Review

Microbiota-targeted therapies in inflammation resolution

Francesco Strati [a,b], Georgia Lattanzi [a], Chiara Amoroso [c], Federica Facciotti [a,b,*]

[a] *Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy*
[b] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[c] *Gastroenterology and Endoscopy Unit, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy*

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ABSTRACT

Gut microbiota has been shown to systemically shape the immunological landscape, modulate homeostasis and play a role in both health and disease. Dysbiosis of gut microbiota promotes inflammation and contributes to the pathogenesis of several major disorders in gastrointestinal tract, metabolic, neurological and respiratory diseases. Much effort is now focused on understanding host-microbes interactions and new microbiota-targeted therapies are deeply investigated as a means to restore health or prevent disease.

This review details the immunoregulatory role of the gut microbiota in health and disease and discusses the most recent strategies in manipulating individual patient's microbiota for the management and prevention of inflammatory conditions.

1. Introduction

The dualistic crosstalk between the microbiota and the immune system starts before birth and it is shaped throughout life as a consequence of geographic, cultural and dietary habits as well as individual's genetic background. For this reason, each individual harbors its own normobiotic microbiota, making difficult the identification of a fixed health-associated microbial ecology. The alteration of the gut microbiota composition leads to a condition called "dysbiosis". Dysbiotic events occur throughout life (*i.e.* upon antibiotic usage, as a consequence of infections, or upon drugs administration). However, microbiota's resilience restores the normobiotic state, possibly aided by the immune system. Though, if repeated dysbiosis-leading events occur and the newly-shaped microbiota diverges too much from the healthy status, then microbiota-modifying interventions are necessary to restore normobiosis. Interestingly, dysbiotic individuals vary more in microbial community composition than healthy individuals [1]. This concept reminds of the Anna Karenina's incipit: 'All happy families look alike; each unhappy family is unhappy in its own way'. The original cause of dysbiosis induces different types of microbiome instability. Upon these premises and according to the nature of dysbiosis, different therapeutic approaches aimed at restoring the normobiosis can be envisaged. When health-associated bacteria are depleted, as observed in autoimmune diseases, supplementation of missing bacteria may be a solution. Similarly, *Clostridium (C.) difficile*-induced colitis is efficiently treated by completely reshuffling the microbial composition *via* fecal transplant. On the contrary, if the microbial dysbiosis is associated with the enrichment of specific pathogens, antimicrobials such as antibiotics, bacteriocins and bacteriophages may be used. Another potential strategy is represented by probiotics, living microorganisms which can

confer positive effects on health by impacting on the resident microbiota, intestinal epithelium cells, and, globally, the immune system [2]. Among them, lactic acid bacteria are the most frequently used as non-pharmacological methods to promote gut health and potentially modulate dysbiosis in inflammatory bowel diseases (IBD) [3].

Here, we review the possible usage of microbiota-targeted therapies to restore normobiosis and resolve inflammation in several major inflammatory conditions (Tables 1–5).

2. Microbiota-targeted approaches to resolve infection-related inflammation

Dysbiosis may be both a cause and a consequence of enteral infections. Many enteric parasites, including *Trichuris* [4], *Heligmosomoides* [5], *Giardia* [6], *Blastocysts* [7], *Cryptosporidium* [8] and *Entamoeba* [9] induce marked changes in the structure of the gut microbiota [10]. For instance, infection with *Toxoplasma (T.) gondii* is accompanied by reduced bacterial diversity, expansion of facultative anaerobes (*i.e.* members of the family *Enterobacteriaceae*), loss of barrier integrity in the gut and bacterial translocation, that collectively contribute to immune-mediated pathologies [11]. During enteric infections, anti-microbial peptides (AMPs) and toxin delivery secretory systems (T6SS, T3SS) are upregulated and released [12]. As a

Table 1
List of microbiota-modulating tools in animal models and patients affected by infective diseases.

Type of infection	Microbiota modulation tool	Studies in animal models	Studies in patients
Clostridium difficile	Antibiotics	Vancomycin; Fidaxomycin [27]	Vancomycin; Fidaxomycin [27] (28, 29, reviewed in 30)
	FMT	–	Fatal adverse events in two cases (*Escherichia coli* contaminated FMT [32] RBX2660 (31, PUNCHCD3, NCT03244644) Oral capsule: CP101 (NCT03110133) RBX7455 (NCT02981316)
	Microbiota-based products	–	SER-109 (33, 34, NCT03183128) Multi-strain probiotics consortium [35] SER-262 (NCT02830542) VE303 (NCT03788434)
Mycobacterium tuberculosis	Combinatory therapies with probiotics	–	Antibiotics or vitamins plus probiotics (*Bifidobacterium* spp. and *Lactobacillus* spp.) [44,45]
	Next-generation probiotics	–	*Bifidobacterium adolescentis* resistant to anti-TB drugs [46]
	FMT	[47]	–
	Postbiotics	Indolepropionic acid [38,39] Bacteria-derived AMPs [42,43]	–
Sars-Cov2	FMT	–	Pre-COVID19 as preventive setting [50, 51] Post-COVID19 as therapeutic for the cytokine storm (NCT04824222)

Table 2
List of microbiota-modulating tools in animal models and patients in immune-compromised conditions.

Immune compromised condition	Microbiota modulation tool	Studies in animal models	Studies in patients
Cancer therapies-associated Mucositis and Colitis	Probiotics	[63]	(Reviewed in 64)
	FMT	[65,66,67,68,69, 70], *Bifidobacterium* spp. and *Enterococcus gallinarum* [69,72]	(71, NCT03772899; NCT03637803)
HSCT and GvHD	Probiotics	*L. rhamnosus* GG [79]	*L. rhamnosus* GG [80] was inefficient (NCT027630331, NCT02805075, 81)
	Prebiotics	–	
	FMT	–	(83–86, NCT02269150; NCT03359980; NCT03492502; NCT03549676; NCT03720392)
HIV and AIDS	Probiotics	–	[100,101,102]
	FMT	In SIV infected primates [103]	(104, 105, NCT03008941)

Table 3
List of microbiota-modulating tools in animal models and patients having dysbiosis-induced inflammation in CNS disorders.

CNS disorders	Microbiota modulation tool	Studies in animal models	Studies in patients
Multiple Sclerosis	Probiotics	PSA+ *B. fragilis* or *Bifidobacterium* [122]	Multi-strain probiotics consortium [124]
	FMT	[120,123]	(125, NCT04150549; NCT03975413 and NCT03183869)
Parkinson's disease	Probiotics	*Bifidobacterium* spp., *Lactobacillus* spp. and *Lactococcus* spp. [133]	Planned clinical trials with; MRx0005 (*Parabacteroides distasonis*) and MRx0029 (*Megasphaera massiliensis*)
Alzheimer's Disease	Antibiotics	[140]	–
	Probiotics	Human isolates of *Bifidobacterium longum* [142]	*Lactobacillus* and *Bifidobacterium* multistrain probiotic [143,144]
	FMT	Transplant from diseased mice into GM one [139]	–

consequence, colonization resistance by the gut microbiota is disrupted and dysbiosis occurs. Protective species belonging to the phyla *Bacteroidetes* and *Firmicutes* are strongly reduced while species belonging to the phylum *Proteobacteria* largely expand [13,14]. Additionally, the antibiotics administered to eliminate pathogens further modify the structure of the microbiota and drastically disturb the process of colonization resistance [15]. Antibiotics may also favor the development of multidrug-resistance (MDR) or invasive species [16], contributing to the exacerbation of intestinal inflammation.

