IBD (CD and UC) (ClinicalTrials.gov Identifier: NCT03863704).

Interestingly, patients with CD could also benefit from alternative ways to stimulate the VN and profit its anti-inflammatory effects like physical activity (197), fasting (198), yoga (199) or mindfulness meditation (200).

Conclusions and Future directions

In this review, we summarized the current status of the most promising "out of the box" therapeutic options for CD, beyond biologics and established treatments.

This is an area of growing interest, since the possibility of tackling chronic gut inflammation avoiding immunosuppression (with known long-term side effects like increased risk for infections and some cancers) could be an attractive option for some CD patients. In addition, some of these new possibilities have demonstrated to be safe in preliminary trials, which could make them suitable to complement current approved therapies. However, some of these novel approaches are in early stages of development and their real-life effectiveness and safety profile still need to be tested in larger controlled trials.

One of the most straightforward attempts to control inflammation is the implementation of specific diets. Although the recommendation of following a healthy diet (rich in fruit and vegetables, low in levels of processed food and low in sugar, fat and red meat) has been traditionally recommended for CD patients, recent rigorous evidence has showed the potent anti-inflammatory effects of certain diets, like the Mediterranean diet. Other specific diets like the Crohn´s disease exclusion diet, the specific carbohydrate diet or CD-TREAT have also demonstrated promising results and their role needs to be clarified in larger studies.

The use of periods of exclusive enteral nutrition is another attractive possibility also in adults with CD, since it was proven to be effective in the induction of remission. In addition, EEN has an excellent safety profile and the possibility to combine it with current drugs (like anti-TNF) has shown promising results. In fact, some European centers are currently using EEN in pre-operative settings or in complicated patients like those with complicated fistulae. Although compliance is still an issue in adults, the use of EEN use in combination with

regular healthy food and the attempt to find formulas for better palatability can improve the use of this potent tool in clinical practice.

The possibility to use T regs to attenuate the inflammatory response in the gut has shown promising preliminary results with a good safety profile and several clinical trials are underway in CD. However, a better understanding of the protocol for the expansion of T regs and the specific ideal subtype to be used as well as the proper regimen of administration in the particular clinical scenarios needs to be further addressed. Hyperbaric oxygen therapy applied via pressurized chambers is a well-stablished method with anti-inflammatory, antioxidant and healing properties that has proved to be beneficial in some CD patients and very safe in small clinical trials. These promising results could be especially relevant for patients with perianal or fistulizing CD, but larger trials are warranted.

Other microbiome-targeting therapies are underway for CD. Recent studies suggest that Fecal Microbiota Transplantation could be a safe, cheap and effective option in selected CD patients. Some relevant questions like donor selection, the manipulation of microbiome composition pre-instillation, the most effective administration route or periodicity of treatment needs to be further investigated. Attempts to use a well-defined bacterial cocktail as a commercial product are currently being tested. Other original ways of modifying the dysbiosis present in CD to more beneficial bacterial phenotypes with anti-inflammatory properties, like the use of bacteriophages have shown promising results, but a better understanding of the role of the virome in IBD is needed. In addition, larger trials are lacking to date.

The use of cannabis in IBD has also been explored. Cannabinoids exert biological effects through the endocannabinoid system resulting in pain-modulation, anti-emetic properties and certain anti-inflammatory effects. However, although symptomatic relief has been reported, there is conflicting data regarding the efficacy of cannabis in CD. Notably, the chronic use of cannabis rises serious concerns regarding mental-health issues, dependency etc., suggesting that its use should be restricted to individual selected patients.

Helminth-based therapies constitute an elegant strategy to modulate the pro-inflammatory Th1/th17 immune response in CD. Helminths and helminth-derived molecules have the natural capacity to suppress the excessive inflammatory response in the gut, promoting Th2 immunity. Although *T. suis* ova did not do better than placebo in the induction of remission

in CD, their use in maintenance of remission or in the post-operative setting has not been tested yet. Furthermore, the use of helminth derived molecules with anti-inflammatory proven effects in animal studies (instead of eggs or attenuated helminths), might be an interesting possibility for CD in the future. Finally, the systemic beneficial effects obtained by stimulation of the vagus nerve in other diseases is also promising approach. This could be obtained via device implantation or with the less invasive transcutaneous stimulation. The positive results of the latter in rheumatoid arthritis are encouraging and should stimulate further study in CD, taking into account its good safety profile.

