Microbiome-based therapeutics

Matthew T. Sorbara and Eric G. Pamer ✉

Abstract | Symbiotic microorganisms inhabiting the gastrointestinal tract promote health by decreasing susceptibility to infection and enhancing resistance to a range of diseases. In this Review, we discuss our increasing understanding of the impact of the microbiome on the mammalian host and recent efforts to culture and characterize intestinal symbiotic microorganisms that produce or modify metabolites that impact disease pathology. Manipulation of the intestinal microbiome has great potential to reduce the incidence and/or severity of a wide range of human conditions and diseases, and the biomedical research community now faces the challenge of translating our understanding of the microbiome into beneficial medical therapies. Our increasing understanding of symbiotic microbial species and the application of ecological principles and machine learning are providing exciting opportunities for microbiome-based therapeutics to progress from faecal microbiota transplantation to the administration of precisely defined and clinically validated symbiotic microbial consortia that optimize disease resistance.

Microbiota restoration
Re-establishment of the diversity, density and beneficial functions of the microbiota following perturbation.

Duchossois Family Institute, University of Chicago, Chicago, IL, USA.

✉*e-mail: egpamer@uchicago.edu*

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Microbial populations colonizing humans (the microbiota), and their aggregate genome (the microbiome), impact every organ system and influence disease resistance and susceptibility[1]. The microbial populations that inhabit the intestines of humans vary in composition and are impacted by diet and exposure to antibiotics[2]. A reduced microbiota diversity resulting from fibre-deficient diets and antibiotic treatment has been associated with diseases ranging from atopy and allergy to susceptibility to viral, bacterial and fungal infections[3,4]. The discovery of associations between microbiota compositions and disease susceptibility and our growing understanding of the mechanisms by which symbiotic microorganisms and their metabolites impact human health are inching us towards the development of targeted therapies that will optimize microbiota composition and increase disease resistance. Approaches to manipulate the microbiota in a way that enhances disease resistance or cures disease currently remain limited.

An important potential target for microbiota restoration is the large population of individuals treated with broad-spectrum antibiotics. Although loss of beneficial symbiotic bacteria caused by antibiotic administration contributes to a range of undesirable consequences[2,4], these negatives are outweighed by the positive impacts of antibiotics on reducing infectious disease mortality[5]. Antibiotics remain crucial for optimal health care, so approaches to re-establish symbiotic bacterial populations that are inadvertently depleted are needed, with the goal of averting adverse outcomes associated with microbiota depletion[6]. Indeed, optimization of the microbiome is increasingly recognized as an important potential adjunct to current and evolving medical treatments.

Large-scale, cross-sectional and longitudinal clinical studies have revealed associations between microbiota compositions and various disease states, and, in some cases, these associations have been replicated in animal models, leading to a mechanistic understanding of disease resistance or susceptibility[7,8]. The discovery of associations between microbiome defects and disease susceptibility as well as the delineation of underlying mechanisms provides the basis for the development of therapeutic approaches. Our increasing understanding of the symbiotic members of the microbiota, their responses to diet, their interspecies interactions and their impact on the mammalian host is providing exciting opportunities to assemble therapeutic bacterial consortia that enhance health.

Faecal microbiota transplantation (FMT) is being studied as a treatment for a range of diseases; however, concerns about pathogen transmission and the inability to completely define the composition of faecal samples have limited its more general use[9,10]. Microbiome-directed therapeutics as an alternative to FMT fall into four categories (FIG. 1). The first, diet and prebiotics, can alter microbiota composition and ranges from microbiota-targeting foods to dietary fibre supplementation and complex polysaccharides that select for the expansion of specific bacterial taxa. Prebiotics are most likely to be effective if the building blocks (that is, symbiotic bacterial strains) of a healthy microbiota are present or accessible from the environment; however, if they are absent, as can occur in a broad range of clinical circumstances, the administration of the missing symbiotic bacterial strains will be necessary. Although it was long believed that most intestinal microorganisms