Infection-induced dysbiosis provokes the loss of commensals synthesizing bacteriocins and short-chain fatty acids (SCFAs) [17]. Similarly, dysbiosis reduces health-promoting *Clostridia* [18–20] and barrier-protecting species including *Bacteroides thetaiotaomicron* and *Akkermansia muciniphila* [21,22]. Pathobionts normally present in low abundance in healthy individuals expand during infections, becoming

Table 4
List of microbiota-modulating tools in animal models and patients suffering from cardiovascular diseases.

Cardiovascular disease	Microbiota modulation tool	Studies in animal models	Studies in patients
Atherosclerosis and coronary artery disease	Probiotics	*L. fermentum* MTCC:5898 [162] *S. thermophilis, L. acidophilus* LA-5 and *B. bifidum* BG-12 [163] *L. plantarum* DR7 or *L. plantarum* PH40 [165]	*Lactobacillus* and *Bifidobacterium* (NCT05095350)
	FMT	[151,166,167]	https://www.trialregister.nl/trial/4188, NTR4338 [168] (NCT04406129)
Chronic and acute heart failure	Diet	Choline-rich, TMAO-containing diet [174]	–
	Probiotic	–	*L. rhamnosus* GG [175] *L. acidophilus* (NCT03968549)
	FMT	[177]	–

dominant species [23,24].

In these contexts, restoration of the gut microbiota may be effective to support the resolution of inflammatory processes.

2.1. Community acquired bacterial infections

Microbiota-targeted therapies are highly effective for the treatment of *C. difficile* infection (CDI). *C. difficile* uses toxin-derived quorum sensing (QS) signals to regulate its persistence in the gastrointestinal (GI) tract, leading to dysbiosis [25]. Indeed, through this mechanism of action, *C. difficile* sustains the enrichment of indole-producing bacteria at the expense of beneficial indole-sensitive bacteria [26]. Antibiotics, including vancomycin and fidaxomicin, are an effective method to prevent and treat CDI, although an inappropriate usage contributes to CDI by compromising gut microbiota composition and colonization resistance [27].

Results from randomized clinical trials (RCTs) and metanalyses demonstrated the superiority of fecal microbiota transplantation (FMT) for recurrent and antibiotic resistant infections [28]. The seminal comparative study of Van Nood [29] demonstrated that 81 % of patients had resolution of recurrent CDI (rCDI) after a single duodenal fecal infusion compared with 31 % in the vancomycin group [29]. A systematic review of 2017, including seven randomized controlled trials and 30 case series, showed that clinical resolution occurred in 92 % of patients treated with FMT [30]. One caveat of FMT usage is the lack of standardization in the methods of collection, storage and administration to patients [28]. Different companies are trying to standardize FMT. Rebiotix-Ferring started the experimentation of the product RBX2660, a microbiota-based suspension derived from healthy donors' stools. This microbial product successfully met the primary endpoint in a phase 3 prospective, multicenter, randomized, double-blinded, placebo-controlled trial presented at DDW 2021 (PUNCHCD3, NCT03244644). The product had already demonstrated superiority to placebo in previous phase 2 trials in rCDI with no adverse effects [31]. However, two reported fatal cases of CDI patients treated with an FMT contaminated with a beta-lactamase producing *E. coli* led the Food and Drug Administration agency to generate a safety warning document and pushed the research of alternatives to FMT [32].

Indeed, oral capsules have been largely implemented as alternatives to endoscopy-administered FMT. The Openbiome initiative (https://www.openbiome.org) is pioneering this application field providing formulations of full spectrum lyophilized incapsulated microbiota. CP101 (Finch Therapeutics) is an oral capsule containing full-spectrum microbiota derived from donor stool. It is being investigated in a phase 2 double-blind, placebo-controlled, dose-finding trial in patients with rCDI (NCT03110133). RBX7455 (Rebiotix) is a lyophilized, broad-spectrum gut microbiota preparation in a room-temperature stable oral capsule. A single-center, three-arm phase 1 clinical trial of RBX7455 for treatment of rCDI is under way (NCT02981316). Seres Therapeutics investigated the efficacy of encapsulated spores derived from stool of healthy donors. In an exploratory study, 30 patients with rCDI were treated with SER-109 after they had a therapeutic response with oral antibiotics; CDI resolved in 96.7 % of patients [33] and gut microbial diversity increased significantly. In addition to the growth of organisms included in SER-109, nonSER-109 bacteria also increased in prevalence. For example, *Bacteroides* present normally in healthy individuals but not in the SER-109 product were augmented in 38 % of patients while *Klebsiella*, a pathogenic bacterium, decreased by 92 % already at week 4 [33]. However, a phase 2 study did not show that SER-109 was superior to placebo (44 % of patients in the SER-109 group had rCDI compared with 53.3 % in the placebo group; the difference was not statistically significant) [34]. A phase 3 multicenter, randomized, double-blind, placebo-controlled trial is under way (NCT03183128; Seres Therapeutics, Inc).

Recent alternatives to FMT include rationally designed microbial consortia growth in the laboratory under specific pathogen free conditions and assembled in defined proportions in commercially available products. Pioneer of the field is surely the a multi-strain probiotics consortium, composed by lactobacilli and bifidobacteria which also showed efficacy in reducing the incidence of antibiotic-associated diarrhea (AAD) in average-risk hospital patients exposed to systemic antibiotics [35]. More recently data for SER-262 (Seres Therapeutics, Inc) were presented at DDW2021. SER-262 is a fermentation-derived, rationally designed, oral microbiome therapeutic that contains bacteria from clades of healthy persons. The ability of SER-262 to prevent a first recurrence of CDI after appropriate antibiotic therapy for primary CDI was assessed in a recently completed phase 1b trial (NCT02830542). Similarly, VE303 (Vedanta Biosciences, Inc), an orally administered live bacterial consortium in powder form, is undergoing assessment for prevention of rCDI in a phase 2 clinical trial (NCT03788434); results are expected soon as the study finished in September 2021.

2.2. Mycobacterium (M.) tuberculosis

Tuberculosis (TB) is still one of the most deadly disease worldwide with an estimated 1.4 million deaths annually [36]. First line treatments include narrow spectrum antibiotics (*i.e.* isoniazid, pyrazinamide and ethambuthol) showing little or no activity outside the mycobacterial genus, often combined with the broad-spectrum antibiotic rifampin. However, prolonged TB-antibiotic treatments are often associated with structural changes in the microbiota [37]. Therefore, strategies aimed at promoting the restoration of a normobiotic microbiota such as dietary interventions and defined probiotic administration have been proposed.

Microbial metabolites and actively secreted bioactive molecules administered through dietary interventions are currently under evaluation in antibiotic treated-TB patients. Among them, microbiota-derived indolepropionic acid (IPA) has been shown to inhibit *M. tuberculosis* by antagonizing its tryptophan biosynthesis [38,39]. Additional positive effects of IPA on epithelial barrier restitution and on the activation of innate and adaptive immune responses have been proposed [40,41]. Bacteriocins isolated from *L. salivarius, Streptococcus cricetus*, and

Table 5
List of microbiota-modulating tools in animal models and patients suffering from chronic inflammatory disorders.