In conclusion, novel approaches are urgently needed to break the therapeutic ceiling in CD. Patients living with CD are demanding new and better solutions with fewer side effects that could complement the current immunosuppressant drugs. However, although some of these "out of the box" treatments are promising and have a good safety profile in preliminary trials, they need to be validated in larger controlled trials to help the clinicians to position them in the current treatment algorithms for CD.

We need to stay curious and open to study efficiently different possible ways to control the mucosal and systemic inflammation in CD. A multidisciplinary approach that allows for information exchange with other disciplines, a better understanding of the pathophysiology of CD and a translational team effort between open-minded basic scientists and IBD clinicians should guide the effort in following years to improve the quality of life of patients living with CD.

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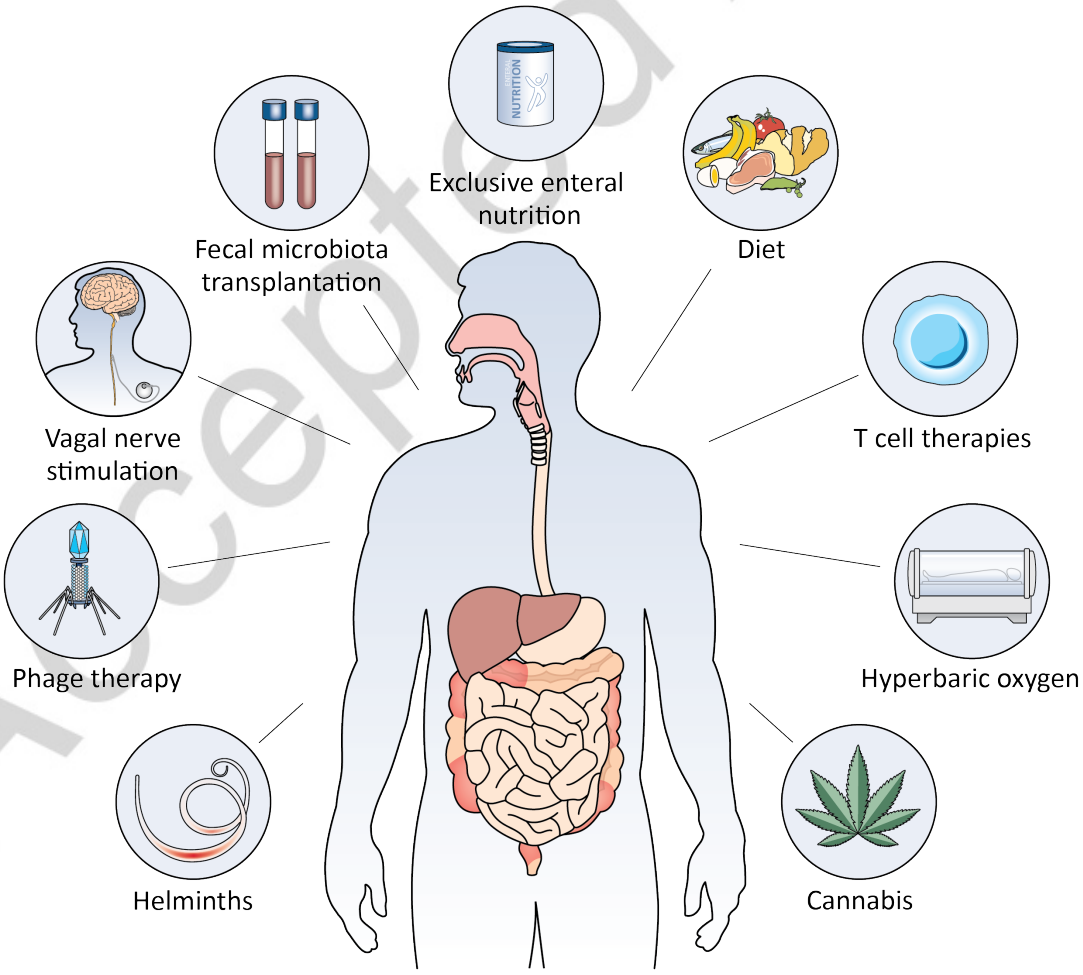

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Figure 1: Illustration of the most promising "out of the box" treatment options under study for Crohn´s disease

TABLES:

Table 1: Main clinical trials testing novel therapies in Crohn´s disease

Author	Year	Methods	Results
Lochs et al (32)	1991	EEN therapy: Randomized controlled trial. 107 adult active CD patients, 6 weeks of therapy: 1) EEN liquid oligopeptide diet; 2) Corticosteroids	Climniacl remission in 53% (EEN) vs 79% (corticosteroids) Median time to remission 30.7 days EEN vs 8.2 days (corticosteroids)
Gasull et al (29)	2002	EEN therapy : Randomized controlled trial. 62 adult active CD patients, 4 weeks of treatment: 1) EEN high in oleate and low in linoelate vs. 2) EEN high in linoelate and low in oleate vs.3) Corticosteroids	Clinical remission was achieved in 63% of patients receiving EEN (high in lineolate, low in oleate) at the end of the study.
Yang et al (30)	2017	EEN therapy : Prospective open study. 41 adult CD patients with intestinal fistula/abdominal abscess or inflammatory intestinal stricture. 12 weeks of EEN treatment.	Clinical remission in 80.5%, fistula closure in 75%. Partial or total stricture healing response in 80%. Resolved intra-abdominal abscess in 76%.Mucosal healing in 47%.
Hisamatsu et al (41)	2018	EN therapy: Randomized controlled trial. 20 adult CD patients refractory to infliximab 5 mg/kg monotherapy, 56 weeks of treatment: 1) Infliximab monotherapy (10 mg/kg) (n=6); 2) Infliximab (10 mg/kg) + EN (n=14)	Clinical remission or response in 78.6% (Infliximab + EN) versus 50.0% (Infliximab) group.
Ge et al (37)	2019	EEN therapy: Retrospective study. 120 adult CD patients, 45 receiving at least 4 weeks of EEN before undergoing laparoscopic bowel resection, 75 patients received no EEN.	EEN treatment improved preoperative levels of albumin, haemoglobin and CRP. EEN treatment led to fewer postoperative complications and surgical siteinfections vs. no EEN treatment.

Kakodkar et al (65)	2015	SCD. Survey study. 50 adult and pediatric patients with IBD in remission (36 CD, 9 UC and 5 indeterminate colitis). Mean time the SCD was followed was 35.4 months (range 1 to 216 months),	33 subjects (66%) noted complete symptom resolution after a mean of 9.9 months (range 1 to 60 months). Subjects reported a mean of 40% difficulty rating in following the diet (range 0% to 100%).
Suskind et al (66)	2016	SCD. Survey study. 417 adult and pediatric patients with IBD (47 % CD/43 % UC/10 % ID). Mean duration on the SCD was 31.6 ± 54.9 months with the range of 0.25-780 months	Symptomatic remission reported by 33 % after 2 months and by 42 % at both 6 and 12 months. Time to achieve remission: <2 weeks in 13 %, 2 weeks to a month in 17 %, 1-3 months in 36 %, and >3 months in 34 %.
Levine et al (48)	2019	CDED with PEN. Prospective trial. 78 children with mild to moderate CD. Treatment for 12 weeks. 1) CDED with PEN; 2) EEN	CDED with PEN was tolerated in 39 children (97.5%) vs. EEN was tolerated by 28 children (73.6%) (p=0.002). At week 6, 75 % given CDED plus PEN were in corticosteroid-free remission vs. 59 % given EEN (p=0.38). At week 12, 75.6 % of children given CDED plus PEN were in corticosteroid-free remission vs. with 45.1 % of 31 children given EEN and then PEN (p=0.01
Cox et al (61)	2020	Low FODMAP diet. RCT. 52 adult patients with quiescent IBD (26 CD and 26 UC). Treatment for 4 weeks.1) Low FODMAP diet; 2) Control diet	Adequate relief of persistent gut symptoms (52 % vs. 16 % (p=0.007)). Reduction in IBS severity scores in low FODMAP diet did not meet statistical significance (P=0.075). Reduction of fecal abundance of microbes believed to regulate the immune response. No significant effect on markers of inflammation.
Chicco et al	2021	MD. Prospective, interventional study. 142 patients	Fewer patients with stable therapy had

(74)		with IBD (84 UC and 58 CD). Intervention for 6 months	active disease (UC 23.7 % vs. 6.8 %, p=0.004; CD 17.6 % vs 3.0 %, p=0.011). Fewer patients with stable therapy had elevated inflammatory biomarkers.