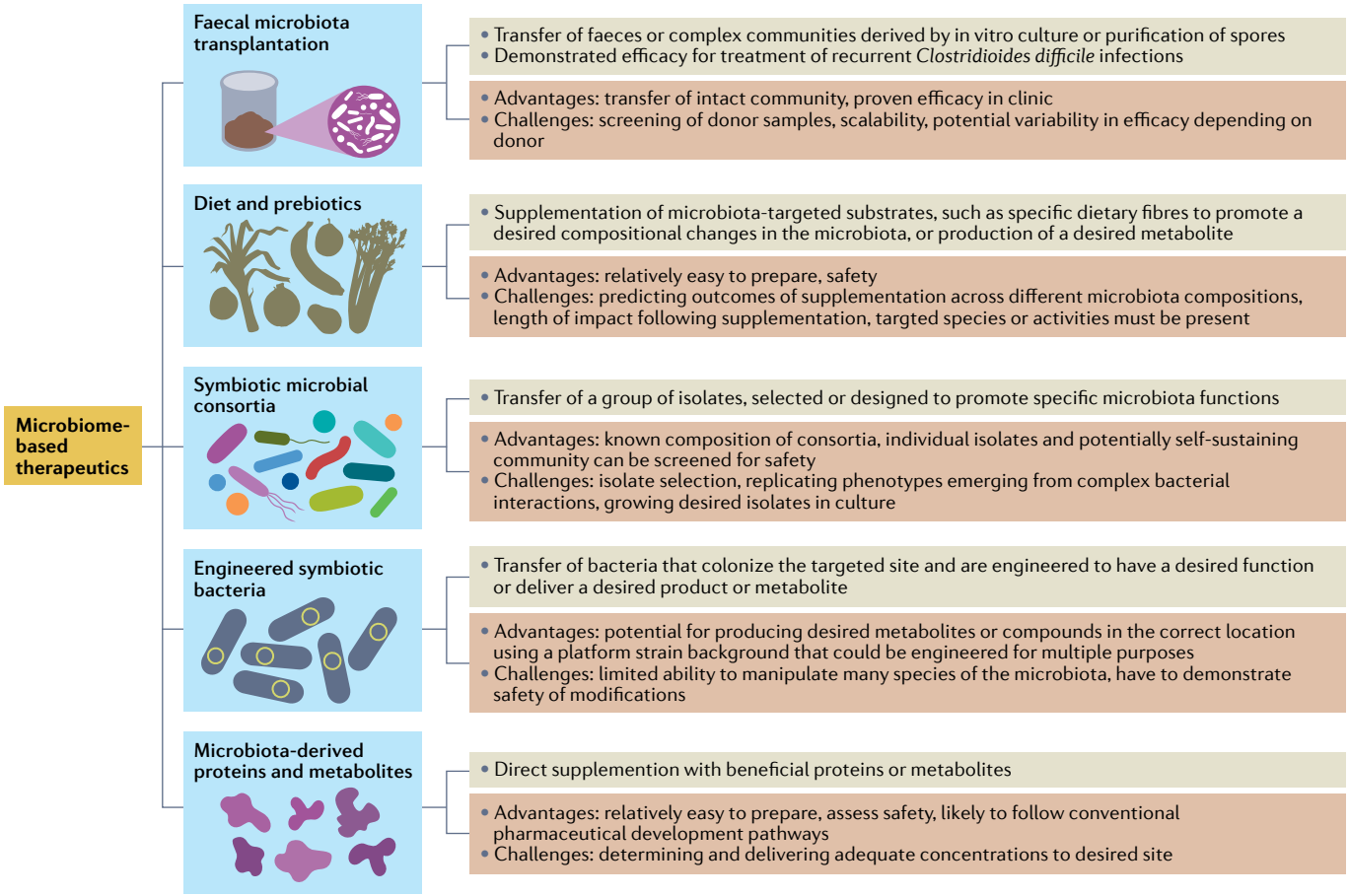

Fig. 1 | **Classes of microbiome-based therapeutics.** The crucial roles the microbiome has in host health has generated substantial interest, both in academic settings and within new and established companies, in the development of therapeutics designed to selectively restore or promote the beneficial functions of the microbiota. A range of approaches to accomplish this are under investigation, including faecal microbiota transplantation, supplementation with prebiotics, transfer of symbiotic microbial consortia or engineered strains, and directly providing microbiota-derived proteins and metabolites.

are unculturable, advances have enabled a large fraction of microbiota members to be grown in vitro[11]. This ability has facilitated the characterization of many different symbiotic bacterial strains at the genomic and metabolic levels, revealing a remarkable diversity between and within bacterial families, genera and species[12,13]. This second category of microbiome-targeted therapeutics, enteral reconstitution with symbiotic bacterial consortia, has the potential to re-establish microbiota compositions by reintroducing essential species that have been lost. A third category of microbiome modification involves the use of engineered microbiota members (that is, symbiotic bacterial strains that have been genetically modified to produce substances or to associate with specific targets) to provide a therapeutic benefit. This category will require tools to manipulate many members of the gut microbiota that remain genetically intractable, which is a formidable challenge[14,15]. The fourth category involves the isolation and production of newly discovered bioactive compounds derived from the microbiota for the development of novel therapeutics.

In this Review, we summarize recent advances in our understanding of the therapeutic potential of symbiotic bacterial species inhabiting the intestine and ongoing clinical studies to optimize the human microbiome with the aim of reducing disease susceptibility or enhancing disease resistance. We begin by reviewing clinical studies of FMT, which remains the most direct demonstration of the impact of microbiota manipulation on disease resistance. We then discuss the major phyla and bacterial families associated with health benefits, their cultivation and their assembly into consortia that function synergistically with the host. Finally, we discuss the challenges confronting the development of microbiota-targeted therapeutics at the level of academic and commercial investigation as well as regulatory hurdles for the conduct of clinical trials and, ultimately, the manufacture and delivery of safe, effective and affordable therapeutic products.

Faecal microbiota transplantation

FMT is an actively investigated and, in limited clinical circumstances, effective microbiome-altering treatment that involves the transfer of a complex, incompletely defined community of microorganisms from a healthy donor screened for the absence of pathogens to a recipient. Ideally, the re-establishment of a healthy community leads to the restoration of beneficial functions of the

Colonization resistance
Inhibition of exogenous species, including pathogens, by the resident microbiota.

microbiome, including providing colonization resistance, producing beneficial metabolites and restoring cross-talk with the mucosal immune system (FIG. 2). Variations in the FMT approach, including the type of donor or the route of administration, have the potential to impact clinical outcomes (FIG. 2). As recently reviewed, many factors, including the inflammatory state and the microbiota composition of the FMT recipient, influence the function and persistence of transplanted microorganisms[16]. In the following sections, we describe