Chronic inflammatory disorders	Microbiota modulation tool	Studies in animal models	Studies in patients
Rheumatoid Arthritis	Probiotics	*L. casei* [179,180]	*L. casei* [181,182] *L. rhamnosus* GR-1 and *L. reuteri* RC-14 [183] (NCT03944096)
	FMT	–	*L. rhamnosus* and *B. lactis* Bb12 (189, NCT03032354)
Type 1 Diabetes	Probiotics	–	*L. salivarius, L. johnsonii* and *B. lactis* (NCT03880760) Multi-strain probiotics consortium (NCT03423589) None of these trails have published results yet
	Synbiotic	–	*L. sporogenes* plus FOS [185]. (NCT03961347)
	FMT	–	Ongoing clinical trial (NCT04124211).
	GM bacteria	GM *Lactococcus lactis* secreting proinsulin and IL10 [188]	GM *Lactococcus lactis;* ongoing clinical trial (NCT03751007).
Inflammatory Bowel Disease	Antibiotics	Broad spectrum antibiotics [198] Metronidazole [199,200]	Ciprofloxacin, metronidazole and rifaximin [190,191,192,193,194,195]
	Probiotics	Multi-strain probiotics consortium [208] *Saccharomyces boulardii* [212]	*E. coli Nissle 1917* [206,207] Multi-strain probiotics consortium in UC patients [209,210] *Saccharomyces boulardii* (213−215)
	FMT	[199,219]	Clinical trials in mild-moderate UC patients (218, 220, 224, 225, STOP-Colitis, NCT03016780, NCT02390726) IMPACT-Crohn study [223]
	GM bacteria	GM *Lactococcus lactis* [226] GM *E. coli* Nissle [236] *E.Coli* MDS42 -mediated RNA interference [237]	GM *Lactococcus lactis* (227, 228, NCT00729872)
	PT	PT toward *E. Coli* AIEC [243]	PT toward *K. pneumoniae* BX002-A, BX003 (NCT04737876) PT toward *E. coli* AIEC (NCT03808103)

Enterococcus faecalis demonstrated *in vitro* antimycobacterial activity which exceeds that of the TB antibiotic rifampicin [42], with nisin and lacticin being effective towards *M. tuberculosis*, *M. kansasii*, *M. smegmatis*, and *M. avium* subspecies *paratuberculosis* [43]. Synergism with TB antimicrobials may therefore allow for shortening of current antibiotics regimens or reducing antibiotic dosing to limit toxic side effects.

Combinatory therapy of antibiotics and probiotics such as *Bifidobacterium* spp. are reported to restore normobiosis in TB patients [44, 45]. A longitudinal study reported that a multi-strain probiotic formulation (*Lactobacillus (L.) acidophilus*, *L. casei*, *L. rhamnosus*, *L. bulgaricus*, *Bifidobacterium (B.) breve*, *B. longum*, and *Streptococcus thermophilus*) combined with supplementation of vitamins B1, B6, and B12 increased serum concentrations of IFNγ and interleukin (IL-)12, needed for the resolution of infection. Next generation probiotics, including rationally designed strains resistant to TB therapy are currently under study [46].

FMT demonstrated to reverse the increased susceptibility of antibiotic-treated mice to *M. tuberculosis* infection [47], but unfortunately no data are currently available for FMT administration in TB patients.

2.3. SARS-CoV2

Microbiota-replacing therapies have been proposed in patients infected with SARS-CoV2. Recent pivotal papers investigated the connection between gut microbiome dysbiosis and COVID-19 severity and proposed that SARS-CoV-2 infection may also disturb the intestinal microbial ecology. Interestingly, it has been shown that SARS-CoV-2 can undergo prolonged shedding in stool and that gut microbiome perturbations may associate with COVID-19 severity [48,49]. Recently, FMT treatment contributed to reduce the severity risk of COVID19 [50]. Indeed, FMT-treated CDI patients subsequently infected by SARS-CoV2 experienced only mild clinical course of COVID-19, albeit they had risk factors for severe features/adverse outcomes of COVID-19 (*i.e.* frailty/comorbidities and immunosuppression) [50]. Authors hypothesized that FMT might have mitigated more adverse outcomes, potentially through impacting microbiome-immune interactions [50]. Similar results were obtained by Gasbarrini and colleagues [51]. An RCT has

been recently submitted in clinicaltrial.gov, aimed at evaluating the impact of FMT for risk reduction of COVID-19 disease progression (FeMToCOVID) (NCT04824222).

3. Microbiota-targeted approaches to resolve dysbiosis-induced inflammation in immune-compromised individuals

Individuals receiving immunosuppressive agents or diagnosed with human immunodeficiency virus (HIV) infection, acquired immune deficiency syndrome (AIDS), inherited or primary immunodeficiency syndromes and hematologic or solid malignancies are considered immunocompromised. All these individuals carry different types of dysbiosis, which may influence therapies outcomes.

In this context, microbial-modifying treatments may help in restoring normobiosis, sustaining the immune system functions. Among them, FMT is identified as the most promising therapeutic approach, but its use has been limited due to the perceived risk of transferring microorganisms with potentially unknown functions in these categories of fragile individuals [52]. In light of these concerns autologous FMT or rationally designed consortia may offer a solution with lower risks.

3.1. Cancer therapies-associated mucositis and colitis

Prolonged anti-cancer therapies generate chronic toxicities inducing fibrosis, vascular damage and atrophy of the affected tissue or organ [53]. Moreover, it is well demonstrated that numerous anti-cancer agents significantly disrupt the intestinal microbiota, shifting its diversity towards a Gram- enterotype [54]. The cytotoxic damage to the intestinal lining (mucositis) induced by anti-cancer therapies combined with the outgrowth of opportunistic Gram- microbes predisposes patients to secondary infections and various complications. On top of that, when mucosal ulceration occurs inflammation is propagated by bacteria translocation. Microbial interactions with immune cells in the breached epithelium modulate oxidative stress responses, inflammatory processes and intestinal permeability [55]. However, the gut microbiota can contribute to the mucus layer regeneration and epithelial repair [56]. Thus, microbiota-targeted intervention may have a supportive role by preventing acute toxicities and secondary complications.

Radiotherapy causes major changes in the gut microbial composition [57,58] and germ free (GF) mice are resistant to lethal radiation enteritis, indicating that microbiota controls intestinal disease processes consequent to radiation-induced damage [59]. Antibiotics-treated mice show a higher survival rate upon irradiation than not treated mice [60]. Cytotoxic chemotherapy or radiotherapy generally induces increases in *Bacteroides* and *Enterobacteriaceae* and decreases in *Bifidobacterium*, *Faecalibacterium prausnitzii*, and *Clostridium cluster XIVa* [61]. Pelvic radiotherapy can substantially decrease *Firmicutes* and increase *Fusobacterium* phyla [57], while patients with rectal cancer and partial response to chemo-radiation show an increase of *Bacteroidales* [57]. Patients who experienced diarrhea, a severe side effect of anticancer therapies, were shown to have increased *Bacteroides, Dialister, Veillonella* and reduced *Clostridium XI* and *XVIII, Faecalibacterium, Oscillibacter, Parabacteroides*, and *Prevotella* [57]. Some evidences also suggests that patients undergoing radiotherapy have a high incidence of CDI, which is associated with high mortality rates [62].

Despite strong preclinical support in animal models [63], the efficacy of probiotic formulations to counterbalance cancer therapies-derived toxicities in humans has been limited and data are controversial. A recent meta-analysis of six randomized controlled trials investigated the activity of oral probiotics in limiting post-radiotherapy diarrhea [64]. However, the heterogeneity of patients-inclusion criteria, the presence or absence of concomitant chemotherapy, the end-point assessment and the types of bacteria used as probiotics limit the possibility to draw definitive conclusions. Few studies evaluated the contribution of FMT in the resolution of radiation-induced dysbiosis and inflammation. In a preclinical setting, FMT reversed antibiotic- and chemotherapy-induced gut dysbiosis in mice [65]. Another preclinical study investigated the effect of FMT on FOLFOX-induced mucosal injury. BALB/c mice implanted with syngeneic CT26 colorectal adenocarcinoma cells were injected with FOLFOX concomitantly with FMT. Normobiosis restoration by FMT reduced the severity of chemotherapy-induced diarrhea and intestinal mucositis [66].