Chicco et al (74)			
Lewis et al (67)	2021	SCD vs. MD. RCT. 194 adult CD patients with mild-to-moderate symptoms. Treatment for 12 weeks:1) SCD; 2) MD	Symptomatic remission was obtained in 46.5 % with SCD vs. 43.5 % with MD (p=0.77). FC response was achieved in 34.8 % with SCD vs. 30.8 % with MD (p=0.83). CRP response was achieved in 5.4 % with SCD vs. 3.6 % with MD (p=0.68).
Szczubelek et al (78)	2021	CDED. Case series. 32 adult CD patients. Treatment for 12 weeks.	Clinical remission was obtained in 76.7 % patients after 6 weeks of therapy. Clinical remission was obtained in 82.1 % patients after 12 weeks of therapy. Calprotectin levels were significantly lower in the second follow-up compared with baseline (p=0.021).
Yanai et al (79)	2022	CDED with PEN. Open-label pilot study. 40 adult CD patients.Treatment for six weeks: 1) CDED with PEN; 2) CDED alone. 56 weeks of treatment:1) Infliximab monotherapy (10 mg/kg) (n=6); 2) Infliximab (10 mg/kg) + EN (n=14)	Clinical remission was obtained in 68 % in the CDED plus PEN group vs. 57 % CDED group (p=0.4618) after 6 weeks of therapy. Among the patients in remission at week 6, 80 % were in sustained remission at week 24 (12 CDED plus PEN/8 CDED alone). At week 24 35 % were in endoscopic remission (8 CDED plus PEN/6 CDED alone)
Desreumaux et al (101)	2012	Phase 1/2a 12-week open-label study, ovalbumin-specific Treg cells were isolated from patients' peripheral blood mononuclear cells, exposed to ovalbumin, and administrated in a single intravenous infusion. 20 CD refractory patinets included	40% of CD patients showed clinical respoinse (CDAI reduction of 100 points) at weeks 5 and 8. Dose-realted effiucacy.No major safety issues.

Canavan et al (102)	2016	Clinical study to define the optimum population for Treg cell therapy in CD	Tregs could be expanded to potential target doses. A specific subpopulation of CD45RA+ Tregs was stable, expressed gut homing integrins and was able to home to human small bowel in a xenotransplant mouse model. Expanded T regs could suppress the activation of T cells isolated from CD intestinal mucosa
Feitosa et al (110)	2016	HBOT therapy: Case series. 29 adult CD patients with refractory disease, associated with enterocutaneous fistula, pyoderma gangrenosum and perianal disease. 4 weeks of treatment, 2.4 ATA for 2 hours, 10-86 (median 20) daily sessions.	Overall healing rate: 22/29, 76%. Specific healing rates: Enterocutanous fistula 91%, pyoderma gangrenosum 100%, Perianal diasease 65%.
Lansdorp et al (111)	2020	HBOT therapy: Case series. 20 adult CD patients with perianal fistulas, failing conventional treatment for 6 months, 8 weeks of treatment, 243-253 kPa for 80 minutes, 40 sessions.	Decrease in PDAI score. Decrease in modified van Assche score. Clinical response in 12/20.Clinical remission in 4/20.
Author	Year	Methods	Results
Sokol et al (120)	2020	FMT. RCT. 21 patients with long-standing disease, mainly ileocolonic. 1) Single dose of fresh stool by colonoscopy after an induction therapy with corticosteroids 2) Sham FMT by colonoscopy after an induction therapy with corticosteroids	Steroid-free clinical remission rate at 10 and 24 weeks was 44.4 % (4/9) and 33.3 % (3/9) in the sham FMT group and 87.5 % (7/8) vs. 50.0 % (4/8) in the FMT group. Primary endpoint, implantation of donor microbiota at week 6 was not reached for any patient .
Yang et al (121)	2020	FMT. RCT. 31 patients with mild to moderate colonic disease on stable medication. 1) Two doses of fresh stool on two consecutive weeks by gastroscopy; 2) Two doses of fresh stool on two consecutive weeks by colonoscopy	Significant increase in OTU and Shannon diversity index after two weeks. Clinical remission was achieved in 66.7 % (18/27). In the gastroscopy group 76.9 % and in the colonoscopy group 64.3 % experienced mild adverse events during or