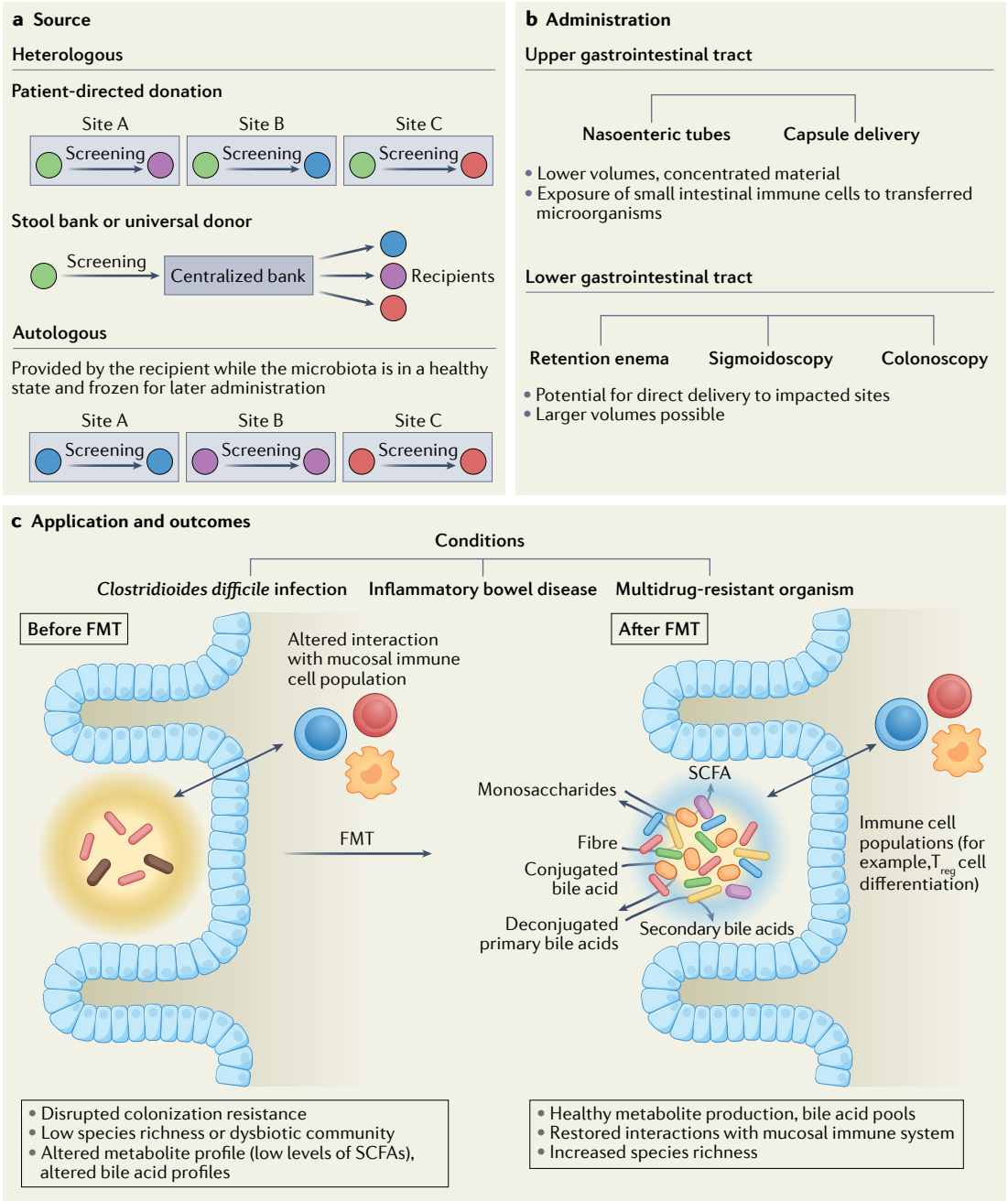

Fig. 2 | **Major factors in using faecal microbiota transplantation treatment to restore the microbiome. a** | In a patient-directed model, transplant centres identify and screen donors for individual faecal microbiota transplantation (FMT) recipients. FMT source material can also come from screened donors as part of a stool bank. The use of stool banks allows greater scalability but at the risk that a single donor with an undetected pathogen could transmit that pathogen to many recipients. Alternatively, if a patient with a healthy microbiome starts a medical procedure that will disrupt the microbiome, earlier samples can also be used as an FMT source in an autologous transfer. **b** | FMT to either the upper gastrointestinal tract or the lower gastrointestinal tract has several implications for FMT preparation (volumes and concentrations) and biological effects (exposure of different immune cell populations). **c** | FMT is being investigated in the treatment of a wide range of microbiota-involved conditions, with the goal of restoring the microbial community and their collective beneficial functions, including short-chain fatty acid (SCFA) and secondary bile acid production, and crosstalk with the mucosal immune system. T_{reg} cell, regulatory T cell.

Colitis
Inflammation occurring in the large intestine.

Engraftment
Establishment of a replicating, self-sustaining population of an introduced species or strain in a community.

Alpha diversity
Measure of community diversity within a sample based on taxonomic richness (number of species) and evenness.

clinical FMT trials that focus on individuals with different diseases. It is important to keep in mind that these trials range from randomized, placebo-controlled trials to single-arm studies with widely differing numbers of individuals, making general or broad conclusions about the effectiveness of FMT for different clinical conditions impossible at this time.

Clostridioides difficile colitis

The potential impact of microbiome reconstitution was demonstrated in humans by transfer of faeces from healthy individuals to individuals with recurrent colitis caused by *Clostridioides difficile*, a spore-forming, obligate anaerobic, toxin-producing intestinal pathogen[17]. *C. difficile* colitis is a disease that results from the inability of the host microbiota to suppress pathogen growth in the gut lumen, a function that the diverse microbiota of nearly all individuals who have not been treated with antibiotics can provide. Treatment of *C. difficile* colitis, which has consisted of antibiotic administration, has a high failure rate, in part because resistance to reinfection requires an intact microbiome. This problem, recognized more than 60 years ago, led a few pioneering physicians to perform curative faecal transplantations from healthy donors to individuals with recurrent *C. difficile* infections[18]. The results were drastic, and subsequent experience demonstrated that faecal transplantation is highly effective, with 90% success rates[19,20]. General acceptance of FMT as a treatment for recurrent *C. difficile* colitis did not occur until a controlled study demonstrated the superiority of FMT to conventional antibiotic administration[21]. More recent studies have demonstrated, down to the strain level, that the success of FMT in the treatment of *C. difficile* colitis is associated with the engraftment of donor symbiotic bacterial strains in the recipient[22].

Graft-versus-host disease

The remarkable effectiveness of FMT as a treatment for recurrent *C. difficile* colitis served as a stimulus for studies of the potential effectiveness of FMT in other diseases associated with microbiota compositions or that are known, from animal studies, to be influenced by intestinal microorganisms (FIG. 3). The resulting clinical trials have demonstrated some benefits of FMT; however, the results have generally fallen short of the remarkable effectiveness of FMT for treatment of recurrent *C. difficile* colitis. Some encouraging results have been obtained from studies involving individuals who underwent allogeneic haematopoietic cell transplantation and subsequently developed severe graft-versus-host disease (GVHD). FMT from a healthy donor, administered to the duodenum, resulted in complete resolution of GVHD in three of four individuals[23]. In a larger study involving 15 individuals who underwent allogeneic haematopoietic cell transplantation and developed GVHD, 10 had complete responses, with resolution of GVHD, increased alpha diversity of the recipient's microbiota and re-establishment of bacterial strains belonging to the order Clostridiales[24]. A study including 55 individuals with steroid-refractory GVHD also demonstrated increased clinical remission after FMT[25].

Metabolic syndrome and diabetes

Clinical and experimental studies have established correlations between the intestinal microbiota and metabolic syndrome, including diabetes and obesity. An innovative study design to determine the impact of FMT on disease status is to compare the impact of duodenal infusion of faecal suspension obtained from lean donors (allogeneic FMT (allo-FMT)) with that of infusion of the patient's own faeces (autologous FMT (auto-FMT)). This type of study was performed and demonstrated transient enhanced insulin sensitivity 6 weeks after allo-FMT, which was associated with increased frequencies of *Akkermansia muciniphila*[26]. By contrast, in individuals with early onset type 1 diabetes, there was better preservation of β-cell function in individuals receiving auto-FMT than in those receiving allo-FMT[27]. Presumably, introduction of faecal microbiota into the upper gastrointestinal tract reduces immune-mediated islet cell damage, and the authors argue that pre-existing immune responses to the autologous microorganisms might enhance the benefits of FMT. Not all studies, however, demonstrate FMT effectiveness in individuals with obesity and metabolic syndrome. With use of four different, lean faecal donors, faecal capsules were prepared and administered to study participants without prior bowel preparation or antibiotic treatment[28]. There were differences in microbiota engraftment from the different donors, and the fraction of engrafted symbiotic bacteria from three of the four donors was quite small[28]. There was no significant impact on weight or metabolic parameters.

Inflammatory bowel disease

Ulcerative colitis and Crohn's disease are complex diseases with variable phenotypes that are, at least in part, induced and sustained by the intestinal microbiota. An array of mouse models, although imperfect at replicating human disease, clearly demonstrate the impact of intestinal microorganisms on inflammatory bowel disease (IBD). Associations between the presence or absence of specific bacterial strains have been uncovered by sequence analyses of faecal samples from individuals with IBD, suggesting that introduction of the microbiota from healthy donors might ameliorate disease[29,30], and a large number of trials targeting Crohn's disease or ulcerative colitis have been initiated (FIG. 3). A study of allo-FMT versus auto-FMT by nasoduodenal infusion after bowel cleansing did not detect a statistically significant impact on clinical or endoscopic remission of ulcerative colitis[31]. However, there was a trend in the direction of enhanced remission in individuals receiving allo-FMT versus individuals receiving auto-FMT, with a correlation between response to treatment and microbiota composition[31]. Responders who received allo-FMT had greater similarity to the donor's microbiota, suggesting that engraftment of donor species contributed to improvement. Multiple healthy donors were used in that study, and it is possible that the impact of allo-FMT might differ, depending on donor microbiota composition[31]. In another clinical trial, FMT was compared with water enema, administered weekly for 6 weeks in individuals with ulcerative colitis, and demonstrated 24% remission in FMT recipients compared with

Hepatic steatosis
A condition of non-alcoholic fatty liver disease defined by the accumulation of intrahepatic fat to greater than 5% of liver weight.