Immunotherapies targeting negative regulators of T cell activation, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are associated with a higher risk of developing severe colitis [67]. Certain bacterial signatures have been shown to associate with differential responses to immunotherapies [67,68], both in terms of treatment efficacy and toxicity. Indeed, targeting of CTLA-4 was not efficient in the treatment of tumors in antibiotic-treated or GF animals [68,69]. Since the antitumor efficacy of the CTLA-4 blockade was dependent on distinct *Bacteroides* spp., microbial fecal transplantation of *Bacteroides* spp.-rich feces from humans into GF mice induced a significant response to CTLA-4 blockade and negatively correlated with tumor size in recipient mice [68,69]. Moreover, modulation of the gut microbiota by patient's derived FMT alters antitumor immunity and response to immunotherapy in gnotobiotic mice [70]. Altogether these evidences suggest that FMT may be a valid therapeutic option for refractory immunotherapy-induced colitis [71]. In 2018, the first case series of immune checkpoint inhibitor- associated colitis successfully treated with FMT was published and identified an increase in the proportion of regulatory T cells (Tregs) post FMT treatment [71]. A phase 1, unblinded single-arm study is currently underway to investigate the efficacy of donor FMT as a prophylactic, adjunct supportive care measure in melanoma patients (NCT03772899). Recently, the candidate live biotherapeutic MRx0518 was studied for its proven anti-tumorigenic effects and immune-stimulatory properties. Indeed, the strain MRx0518 *Enterococcus gallinarum* elicits a strong pro-inflammatory response in key components of the innate immune system, including toll-like receptor (TLR) 5 and NFkB, but also in intestinal epithelial cells inducing IL-8 production. Mechanistic studies indicated that flagellin produced by MRx0518 may play a role in its therapeutic properties [72]. MRx0518 is now being tested in a phase 1/2 trial in combination with pembrolizumab for solid tumors with acquired resistance to checkpoint immunotherapy (NCT03637803).

3.2. Hematopoietic stem cell transplantation (HSCT) & Graft versus host disease (GvHD)

In patients undergoing extensive conditioning regimens for HSCT, which include high dose chemotherapy and radiation therapy, mucosal barrier disruption allows translocation of bacteria into the blood circulation resulting in bloodstream infections (BSI) and sepsis. *Enterobacteriaceae, Staphylococci, Enterococci, P. aeruginosa*, and *Streptococci* represent the most common BSI pathogens [73]. Microbiota-modulating therapies in these patients may thus not only reverse dysbiosis but also decrease the risk of infectious complications. Longitudinal analysis of the microbiome of HSCT recipients showed a reduction in obligate anaerobes, associated with an expansion of pathogenic species (including *Viridans*-group *Streptococci* and vancomycin-resistant *Enterococcus*) [74]. Conversely, increased bacterial donor diversity reduced the risk of allogenic-(a) GvHD [75] and associated to lower mortality rate [76]. Probiotic usage in HSCT patients has been proposed and tested, with no patients developing BSI even though the clinical outcomes were mostly inconsistent [77,78]. Preclinical studies in mice indicated that administration of the probiotic *L. rhamnosus* GG reduced the incidence of aGvHD [79], but the first randomized clinical trial on allo-HSCT patients supplemented with *L. rhamnosus* GG did not show changes in the incidence of GvHD [80].

Dietary fibers fermented by gut microbiota (such as starches, fructooligosaccharides (FOS) and galactooligosaccharides (GOS)), commonly known as prebiotics, can alter microbiota composition while minimizing the risk of bacteremia in immunocompromised populations. A retrospective study showed that the combined supplementation of glutamine, fiber and FOS in HSCT patients effectively decreased the severity of mucosal damage post-transplant, mucositis and diarrhea. Accordingly, survival at day 100, weight loss and the number of intravenous hyperalimentation were better in patients on prebiotics compared to those who did not receive the supplementation [81]. Two clinical trials are currently investigating the association between potato starch and risk of GvHD (NCT027630331) and tolerability of HSCT patients to FOS (NCT02805075) [82]. Several small studies have demonstrated a beneficial effect of FMT on remission of GvHD in allo-HSCT patients [83–86]. A case study with 4 patients has shown donor FMT was efficient to control acute intestinal steroid-refractory and -dependent GvHD, with 3 out of 4 patients showing complete resolution [83]. Encapsulated FMT, as well as nasogastric tube delivery, were also successful in treating GvHD [84,85]. FMT was capable to restore microbiota composition and increase diversity with the abundance of *Bacteroidetes, Bacteroidaceae, Ruminococcaeae* and *Desulfovibrionaceae* accompanied by amelioration of clinical symptoms including stool volume, abdominal pain and longer progression-free survival [86]. Auto-FMT may be preferable to allo-FMT due to a lower risk of GvHD insurgence, albeit risks associated with the reintroduction of HSCT-associated pathobionts exist. FMT demonstrated efficacy in the safe eradication of MDR organisms (MDRO), as shown in small study involving 10 allo-HSCT patients [87]. Additional ongoing clinical trials (NCT02269150; NCT03359980; NCT03492502; NCT03549676; NCT03720392) are currently evaluating the impact of FMT on HSCT patients [87].

3.3. Human immunodeficiency virus (HIV) & acquired immunodeficiency virus (AIDS)

HIV is a retrovirus with CD4 + T cell tropism leading to a severe immunodeficiency, alteration of the intestinal barrier [88] and rapid and massive destruction of CD4 + T lymphocytes. Infected individuals manifest HIV-associated intestinal lesions ("HIV enteropathy") characterized by inflammation and mucosal atrophy [89], which persist despite antiretroviral therapy (ART) [90,91]. During HIV enteropathy,

intraepithelial cells display a downregulation of host genes associated to epithelial barrier and gut microbiota regulation [89,92].

Report of HIV-associated dysbiosis are discordant, yet available data show a major depletion of *Bacteroides* in favor of *Proteobacteria* [93], an enrichment of pathobionts (*Enterococcus, Streptococcus, Staphylococcus, Salmonella,* and *Escherichia* species) and a significant reduction of symbionts throughout the entire intestinal tract [94]. Importantly, dysbiosis seems to be aggravated by ART [95,96]. Patients with immunologic failure on ART manifest an increase of *Enterobacteriaceae* instead of *Lactobacillus* [97]. Moreover, butyrate synthesized by dominant species (*Fusobacterium nucleatum, Clostridium cochlearium,* and *Eubacterium multiforme*) in HIV patients is capable to reactivate both unintegrated HIV-1 genomes and latent HIV proviruses through histone deacetylase (HDAC) inhibition [98,99].

Current microbial modulating therapies in HIV involve the administration of probiotics [100,101] [102] and FMT [103–105]. Administration of probiotics has been tested in several studies on people living with HIV (PLHIV) [106,107]. However, PLHIV are continuously on ART [108], which showed improved prognosis if compared to probiotics alone, therefore hindering the direct evaluation of probiotics efficacy in this context.

Few small studies investigated the administration of FMT in HIV infected subjects or SIV-infected primates [103–105]. Among them, the most successful and recent study [105] involved 30 HIV-infected subjects on ART with a CD4/CD8 ratio < 1 that were administered weekly fecal microbiota capsules or placebo for 8 weeks. FMT attenuated HIV-associated dysbiosis increasing significantly the α-diversity of FMT-treated patients. The *Lachnospiraceae* and *Ruminococcaceae* families, which are typically depleted in people with HIV, were the taxa more robustly engrafted across time-points (NCT03008941).