			shortly after treatment, but no significant differnces were seen.
Febvre et al (136)	2019	Phage therapy: Double-blinded, placebo-controlled crossover trial. 43 adult healthy individuals with mild to moderate gastrointestinal symptoms. Orally administered cocktail (4 different phages) targeting E.coli, for 4 weeks, 2 week wash-out period.	E. coli was reduced or not detectable in 71% of participants after treatment. IL-4 levels were reduced.
Summers et al (157)	2003	Open trial to study safety and effctiveness of single dose of 2500 live Trichuris suis eggs was given orally. UC and CD patients were followed every 2 wk for 12 wk.	it is safe to administer eggs from Trichuris suis to patients with CD and UC. Improvement shown in the common clinical indices used to describe disease activity. 3 out of 4 CD patiuents entered clinical remission.
Summers et al (160)	2005	Open label trial in 29 moderate CD patients treated with ngested 2500 live T suis ova every three weeks for 24 weeks	Treatment with T suis ova for 24 weeks yielded a response rate of nearly 80% and a remission rate (CDAI less than 150) of nearly 73%. Limited by the abscence of placebo arm
Sandborn et al (159)	2013	Randomised, double-blind, placebo-controlled study to evaluate the safetyof a single dose of oral T suis ova in 36 patients with Crohn's disease. Sequential dose-escalation (500, 2500 and 7500 viable embryonated T. Suis ova)	A single dose of up to 7500 T suis ova was well tolerated and did not result in short- or long-term (6 months) side effects. No data provided for efficacy. Limited by the lack of assessment of multiple doses.
Schölmerich et al (161)	2017	Randomised, double-blind, placebo-controlled, multicentre Phase II study undertaken to evaluate the efficacy and safety of three different dosages (50, 2500, or 7500 TSO) of T suis ova versus placebo in 252 mildly-to-moderately active CD patients	No dose showed a clinically relevant effect over placebo for induction of clinical remission or response at week 12. Good safety profile. Dose-dependent immunological response shown

Capron M et al (168)	2020	Phase 2a multicenter, open-label, pilot study to evaluate the safety of Schistosoma derived recombinant P28GST protein administered to 10 patients with mild CD	Effect on CDAI scores and calprotectin levels decreasing first 3 months. Generally safe. More adverse events in the treatment arm, mostly loco-regional reactions at injection site
Naftali et al (177). 2013		Cannabis therapy: Prospective placebo-controlled study. 21 adult active CD patients failing conventional treatment. Treatment for 8 weeks : 1) 115 mg tetrahydrocannabinol (THC) (cigarettes) twice daily (n=11); 2) Placebo (cigarettes) twice daily (n=10)	Clinical response in 10/11 (patients) vs. 4/10 (placebo). Complete remission in 5/11 (patients) vs. 1/10 (placebo).
Naftali et al (178)	2017	Cannabis therapy. Randomized controlled trial. 20 adult active CD patients failing conventional therapy.Treatment for 8 weeks: 1) 10 mg cannabidiol CBD (sublingual droplets) twice daily; 2) Placebo twice daily	No difference in clinical symptoms (CDAI scores).
Bonaz et al (193)	2016	Prospective. Open pilot study of VNS in 7 patients with moderately-active CD. Device surgically implanted and continuos stimulation. Results at 6 months	2 patients removed because of disease worsening. 5/7 achieved clinical remission. VNS well tolerated.
D´Haens et al (195)	2018	Prospective. Open-label study. VNS implanted in 16 Cd patients with active disease, refractory to biologics. VNS used as monotherapy or adjunctive to conventional treatments	Clinical response definedas a reduction in 70 points in CDAI in 7/16 patients with and associated biomarker and endoscopic improvement. All SAEs reslated to CD worsening except for one device-related postoperative infection and one device deficiency.

		in 2 cohorts . Results at week 16.	

		Prospective. Open-label study. VNS implanted in 16 Cd patients with active disease, refractory to biologics. VNS used as monotherapy or adjunctive to conventional treatments in 2 cohorts . Results at week 16.	
Sinninger et al (194)	2020	Prospective. Open pilot study of VNS in patients with moderately-active CD. Follow-up from previous study by Bonaz et al 2016. Results at 12 months	Five out of 9 patients achieved clinical remission. 6 patients achieved endoscopical remission. Effect on cytokine profile. VNS well tolerated