5% remission in placebo recipients[32]. Of note, remission was associated with only one of multiple donors, suggesting that donor microbiota composition determines the effectiveness of FMT. A complex, randomized trial of FMT by colonoscopy followed by 40 self-administered enemas of faecal mixtures pooled from three to seven donors demonstrated that 27% of individuals receiving FMT progressed to steroid-free clinical remission, whereas only 8% of individuals receiving placebo enemas progressed to clinical remission[33]. In that study, remissions were associated with an increased microbiota diversity. A more recent randomized clinical trial compared allo-FMT by colonoscopy followed by two enemas in the following week with a similar regimen using auto-FMT[34] and demonstrated a 32% response in the allo-FMT group versus 9% in the auto-FMT group at 8 weeks. Although most FMT studies have focused on individuals with ulcerative colitis, recent studies suggest that FMT can increase the duration of remission between Crohn's disease flares[35] and is associated with the transfer of donor bacterial strains to recipients[36]. FMT has also been studied as a treatment for hepatic steatosis, and a trial demonstrated a trend towards reduced liver inflammation in recipients of FMT from lean donors compared with recipients of auto-FMT[37].

FMT is a stopgap therapy

These recently published FMT studies illustrate the complexities of FMT and its varied effectiveness depending on the clinical condition being targeted and provide

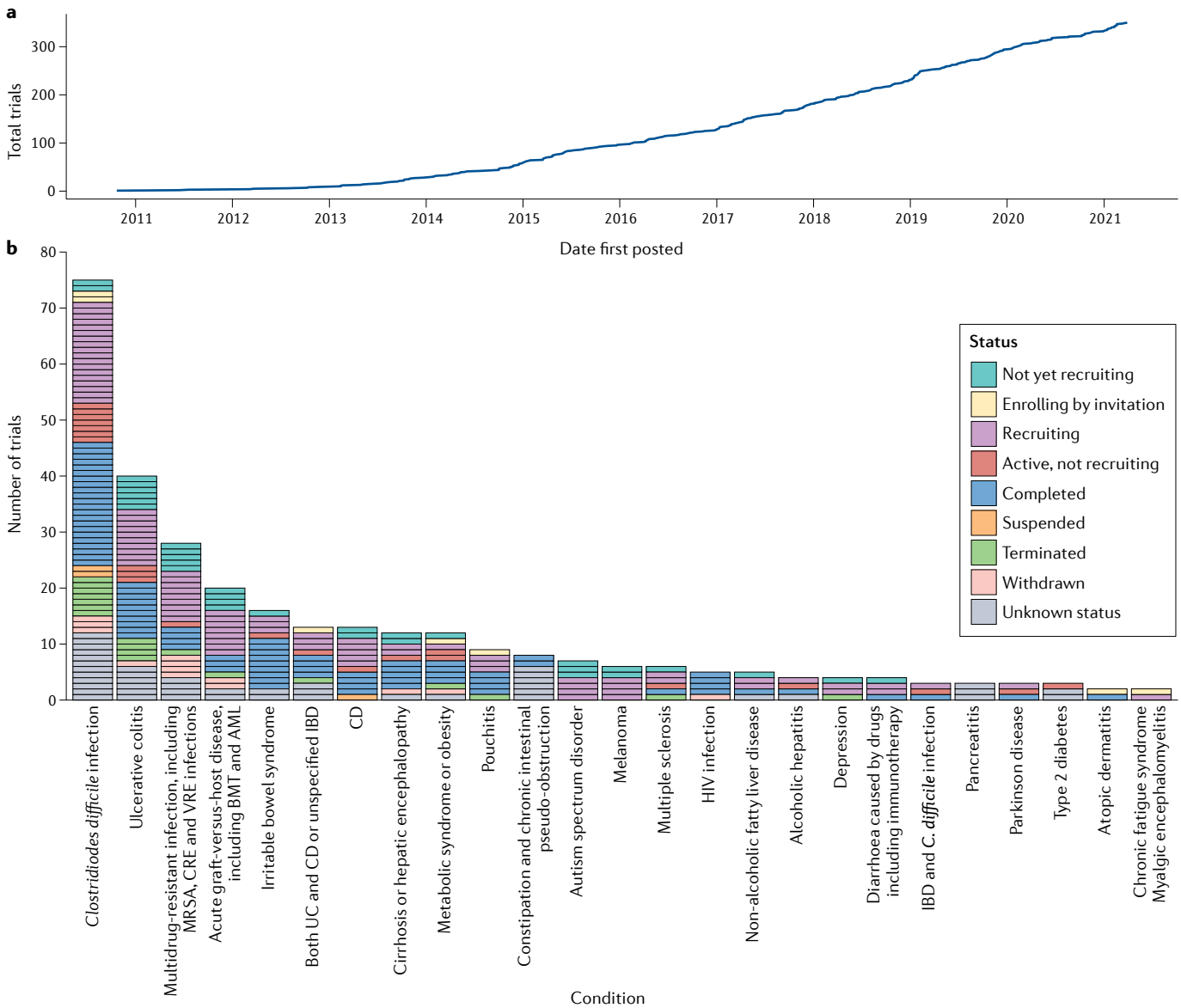

Fig. 3 | **Registered clinical trials using faecal microbiota transplantation.** Registered clinical trials were retrieved from the ClinicalTrials.gov database with the search terms 'FMT', 'fecal microbiota transplantation', 'fecal transplant' and 'fecal transplantation', and are plotted by the date they were first posted to the database in part **a**. The most common conditions targeted are plotted in part **b**. Data retrieved April 2021. AML, acute myeloid leukaemia; BMT, bone marrow transplant; CD, Crohn's disease; CRE, carbapenem-resistant Enterobacteriaceae; IBD, inflammatory bowel disease; MRSA, methicillin-resistant *Staphylococcus aureus*; UC, ulcerative colitis; VRE, vancomycin-resistant *Enterococcus*.