4. Microbiota-targeted approaches to resolve dysbiosis-induced inflammationin Central Nervous System (CNS) disorders

The bidirectional communication between the gut and the CNS is of utmost importance in maintaining homeostasis of the two biological systems [109] Indeed, the gut microbiota affects the development of neural systems that govern the endocrine response to stress. GF mice show exaggerate hypothalamic–pituitary–adrenal responses and reduced brain-derived neurotrophic factor (BDNF) levels compared to gnotobiotic mice. This phenomenon can be reversed by treatment with probiotics or by restoring the microbiota during early stages of development [110].

Alterations in the composition of the gut microbiota have been implicated in a wide variety of neurological disorders, among which Multiple Sclerosis (MS), Parkinson's Disease (PD), Alzheimer Disease (AD) and neuropsychiatric disorders like major depressive and mood disorders [111–114]. We are at the infancy of understanding the role of gut microbes in the development of these disorders; we don't know yet if gut dysbiosis is merely a consequence or a cause of these diseases. However, there is increasing evidence that circulating microbial-derived pro-inflammatory mediators may contribute to the progression of CNS disorders [115]. To note, up to 40 % of patients with IBDs reveals psychosocial disturbances [116]. A recent seminal work showed that intestinal inflammation leads to dissemination of microbiota-derived lipopolysaccharides (LPS) due to impairments of the gut vascular barrier, important to control the dissemination of bacteria from the intestine to the liver [117]. In this context, the inflammatory response promotes the concomitant closure of choroid plexus endothelial cells leading to deficit in short-term memory and anxiety-like behavior [118]. Thus, this work suggests that IBD-associated neurological abnormalities are a consequence of deregulated gut–brain vascular axis due to microbiota-mediated systemic inflammation [118] and that manipulation of the microbial dysbiosis may regulate not only inflammatory responses but also counterbalance neurological disturbances.

4.1. Multiple sclerosis (MS)

MS is a CNS-related autoimmune disorder characterized by neuroinflammation, infiltration of lymphocytes into the CNS, demyelination and axonal loss. The gut microbiota of MS patients significantly differs from that of healthy subjects; even more strikingly, the gut microbiota differs between MS patients with active disease *vs* patients in the remission phase [119,120]. The evidence of a gut-brain connection in MS is provided by different translation approaches. In the experimental autoimmune encephalomyelitis (EAE) model of MS, colonization of the gut microbiota is required for the induction of the EAE. GF mice show less severe EAE whereas colonization with Segmented Filamentous Bacteria results in dysregulated Th17 responses which exacerbate the severity of the disease [121]. On the contrary, treatments with polysaccharide A+ (PSA+) *B. fragilis* or *Bifidobacterium* alleviate EAE symptoms [122]. The strong immunomodulatory role of the gut microbiome in MS was elegantly demonstrated by transplanting feces from diseased individuals into GF animals that exhibited one of the hallmark symptoms of MS, *i.e.* autoimmune encephalomyelitis. The animal's symptoms correlated with an increase in relative abundance of *Akkermansia,* which is significantly higher in MS patients, together with the species *Acinetobacter calcoaceticus* [120,123]. On the other hand, MS patients also exhibited reduced levels of *Parabacteroides distasonis*, a species associated with anti-inflammatory activity. Accordingly, mice "humanized" with microbiota from MS patients had reduced proportions of IL-10-producing Tregs [120,123]. Thus, much interest is now focused on possible therapeutic intervention *via* the gut microbiota. A change in the microbiota composition was observed in MS patients treated with a multi-strain probiotics consortium, but not in healthy controls [124]. FMT in MS patients reverted their neurological symptoms although they were treated for underlying GI symptoms and constipation [125]. Three clinical trials are currently ongoing to test the efficacy of FMT in MS patients (NCT03183869, NCT03975413 and NCT04150549). Overall, these studies demonstrate that different therapeutic strategies targeting the microbiome may be effective in MS.

4.2. Parkinson's disease (PD)

PD is a progressive nervous system disorder that affects voluntary movements. Motor function deterioration is due to accumulation of misfolded α-synuclein (αSyn) and loss of dopamine-producing neurons [126]; patients also typically suffer from GI symptoms that can precede PD diagnoses by many years [127]. Gut inflammation and accumulation of αSyn in the enteric nervous system and vague nerve suggest that the PD etiopathology may start in the gut [128]. Indeed, PD patients have an altered gut microbiota composition compared to heathy individuals characterized by the enrichment of pro-inflammatory *Enterobacteriaceae* and depletion of anti-inflammatory SCFAs-producing bacteria such as taxa from *Lachnospiraceae* family and *Faecalibacterium prausnitzii* [129–131]. The gut microbiota contributes to PD pathophysiology also by reducing the efficacy of anti-PD drugs and increasing the rates of levodopa (L-dopa) drug inactivation [132].

The first study to demonstrate mechanistically the role of the microbiota in PD pathophysiology used a translational approach in which transplantation of gut bacteria from individuals with PD into GF mice replicated some PD-like motor symptoms [111]. Administration of a probiotic mix containing *Bifidobacterium, Lactobacillus* and *Lactococcus* strains significantly attenuated the deterioration of motor dysfunctions in the MitoPark PD mouse model [133]. A novel probiotic formulation (SLAB51) showed to control the toxic effects of 6-hydroxydopamine *in vitro* and *in vivo* in models of PD [134]. Because of the role that the microbiota has in neuroinflammatory processes, 4D is now planning a first-in-man study in patients with PD by using the live biotherapeutic candidates, MRx0005 (*Parabacteroides distasonis*) and MRx0029 (*Megasphaera massiliensis*), which showed *in vitro* neuroprotective properties [135]

4.3. Alzheimer's disease (AD)

The gut microbiota has a role also in AD, the leading cause of dementia worldwide. The brain pathology associated with AD includes the formation of insoluble beta-amyloid precursor protein (Ab) deposits and hyperphosphorylated tau protein in the brain that trigger a cascade of pathological events leading to dementia. Several microbial factors have been linked to AD pathogenesis. In AD patients, alterations in the gut microbiota composition have been observed with decreased abundance of *Firmicutes* and *Bifidobacterium* and increased abundance of *Bacteroidetes* and *Enterobacteriaceae*; these shifts correlate with inflammation and increased expression of amyloid proteins [136,137].

The mouse models APP/PS1 line and the 5XFAD transgenic mice exhibit marked changes in the gut microbiota correlating with neuro-inflammatory markers similar to human AD [138–141]. Fecal transplantation from conventionally-raised APP/PS1 mice into GF APP/PS1 hosts increased cerebral Ab pathology while antibiotic-induced microbiota depletion attenuates inflammation and brain pathology [139,140]. Metabolic analyses revealed significant changes in amino acid–related metabolism affecting principally Th1 immune responses [138]. The translational value of the microbiota in AD is therefore under evaluation. Supplementation of the *Bifidobacterium longum* (NK46) human isolates in 5xFAD-transgenic mice promotes an anti-inflammatory response associated with shifts in gut microbiota composition, reduction of fecal and blood LPS levels, suppression of NF-κB activation and TNF-α expression; NK46 treatment also alleviated cognitive decline in 5XFAD-Tg [142]. A randomized, double-blind, placebo-controlled trial using a *Lactobacillus* and *Bifidobacterium* multistrain probiotic product showed improvements in the Mini Mental State Exam [143]. However, another clinical trial evaluating the efficacy of probiotic supplementation in AD showed no improvement in cognition [144].

5. Microbiota-targeted approaches to resolve dysbiosis-induced inflammation in cardiovascular disease (CVD)

CVD consists of multiple disorders, including atherosclerosis, coronary artery disease (CAD), acute and chronic heart failure (CHF), arrhythmia, atrial fibrillation and heart valve complications with an increasing prevalence in the elderly [145]. Recent studies have highlighted the connection between CVD and the gut microbiota [146,147]. Dysbiosis, along with alterations in microbiota-derived metabolites (*i.e.* choline-derived trimethylamine-N-oxide (TMAO)), represents a newly established risk factor for the development of CVD [148–150] and augments pathologic conditions such as hypertension [151]. Hypertensive subjects display an increase in the *Firmicutes* to *Bacteriodes* ratio of their gut microbiota that is limited by antibiotic treatment in mice, resulting in effective lowering of the blood pressure [152,153]. In light of these findings, targeted therapies aimed at restoring normobiosis, intestinal integrity and microbiota-derived metabolites are conceived. The role of prebiotics, probiotics and synbiotics in CVD are deeply reviewed by Olas and by Oniszczuk et al. [154,155] Thus, here we will focus on the most effective microbiota treatments and the most recent clinical trials.

5.1. Atherosclerosis and coronary artery disease (CAD)

The gut microbiota can be involved in the progression of CAD by accelerating the formation of atherosclerotic plaques upon colonization. Interestingly, bacterial DNA was found in atherosclerotic plaques [156] and patients with symptomatic, stenotic plaques were characterized by the unique presence of the genus *Collinsella* in their intestinal microbiota [157] The gut microbiota of CAD patients has a greater α-diversity than healthy controls, with abundance of *Firmicutes* and *Fusobacterium*, *Enterobacteriaceae* and *Streptococcus* spp. [112,158]. Dysbiosis and disruption of epithelial integrity cause bacterial-metabolites translocation and systemic inflammation. The microbial-derived metabolite

TMAO plays a major role in the formation of atherosclerotic plaques, showing dose-dependent association with plaques size, myocardial infarction and it represents a long-term risk factor for adverse cardiac events [159,160] On the contrary, SCFAs exhibit anti-inflammatory properties, maintain barrier integrity, and regulate blood pressure [161] Administration of several composition of fermented milk (*i.e. L. fermentum* MTCC:5898) in rodents showed significant effectiveness in the treatment of CAD by lowering the systemic inflammation, the systolic blood pressure and the atherogenic index [162]. Moreover, administration of a probiotic consortium containing *S. thermophilis*, *L. acidophilus* LA-5 and *B. bifidum* BG-12, as well as administration of *L. plantarum* DR7 or *L. plantarum* PH40 showed cholesterol lowering properties [163–165] An interventional double-blind clinical trial (NCT05095350) is now recruiting to assess the effect of a probiotic powder composed of *Lactobacillus* and *Bifidobacterium* in the management of hypertension.

Microbiota transplant from hypertensive donors into GF mice exacerbated CVD symptoms such as blood pressure and vascular inflammation [151,166]. On the contrary, FMT from healthy donors' stool to hypertensive rodents decreased their disease state modulating the sympathetic nervous activity, the production of arachidonic acid and increasing the presence of healthy gut bacterial species such as *Bacteroides fragilis* [166,167]. A first interventional clinical trial tested the effect of vegan/vegetarian donor FMT on the production of TMAO and subsequently on the vascular inflammation in patients with metabolic syndrome (https://www.trialregister.nl/trial/4188, NTR4338). Vegan/vegetarian FMT resulted in detectable changes in the recipient gut microbiota, however it failed to change levels of TMAO [168]. Currently restoration of the gut microbiota composition on primary hypertension upon FMT is under evaluation in a double-blind clinical trial (NCT04406129), results are expected by mid 2022.

5.2. Chronic and acute heart failure (HF)

An innovative theory connects heart failure with dysbiosis and damaged intestinal permeability. The heart-intestine axis hypothesis suggests that reduced cardiac outflow in CHF causes a lower blood flow in the intestine, modifying intestinal permeability, absorption and inflammation [169,170] In a first observational study, patients with severe CHF were characterized by a dysbiotic gut microbiota rich in the pathogenic bacteria *Candida Campylobacter* and *Shigella*, that correlated with disease severity, intestinal permeability and inflammation [147]. Other studies confirmed the dysbiotic state of CHF patients with the increase of *Ruminococcus gnavus* and the decrease of *Faecalibacterium prausnitzii* [171,172]. Moreover, there is an imbalance in the production of TMAO in CHF patients [171]. TMAO is now considered a valuable prognostic indicator of major adverse cardiac events in heart failure (HF), with elevated TMAO levels in both chronic and acute HF patients [173]. Administration of choline-rich or TMAO-containing diet to C57BL6/J mice significantly enhanced HF severity and myocardial fibrosis, affirming their role in CVD progression [174]. The probiotic administration of *L. rhamnosus* GG to patients with coronary artery disease resulted in improved cardiovascular-related factors and decreased serum levels of the inflammatory cytokine IL1β [175]. An interventional clinical study is assessing the effect of the probiotic strain *L. acidophilus* in patients with HF in terms of dysbiosis reconstitution and inflammatory index (NCT03968549). However, no results have been published yet. Interestingly, also myocarditis, a condition caused by inflammation of the heart muscle that ultimately leads to HF [176], is characterized by a dysbiotic gut microbiota [177]. Hu et al. have recently investigated the gut microbiota composition of experimental autoimmune myocarditis (EAM) mouse model and found an increase in microbial richness, diversity and in the *Firmicutes* to *Bacteroides* ratio [177]. They also evaluated the efficacy of FMT in the myocarditis setting by administration of healthy donor stools to EAM mice. FMT-treated EAM animals showed a rebalanced *Firmicutes/Bacteroides* ratio, a

decreased inflammatory condition with lower expression of *IFN-Y* in the heart and a general amelioration of the myocarditis [177].

6. Microbiota-targeted approaches to resolve dysbiosis-induced inflammation in chronic disorders

The composition and function of the intestinal microbiota of patients suffering from autoimmune diseases, including IBD, Rheumatoid Arthritis (RA), and Type1 Diabetes (T1D) is profoundly altered. Although each immune disease carries a specific dysbiotic signature, their luminal or mucosal microbiome is steadily different from that of first-degree relatives or healthy controls and their microbial dysbiosis often precedes the onset of the disease [178]. In these patients the genetic predisposition, the environmental cues, the pharmacological treatments as well as the aberrant chronically activated immune system, along with alteration in the epithelial barrier functions contribute to generate and fuel intestinal dysbiosis.

Targeting gut dysbiosis may be pivotal to end the vicious circle of inflammation-dysbiosis observed in these patients. Currently, the majority of trials targets the inflammation/dysbiosis axis restoration by probiotics (both consortia and single strains) and by FMT. Some new products consist of bioengineered bacteria secreting proteins, such as interleukins, to reduce autoimmune responses and are currently under evaluation mainly in preclinical models of IBD.