Bacteroicins
Short ribosomally synthesized antimicrobial peptides produced by bacteria that often target closely related species but are, in general, not harmful to producing bacteria owing to immune mechanisms.

important insights into the challenges of using donor faeces to improve the health of individuals with diseases that are driven or influenced by the intestinal microbiota. Aside from important concerns about transfer of potential intestinal pathogens, as recently documented[38,39], variable microbiota compositions between donors and even compositional changes that occur in individual donors over time are likely to impact the effectiveness of FMT in ways that are difficult to predict. On the recipient side, many microbiota-driven diseases, such as metabolic syndrome, IBD and hepatitis, are likely associated with microbiota compositions that provide colonization resistance, in contrast to *C. difficile* colitis, which results from the loss of microbiota-mediated colonization resistance. Although it may, eventually, be possible to use metagenomic sequencing platforms to determine the compatibility of donor and recipient microbiota compositions, we remain a long way from being able to do this currently. Advances in the characterization of symbiotic bacterial strains inhabiting the intestine and the ability to culture these organisms suggest that symbiotic bacterial species and their products can be developed with precision, purity and adherence to stringent health and safety regulations required for the manufacture of therapeutics.

Beyond FMT: microbiome-directed therapies

Reconstitution or alteration of the intestinal microbiome with specific bacterial species, although straightforward in principle, requires consideration of bacterial nutrient requirements, their compatibility with other symbiotic bacterial strains, including expression of bacteriocins and other toxins, and their impact on the host and potential intestinal pathogens. Attempts to rebuild microbiota compositions following antibiotic treatment and/or augmentation of the microbiota to reduce disease susceptibility will require an understanding of how different symbiotic bacterial species and strains support, tolerate or reject each other in the intestinal ecosystem. These issues, in the context of the gut ecosystem and its hundreds of microbial species, make the assembly of consortia of symbiotic bacterial strains complex. Nevertheless, as our understanding of the metabolic requirements and products of different symbiotic bacterial species increases, the ability to create assemblies of strains that can function cooperatively, persist in the gut and enhance disease resistance is certain to improve.

Commensal and symbiotic bacteria

Bacterial species are classified into distinct phyla that differ in complexity and breadth, making broad conclusions about the health impacts of one bacterial phylum versus another impossible. As bacteria belonging to specific phyla share characteristics that can impact health, we review the most prevalent phyla inhabiting the human intestine in the following sections. Bacteria belonging to the phyla Bacteroidetes and Firmicutes represent most species inhabiting the colon, whereas the phyla Verrucomicrobia, Actinobacteria and Proteobacteria constitute smaller populations. Most of these bacteria are likely to have co-evolved with their mammalian hosts, and the intestine is their principal environment. A growing number of beneficial functions of specific groups of the microbiota have been reported (BOX 1). The following sections briefly explore recent advances in our understanding of five major phyla constituting the gut microbiota, with a focus on those aspects that influence their potential as live biotherapeutics (that is, cooperativity and competition between symbiotic bacterial species in the intestinal environment). It is important to keep in mind that the impact of specific bacteria can be double-edged; that is, benefitting the host in some circumstances (for example, resistance against pathogens), while potentially harming the host (for example, by enhancing deleterious inflammatory responses) in other settings.

Firmicutes. The phylum Firmicutes is highly diverse and includes species in the orders Lactobacillales, Clostridiales, Erysipelotrichia and Negativicutes. The order Lactobacillales includes some of the most commonly used probiotics[40] and its members are facultative anaerobes. By contrast, the Clostridiales are obligate anaerobes that form a large fraction of the anaerobic bacterial population of the cecum and colon. Important beneficial functions have been ascribed to Lactobacillales and specific members of the Clostridiales; therefore, it is likely that members of these orders will feature prominently in future attempts to rebuild microbiota functionality following perturbation. Members of the Clostridiales contribute to colonization resistance against a wide range of enteric pathogens[41–44], ameliorate IBD[29], direct colonocyte metabolism[45,46], promote T cell differentiation[47,48] and protect against food allergy[49,50]. The Clostridiales contribute to the intestinal metabolome, including producing butyrate and secondary bile acids that impact the host[51] and enteric pathogens[44]. Members of the Lactobacillales interact with the mucosal immune system to induce the production of antimicrobial peptides in epithelial cells[52], promote mucosal homeostasis[53] and metabolize amino acids such as tryptophan into bioactive compounds that activate the aryl hydrocarbon receptor (AhR)[52,54]. Some Firmicutes members have been associated with negative health outcomes such as obesity[55] or, in the case of *Ruminococcus gnavus*, exacerbation of IBD[56]. Whole-genome sequencing of a panel of Lachnospiraceae isolates revealed high levels of interspecies and intraspecies diversity[13]. The diverse impacts of the Lachnospiraceae and other Firmicutes members on disease resistance or susceptibility highlight that understanding diversity within taxa of Firmicutes will be important in selecting isolates for microbiome-based therapeutics.

The Firmicutes are involved in complex cross-feeding interactions with other members of the microbiota[57,58], and, because the Clostridiales are obligate anaerobes, prior colonization with facultative anaerobes enhances their ability to colonize the gut[41]. During normal human infant development, a similar programme of developmental succession has been reported[59], suggesting that 'support' symbiotic bacterial strains will likely be necessary for the persistence and effectiveness of the Clostridiales as live biotherapeutics.

Box 1 | **Beneficial members and functions of the microbiota**

Firmicutes

- Clostridiales
  - Clostridia species contribute to colonization resistance against a wide range of enteric pathogens[41–44]
  - Direct colonocyte metabolism towards β-oxidation[45,46]
  - Promote the generation of regulatory T cells[47,48]Protect against food allergy[49,50]
  - The neonatal microbiota supports Clostridiales expansion
  - *Ruminococcus gnavus* associated with inflammatory bowel disease flares[56]
  - Some Firmicutes members associated with obesity[55]
- Lactobacillales
  - Production of antimicrobials in epithelial cells[52]
  - Promote mucosal homeostasis after infection[53]

Bacteroidetes

- Catabolism of ingested complex polysaccharides and fibres
- Up to 25% of the genome encodes polysaccharide utilization loci[64]
- Intraspecies competition in the gut based on polysaccharide utilization locus homologies[69]
- IgA-mediated persistence by association with mucin[70]
- Use type VI secretion system (T6SS) to kill neighbouring bacteria[71]
- T6SS immunity gene clusters are widespread in mobile genetic elements[72]
- Secrete bacteriocins that kill competing bacteria[73,74]
- Mediate colonization resistance against pathogens
- Produce B vitamins
- Support other anaerobic intestinal symbiotic bacteria
- *Prevotella copri* abundance positively correlates with development of rheumatoid arthritis[65]
- *Bacteroides fragilis* enterotoxin triggers inflammation that can promote development of colon cancer in susceptible mouse models[66]
- Bacteroides-produced polysaccharide impacts $CD4^+$ T cell development and ameliorates inflammatory bowel disease[67,68]