6.1. Rheumatoid arthritis (RA)

Although there is evidence that anti-inflammatory therapies combined with microbial targeted approaches strongly complements RA patients' dysbiosis, few studies are currently available. *L. casei* was tested in animal models of arthritis [179,180], showing a decreased incidence of the diseases in association with reduced production of inflammatory cytokines [179,180] In RA patients, supplementation of encapsulated, active *L. casei* [181] induced a reduction of pro-inflammatory cytokines and an increase of anti-inflammatory IL-10, associated with the reduction of the disease activity scores for tender and swollen joints. Combined administration of *L. casei*, *L. acidophilus* and *B. bifidum* in capsules was sufficient to reduce the disease activity score; however, tender and swollen joints scores were not decreased [182]. Administration of both *L. rhamnosus* GR-1 and *L. reuteri* RC-14 in capsules to RA patients in a randomized, double- blind, placebo-controlled clinical trial highlighted a decrease in circulating pro-inflammatory cytokines, although no overall clinical improvement was reported [183]. One clinical trial (NCT03944096, FARM) is currently testing FMT for treating RA patients but no results have been released yet.

6.2. Type I diabetes (T1D)

Currently several studies investigate the possibility to use microbial-modulating therapies to control hyperglycemia. Following successful preclinical studies in animal models of T1D [184], a first clinical trial in children diagnosed with T1D evaluated the efficacy of a synbiotic administration of *L. sporogenes* and FOS for 8 weeks [185]. The synbiotic mix improved glycemic indices as compared to a placebo control group [188]. Another clinical trial (NCT03961347) is currently evaluating the effect of supplementation of *L. johnsonii* to adults with T1D, based on favorable preclinical results [186–188].

Few clinical trials are evaluating the effects of a bacteria consortia on T1D patients. In a first trial (NCT03032354), *L. rhamnosus* and *B. lactis* Bb12 were administered to children with T1D [189]. In a second one (NCT03880760), the mix was composed by *L. salivarius, L. johnsonii* and *B. lactis*. While the third multiple bacterial therapy clinical trial (NCT03423589) was designed to administer the a multi-strain probiotics consortium to T1D patients. None of these trails have published results. Currently there is only one clinical trial evaluating the benefit of FMT in

T1D (NCT04124211), but it is still in the recruitment phase.

To note, an innovative trial (NCT03751007) in the United States and Belgium is testing the use of genetically engineered bacteria, *Lactococcus lactis* secreting proinsulin and IL10 (AG019-Precigen Actobio T1D, LLC). This genetically modified bacterial strain was firstly tested on NOD mice, in which its administration plus anti-CD3 therapy induced a reduction in the hyperglycemia [188].

6.3. Inflammatory bowel disease (IBD)

Given the close interconnection between gut microbiota dysbiosis and the aberrant activation of the mucosal immune system in IBD, microbe-targeted therapies aimed at restoring gut normobiosis and immune homeostasis are promising therapeutic options. Among them, antibiotics, pre and probiotics, FMT and consortia administration are the most widely implemented in preclinical and clinical studies. Antibiotics have been used to control intestinal inflammation by selectively eliminating specific pathobionts; ciprofloxacin, metronidazole or rifaximin are largely used in the clinical practices [190]. Ciprofloxacin and metronidazole are commonly administered to Crohn's Disease (CD) patients [191] and are effective for anal lesions and delay of postoperative recurrence [192] by reducing TNF-α, IL-1β and IL-8 [193] or by inducing long-term changes in the immune phenotype of Treg and naive T-cells [194]. Rifaximin, a non-absorbable antibiotic, showed an excellent safety profile coupled with a reduction of colonic inflammation and bacterial translocation in the mesenteric lymph nodes (mLNs) [195]; however, it does not yet have validated efficacy [196]. Despite some favourable clinical effects, the use of broad-spectrum antibiotics hampers the reconstitution of the gut microbiota and promotes a pro-inflammatory phenotype in the long term. Indeed, we recently demonstrated how a short-term treatment with broad-spectrum antibiotics profoundly affected the frequency and function of intestinal invariant natural killer T (iNKT) cells, a population of T lymphocytes capable of recognising the mucosal microbiota in IBD patients [197], but not of CD4 + T cells in the absence of intestinal inflammation [198]. Reconstitution of the gut microbiota after antibiotic treatment was sufficient to imprint colonic iNKT and CD4 + T cells toward a Th1-Th17 pro-inflammatory phenotype, aggravating clinical conditions upon intestinal inflammation [198]. On the other hand, we recently showed that not all antibiotics are alike and metronidazole administration in colitic mice [199,200] efficiently controls the outgrowth of pathobionts, supporting instead the maintenance of SCFA-producing taxa which, in turn, sustain the activity of anti-inflammatory mucosal T cell populations *in vivo* and *ex vivo* [200].

Similarly, the use of probiotics may modulate dysbiosis in IBD patients [3], through their strain-specific metabolisms and metabolic by-products (*i.e.* SCFAs, bacteriocins, hydroperoxides, secondary bile acids, and lactic acids) [201]. Different strains of *Lactobacillus* and *Bifidobacterium* showed significant capability to reduce pro-inflammatory IL-6 and IL-17 levels [202], restoring the Treg/Th17 balance [203] and to control the overgrowth of pathobionts belonging to *Enterobacteriaceae* [204,205]. *E. coli* Nissle 1917 (*EcN*) can colonize the intestine and perform several documented protective functions, including IL22 -mediated epithelial restitution [206] and Treg expansion [207]. Thus, it's being successfully used to extend remission phases in IBD patients in the clinical practice. The treatment with a multi-strain probiotics consortium has been shown to possess anti-inflammatory properties in experimental colitis [208] and in different randomized, double-blind, placebo-controlled trials. The use of this probiotics mix in Ulcerative Colitis (UC) patients showed significant effects in terms of clinical remission and clinical response during active UC and pouchitis with no side-effects [209,210].

Not only bacteria but also fungi are used as probiotics. *Saccharomyces boulardii* is a well characterized probiotic yeast often used to alleviate GI tract disorders [211]. Moreover it showed efficacy in preclinical models of IBD in alleviating symptoms by trapping pathogenic T cells in the

mLNs [212] and it was tested in both UC [213,214] and CD patients [215].

Nevertheless, probiotics effects are often transient and limited in most IBD subsets because of i) inability to replace/restore the microbial species depleted, ii) colonization resistance, since the individual immunological status and mucosal microbial features are associated with probiotics persistence, iii) treatment timing and proper delivery mode [216].

Although it is clear that in rCDI FMT efficiently eliminates the pathogen and its virulence factors [217], less it is known on the mechanisms behind the therapeutic effects of FMT in IBD. Different RCTs have been performed so far in IBD patients, with those performed in mild-moderate UC giving the best results [218]. Mechanistically, we recently showed that therapeutic FMT administered during acute [199] and chronic [219] experimental colitis restores eubiosis and directly modulates both innate and adaptive mucosal immune responses towards the control of intestinal inflammation. Therapeutic FMT not only is able to reduce colonic inflammation, as demonstrated by decreased levels of the pro-inflammatory cytokines TNFa, IL1-β and IFN-γ, but also initiates the restoration of intestinal homeostasis through the simultaneous activation of different immune-mediated pathways [199]. Indeed, higher amounts of colonic IL-10 as well as increased frequencies of IL-10-producing APC, CD4 + T and iNKT cells were observed in FMT-treated mice as compared to untreated ones [199].