Verrucomicrobia

- Produce acetate and succinate[76]
- Metabolize mucin-derived saccharides
- Frequency increased by mucin glycans or human milk oligosaccharides
- Dietary fats impact the relative frequency of *Akkermansia muciniphila*[77]
- Transplantation of *A. muciniphila*-containing faeces reduces obesity and inflammation[77]
- *A. muciniphila* improves glucose homeostasis later in life[78]
- Pasteurized *A. muciniphila* reduces obesity in mice and reduces inflammation
- Increased insulin sensitivity in individuals receiving pasteurized *A. muciniphila*[80]
- Cancer immunotherapy in mice is enhanced by *A. muciniphila*[81]
- *A. muciniphila* enhances colitis in mice lacking IL-10 and NLRP6 (REF.[82])
- *A. muciniphila* frequency increased in individuals with Parkinson disease and Alzheimer disease[83]

Actinobacteria

- *Bifidobacterium* strains expressing ATP-binding cassette transporters for carbohydrates enhance resistance to enterohaemorrhagic *Escherichia coli* infection[84]
- Express glycoside hydrolases that support growth of other symbiotic species[85]
- Interact with other bacterial taxa to enhance biotin and butyrate production[86]
- Increase intestinal *Lactobacillus* species by stimulating colonic regulatory T cells[87]
- Enhance tumour-specific T cell activation by anti-PDL1 immunotherapy[88]
- May enhance response to anti-PDL1 immunotherapy for metastatic melanoma[89]
- May ameliorate age-related mild cognitive impairment[90]

Proteobacteria

- Expansion is a signature of dysbiosis (reviewed in REFS[91,92])
- Thrive in the presence of oxygen, nitrates or tetrathionate[46,93–96]
- Enteric domination increases the risk of bloodstream infection[100,101]
- Commensal Enterobacteriaceae compete with pathogenic strains for nutrients
- Produce antimicrobials and have been used as probiotics[102]

Bacteroidetes. Bacterial species belonging to this phylum constitute 5–60% of the colonic microbiota[60] and most belong to one of four families: Bacteroidaceae, Prevotellaceae, Rikenellaceae and Porphyromonadaceae. The contributions of Bacteroidetes species to health and disease are complex, and include catabolism of ingested complex polysaccharides, colonization resistance against pathogens, B vitamin production and support of other anaerobic intestinal symbiotic bacteria[61]. Recent studies demonstrate that some members of the order Bacteroidales express 5α-reductase and 3β-hydroxysteroid dehydrogenase, enabling them to modify secondary bile acids into forms that impact T cell differentiation[62,63]. A distinguishing characteristic of the Bacteroidetes is that up to 25% of their genomes, in the form of diverse and numerous polysaccharide utilization loci (PUL), is dedicated to polysaccharide degradation and import, enabling them to break down complex carbohydrates into easier-to-handle monosaccharides and disaccharides[64]. Some Bacteroidetes species, such as *Prevotella copri*, are associated with rheumatoid arthritis[65], and enterotoxin-expressing *Bacteroides fragilis* is associated with colon cancer[66]. However, *B. fragilis* also produces a zwitter-ionic polysaccharide that is immunomodulatory, enhancing $CD4^+$ T cell development and ameliorating IBD[67,68].

Competition between Bacteroidetes strains makes the successful transfer of specific strains into recipients challenging because of defence mechanisms encoded by specific strains. For example, colonization of mice with one strain of *B. fragilis* prevented other strains from colonizing the gut, which was attributed to a gene cluster with homology to PUL[69]. Strain exclusion is dependent on immunoglobulin A, which enhances *B. fragilis* persistence in the gut by facilitating bacterial association with mucins[70]. Bacteroidetes strains also compete by expressing the type VI secretion system (T6SS), which kills competitors in a contact-dependent manner[71] unless they express T6SS-specific immunity gene clusters, which are widespread among human-derived Bacteroidetes strains and are encoded on mobile genetic elements[72]. Additional secreted bacteriocins that mediate competition between Bacteroidetes strains have also been described[73,74]. The mechanisms that Bacteroidetes

Mucins
A family of large, highly glycosylated proteins secreted at mucosal surfaces. In the intestine, secreted mucins form a protective and selective barrier between epithelial cells and luminal contents.

Checkpoint immunotherapy
Cancer therapy that functions by promoting an antitumour immune response by targeting proteins such as CTLA4 or PD1/PDL1 that normally function to limit immune responses.

strains have evolved to gain a competitive advantage[75] add a level of complexity to the design of therapeutic bacterial consortia as the receptivity of host bacterial strains to the introduction of a new member will need to considered when one is selecting off-the-shelf strains. Furthermore, the ability of consortium members to tolerate the acquisition of new and potentially beneficial symbionts should also be factored into strain selection.

Verrucomicrobia. This phylum has only one species, *A. muciniphila*, which is commonly identified in human microbiotas. Among intestinal symbionts, *A. muciniphila* is perhaps the best example of a species with potential therapeutic benefits needing to be balanced against potential detrimental impacts on health. An obligate anaerobe, it produces acetate and succinate[76], enabling other symbionts such as *Anaerostipes caccae* to produce butyrate via the acetyl-CoA pathway[76]. Transplantation of *A. muciniphila*-containing faeces reduces the development of obesity and inflammation in mice[77], and administration of *A. muciniphila* to breastfeeding mouse pups is associated with improved glucose homeostasis later in life[78]. Administration of an *A. muciniphila* surface protein to mice provides similar benefits in a TLR2-dependent fashion[79]. A randomized exploratory clinical trial involving 32 humans with obesity receiving placebo, live *A. muciniphila* or pasteurized *A. muciniphila* detected no short-term adverse events and demonstrated modest reductions in circulating insulin levels as well as increased insulin sensitivity in individuals receiving pasteurized *A. muciniphila* but not live *A. muciniphila*[80]. Responses to cancer checkpoint immunotherapy were enhanced when *A. muciniphila* was present in the microbiota, and administration of *A. muciniphila* to mice improved the response to checkpoint immunotherapy[81]. However, the presence of *A. muciniphila* in mice lacking IL-10 and NLRP6 increased the incidence of colitis[82], and *A. muciniphila* frequencies may be increased in individuals with Parkinson disease, Alzheimer disease and multiple sclerosis[83].

Actinobacteria. The phylum Actinobacteria includes species belonging to the genus *Bifidobacterium* and *Eggerthella lenta*, which colonize the mammalian intestine and are associated with health benefits. *Bifidobacterium* strains are prominent members of the microbiota in infants, and many strains of *Bifidobacterium* have been developed as probiotics, and numerous preclinical and clinical studies have implicated *Bifidobacterium* species in disease resistance and improved cancer treatment responses. *Bifidobacterium* strains expressing ATP-binding cassette transporters for specific monosaccharides support acetate production and, upon administration to mice, enhance resistance to enterohaemorrhagic *Escherichia coli* infection[84]. *Bifidobacterium* species can release monosaccharides, such as fucose and galactose, from mucins and support growth of other species lacking extracellular glycoside hydrolases[85]. Other synergies include promoting biotin production by *Bacteroides caccae* and butyrate production by *Eubacterium rectale* following *Bifidobacterium longum* administration in mice[86]. Administration of *Bifidobacterium breve*, by stimulating regulatory T cells (T_{reg} cells) in the colon, reduces immune checkpoint inhibitor-induced colitis[87]. *B. breve* and *B. longum* administration also enhances tumour-specific T cell activation by anti-PDL1 immunotherapy and suppresses tumour growth in mice[88], and a clinical study demonstrated a correlation between the presence of *B. longum* and *B. breve* and responses to anti-PDL1 immunotherapy in individuals with metastatic melanoma[89]. Daily oral administration of *B. breve* improved cognitive function in individuals with age-related mild cognitive impairment[90].

Proteobacteria. Proteobacteria is a diverse phylum of Gram-negative bacteria. In the gut, members of the class Gammaproteobacteria, which includes the family Enterobacteriaceae, are the most prevalent and most abundant members of Proteobacteria. However, Proteobacteria are generally minority constituents of the microbiota, and expansion of Proteobacteria is a signature of dysbiosis (reviewed in REFS[91,92]). Enterobacteriaceae exploit environmental changes during inflammation, such as increases in luminal oxygen, nitrates or tetrathionate, which enable anaerobic or aerobic respiration[46,93–96]. Intestinal inflammation can also inhibit growth of strict anaerobes in the colon, which normally inhibit the replication of Enterobacteriaceae by producing short-chain fatty acids (SCFAs) and acidifying the gut lumen[97–99]. In allogeneic haematopoietic stem cell transplant recipients, enteric domination by members of Proteobacteria (more than 30% of total bacteria) significantly increases the risk of bloodstream infection[100,101]. In settings of high microbiota diversity, low levels of commensal Enterobacteriaceae strains can benefit the host by directly competing with pathogenic strains for nutrients or by producing antimicrobials. Indeed, *E. coli* Nissle has been used for more than a century as a probiotic[102]. Proteobacteria promote colonization by strict anaerobes by consuming oxygen. From a therapeutic perspective, the ability to extensively modify Proteobacteria members, such as *E. coli*, raises the possibility of creating designer strains that provide specific functionalities.

Food and prebiotics

A better understanding of the nutrient requirements and metabolism of beneficial members of the microbiota opens the possibility of supporting these species through targeted supplementation of preferred growth substrates. Indeed, changes in dietary components, such as the soluble fibres inulin and pectin, altered the microbiota composition in human study participants provided with defined diets[103]. Studies in humans revealed that a plant-based diet resulted in an increased prevalence of Firmicutes members that metabolize fibre and complex polysaccharides, whereas animal-based diets led to increased frequencies of Bacteroidetes and other bile-resistant symbionts[104]. More extreme dietary deprivation of carbohydrates, such as the high-fat ketogenic diet, reduces the level of Bifidobacteria in the microbiota and the development of T helper 17 (T_H17) cells[105]. A recent human intervention study found that a very

low-calorie diet improved metabolic health in individuals with obesity but altered the microbiota composition and decreased colonization resistance against *C. difficile*[106]. The impact of dietary differences, such as the type of bread that is ingested, can differ between individuals and is impacted by microbiota composition[107,108]. Beyond dietary composition, the cooking of foods also impacts microbiota composition, by enhancing starch and fibre digestibility[109].

Candidate microbiota-directed complementary foods can be produced by screening potential ingredients for the desired impact on the microbiota using gnotobiotic mice[110]. This approach has been applied in the setting of childhood undernutrition, where microbiota-directed complementary foods have been generated, tested and demonstrated to increase the abundance of bacterial species associated with normal microbiota maturation and the circulation of factors associated with optimal bone growth, immune defences and neurodevelopment[110]. Furthermore, the treatment of children with moderate acute malnutrition with a microbiota-directed complementary food increased rates of change in weight-for-length and weight-for-age *z* scores compared with a ready-to-use supplementary food[111]. Recently, microbiota-directed fibre snacks have been tested in the context of adult obesity and led to significant alterations in both the microbiome and plasma protein levels[112]. Recent studies have revealed the impact of certain carbohydrates on interactions between specific symbiotic species and strains inhabiting the gut. For example, dietary arabinoxylan derived from complex plant-derived polysaccharides increases the representation of *P. copri* in the microbiota[113]. Substantial progress has recently been made by colonizing mice with human-derived symbiotic bacterial populations and feeding them 34 different types of fibre, and measuring metabolite production and the degradation of fibre by specific bacterial species. These studies are providing guidance for the design of microbiota-directed complementary foods for a range of clinical circumstances[114]. Although the impact of diet and prebiotics on microbiota composition is increasingly clear, dietary changes also have direct effects on the host that can impact health and disease resistance.

Symbiotic microbial consortia

Given the risks and challenges associated with FMT, the notion of assembling well-characterized bacterial strains into consortia that can re-establish microbiota compositions that provide health benefits is appealing (FIG. 4). The idea that microbiota compositions can be improved goes back to the days of Metchnikoff and his fascination with the hypothesized benefits of lactobacilli, and has given rise to the robust probiotics market that has persisted for more than a century[115]. Work in the 1950s characterizing the increased susceptibility of antibiotic-treated individuals to infection demonstrated that disease resistance was principally associated with obligate anaerobic bacteria inhabiting the lower gastrointestinal tract[116,117]. The first efforts to assemble bacterial consortia consisting of species derived from the intestine that approximate the functions of the intestinal microbiota were made by Schaedler in the 1960s[118]; however, the initial eight strains were all facultative anaerobes. An updated version, referred to as 'altered Schaedler's flora' (ASF), was generated in the 1970s and includes three species that are obligate anaerobes[119]. ASF has been widely used in gnotobiotic mouse models to investigate the impact of a narrow range of strains on mouse physiology and resistance to disease. For example, microbiota-dependent maternal transmission of mouse mammary tumour virus to offspring is fully supported by the eight-member ASF[120]. However, in the setting of *Salmonella enterica* subsp. *enterica* serovar Typhimurium infection, ASF, in contrast to a specific-pathogen-free microbiota, does not provide colonization resistance[121].

Studies of symbiotic consortia have used gnotobiotic mouse models to determine the impact of specific combinations of bacterial strains on a specific phenotype (for example, the induction of a T cell subset, the reduction of an inflammatory disease or enhancement of colonization resistance to enteric pathogens). Arguably the most readily measured impact of a diverse microbiota is colonization resistance, the complex process by which enteric pathogens are excluded from the intestine. In the 1970s, investigators in the Netherlands recognized that highly immunocompromised individuals undergoing 'bowel decontamination' by oral administration of non-absorbable antibiotics developed dense intestinal colonization with highly antibiotic-resistant potential pathogens, such as *E. coli* and *Enterococcus faecalis*, and used anaerobic bacterial cultures derived from the faeces of healthy donors to re-establish colonization resistance in humans[122]. The first bold step, however, to assemble symbiotic bacterial strains and administer them to individuals was performed by Tvede and Rask-Madsen in 1989 when they cultured ten strains consisting of facultative and obligate anaerobes, and administered them by enema to five individuals with recurrent *C. difficile* infection[123]. Although that was an uncontrolled study, the absence of recurrences in these five individuals suggested that the consortium had some effectiveness[123].