These preclinical evidence on the anti-inflammatory role of FMT have been confirmed by several RCTs in IBD patients. In a single-centre, prospective, open-label pilot study, the impact of FMT preparation and donor characteristics on the therapy success was evaluated [220]. Immune cell profiling was performed on mucosal biopsies before and after FMT to assess its impact on mucosal T cell immunity. Analysis of CD4 + T cell cytokine production revealed a significant reduction of IFNγ in Tregs at week 4 compared to time of transplant, however no difference in IL4, IL17, IL22 or Th17 was reported [220]. Moreover, specific members of the gut mycobiota can play a protective function in the gut [221]. Getting a better knowledge on host response to these organisms might harbour potential predictive markers on the outcome of microbiome-based therapies and should be further explored in future FMT trials [222]. The IMPACT-Crohn study showed that FMT is efficacious also in CD patients, showing that higher donor colonization associated with maintenance of remission [223]. The STOP-Colitis study is evaluating not only the efficacy and safety of FMT in UC patients, but also the colonic immune profile of recipients before and after FMT. Furthermore, a Chinese FMTFUC study is evaluating local and systemic inflammatory markers in UC FMT-treated patients (NCT03016780). Finally, the University of Vermont Medical Centre (NCT02390726) is assessing inflammatory markers pre- and post-FMT as well as changes in the host immune response *via* measurement of both mucosal and peripheral T-cells populations (Th1, Th2, Th17). Similarly, convincing data have been shown concerning the preliminary results of trials in paediatric UC [224].

In this context, a recent paper by the group of Joel Dore' analysed data from a paediatric trial in which patients received personalized anti-inflammatory treatments over a period of one year [225]. Stool samples at 0, 4, 12 and 52 weeks allowed an estimation of microbiota status (through 16S rRNA gene sequencing) and host inflammatory status (through the measurement of fecal calprotectin levels). Longitudinal data showed that the improvement of inflammatory status is accompanied by an enrichment of microbiota diversity. Their observations strongly suggest that inflammation suppression should be combined with microbiota management, where possible, to improve the efficacy of UC treatment.

7. New frontiers: live biotherapeutics products (LBPs) and Phage Therapy (PT)

A new era in microbial research is addressing specific patient's needs towards the comprehension of a wider microorganism's range with potential health benefits, including Live biotherapeutics products (LBPs) and Phage therapy (PT).

LBPs conform to the normal definition of probiotic, including also genetically modified microorganisms (GMMs) and their use is addressed under a pharmacological point-of-view. GMMs are designed to deliver a range of anti-inflammatory molecules such as anti-TNF, anti-IL17 and IL10 and efficiently alleviate mucosal inflammation by promoting a homeostatic immunologic profile. *L. lactis* is the most prevalent bacteria used as GMM because it is a non-pathogenic, non-invasive, non-colonizing Gram + bacterium. For this reason, it is already used in the dairy food industry. In a pivotal work, intragastric administration of IL10-secreting *L. lactis* controlled intestinal inflammation in a model of DSS-induced chronic colitis [226]. The effect of bacteria-derived IL10 was equal to the daily administration of recombinant IL10 [226]. These encouraging results led to the evaluation of a placebo-uncontrolled phase-I clinical trial, in which 10 CD patients were treated with IL10 producing *L. lactis* [227]. The trial identified the treatment as safe with a decrease in disease activity in 8 out of 10 patients [227]. A larger phase-II, randomized, double-blind placebo-controlled, multi-centre dose-escalation study (NCT00729872) met all three primary endpoints but did not found any statistically significant amelioration of mucosal healing in patients treated with IL10-secreting *L. lactis* [228]. The lack of clinical effect could depend on the limited bacteria viability, which may be enhanced by newly developed technological improvements or to a wrong protein-targeting strategy.

GMM *L. lactis* has been also used as a tool to deliver not only anti-inflammatory cytokines, but also heat-shock proteins (HSPs), cytokine antagonistic receptors, protease inhibitors and antioxidant enzymes, which are known to restrict the exacerbation of IBD [229–231, 231–235]. Interestingly, at DDW21 it was presented an *E. coli* Nissle engineered to secrete IL22 only in the gut thanks to a specific FNR promoter, which is induced in the low-oxigen colonic environment [236].

To solve the issues related with bacterial viability and protein expression and delivery, newly developed approaches are now being tested. A non-pathogenic strain of *E.Coli* MDS42 has been engineered to invade the colon epithelium and release small hairpin RNA (shRNA) interfering *TNF* directly in target cells [237]. The bacterium-mediated RNA interference strategy significantly reduced inflammation in colitic mice, resolving the limitations of protein-based treatments [237].

PT consists in the exploitation of lytic viruses to invade and disrupt bacterial cells and thus it has been re-discovered in therapy of bacteria-associated diseases. However, it was recently shown that bacteriophages themselves could exacerbate intestinal inflammation in colitic mice through the induction of IFNy responses upon TLR-9 binding [238]. Thus, extra care is put in the formulation and administration of PT as well as in its safety regulation [239–241]. Preclinical studies in DSS-treated mice showed that administration of a bacteriophage cocktail targeting *E. coli* AIEC, which is implicated in the pathogenesis of IBD [242], reduced DSS associated colitic symptoms [243]. In humans, a Phase 1a clinical study (NCT04737876) evaluated the safety, tolerability and pharmacokinetics of oral phage therapy (BX002-A) toward *K. pneumoniae*. These results demonstrated safety and tolerability with no serious adverse events, leading to discontinuation of the study for any of the 18 healthy participants. The study is still ongoing with a Phase 1b/2a clinical trial aimed at evaluating the effect of PT (BX003) on people infected by *K. pneumoniae* with results expected by mid-2022. Another phase 1/2a double-blind, randomized, placebo-controlled trial started in 2019 (NCT03808103) and it is assessing the safety and efficacy of PT (EcoActive) toward the *E. coli* AIEC strain in 30 CD patients with inactive disease state; results are expected by the end of 2022. These preliminary results suggest that PT has the potential for being considered a reliable therapy in antibiotic-resistant bacteria-associated diseases, although further research on adverse effects associated to inflammatory events is needed.

8. Conclusions/Discussion

Alterations in the composition of the gut microbiota have been implicated in a wide variety of pathologies, spanning from inflammatory to neurological, metabolic and autoimmune disorders. Both, intra- (*i.e.* IBD, *C.*difficile infection) and extra- intestinal (*i.e.* CVD, CNS, COVID-19) diseases are associated with dysbiosis, with changes in gut permeability and with exacerbated local or systemic immune responses. Thus, modulating patients' dysbiosis is arising as a novel strategy for the treatment of the aforementioned diseases, with particular emphasis on the inflammatory-induced disorders [244,245]. Extra-intestinal diseases are often associated with bacteria metabolites or derivatives which are produced in the intestine but translocated to distant sites due to the rupture of the intestinal barrier during dysbiosis (*i.e.* TMAO production in CVD). Moreover, a casual role of gut microbiome was observed in many extra-intestinal diseases. Therefore, dietary interventions, restoration of gut integrity and normobiosis through prebiotics, probiotics or FMT may indirectly affect extra-intestinal disease outcomes. Many remarkable challenges are common among intra- and extra-intestinal diseases for the efficacy of microbiota-targeted therapy, as choosing the correct bacteria consortium, assessing stable engraftment and delineate safety concern.

FMT represents an alternative for the normalization of dysbiosis among different inflammatory pathologies, however one main pitfall is that specific parameters for collection, storage and administration to patients are still mostly unknown [28]. Clinically, other promising microbiota-associated interventions such as the use of prebiotics, probiotics and live biotherapeutics are under evaluation. However, due to the unique microbiota signature of each individual a personalized approach based on microbiome stratification is needed [246]. Moreover, although discouraging, it is important to realize that this field of research must deal with complex numerous variables, such as general lifestyle habits, diet, age and mode of birth which are further influenced by the host genome. To address this issue, machine-learning represents a promising tool, exploiting existing metagenomic and metabolomic data, integrating personalized therapies based on genetics, environmental factors and the underlying microbial community present within an individual [247]. Undoubtfully, the results of the ongoing RCTs cited in this review will provide invaluable insights on efficacy, long-term safety and durability of microbiota restoration therapies.

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