Many years passed before further studies were performed to identify specific bacterial strains that confer colonization resistance against *C. difficile*. An initial study demonstrated that bacteriotherapy with a consortium of six symbiotic bacterial strains could clear *C. difficile* from the gut of infected mice[124], and subsequent experiments demonstrated that a consortium of four bacterial strains, including *Clostridium scindens*, a species that has the rare ability to convert primary bile acids into secondary bile acids (which have been shown to inhibit the growth of *C. difficile*), markedly enhanced resistance to *C. difficile* colitis in mice[44]. A consortium consisting of four symbiotic bacterial strains can reduce intestinal colonization of mice with vancomycin-resistant *Enterococcus faecium*[125] and *Listeria monocytogenes*[43]. The mechanism mediating clearance of these Gram-positive pathogens involves production of lantibiotics, a class of ribosomally synthesized small peptides that are broadly active against Gram-positive organisms[42]. It is increasingly appreciated that lantibiotics are also produced by bacterial species that reside in the lower gastrointestinal tract; however, their impact on the colonic ecosystem remains to be investigated[126].

Establishing colonization resistance against invasive intestinal pathogens, such as *Salmonella* spp. has been challenging. Recent studies have investigated the impact of a 12-member consortium of bacterial strains referred to as 'Oligo-Mouse-Microbiota' (Oligo-MM12) and found that these strains, even if combined with the eight-member ASF, provided minimal colonization resistance against *Salmonella* Typhimurium[127]. Using metagenomic sequencing to identify metabolic pathways present in colonization resistance-conferring diverse microbiotas but lacking in Oligo-MM12 and ASF, the investigators identified three bacterial strains, *E. coli*, *Streptococcus danieliae* and *Staphylococcus xylosus*, that augment colonization resistance by Oligo-MM12. The combination of 15 strains provided high-level resistance to *Salmonella* Typhimurium infection[127], and a follow-up study demonstrated that galactitol depletion by *E. coli* in combination with C_5 and C_6 sugar depletion by Lachnospiraceae inhibits *Salmonella* Typhimurium[128]. Another study demonstrated that propionate-producing *Bacteroides* species also provide colonization resistance against *Salmonella* Typhimurium[98]. Other mechanisms of colonization resistance against additional Gram-negative pathogens, such as *Citrobacter rodentium*, *E. coli* and *Klebsiella pneumoniae*, include microcin production[129], succinate depletion[130], amino acid consumption[131] and SCFA production with intestinal acidification[97].

Because the microbiota is known to impact T cell differentiation, modifying microbiota composition is a potential approach to modify immune responses, potentially enhancing immune responses for cancer treatment or in settings of immunodeficiency, and attenuating

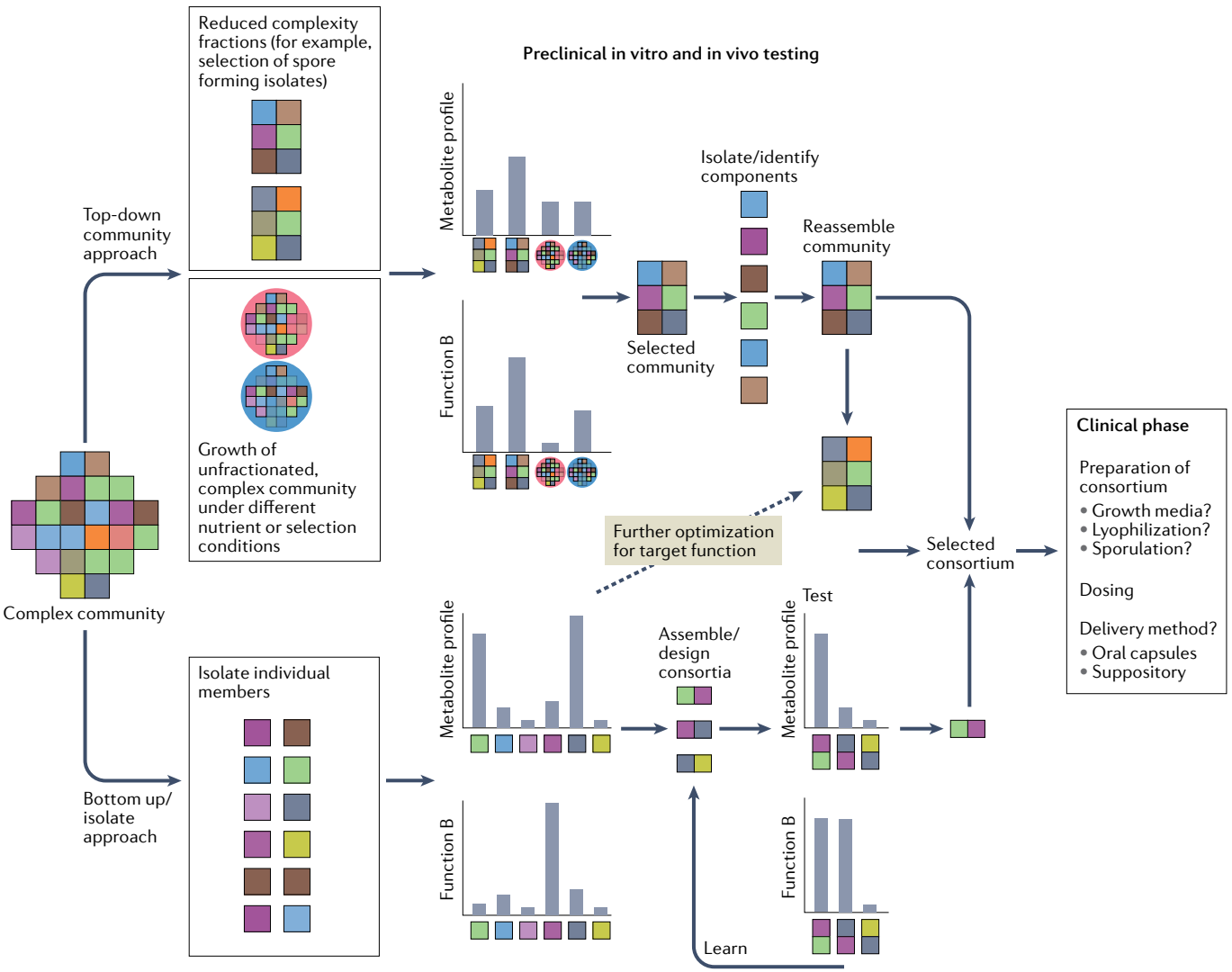

Fig. 4 | **Generation of symbiotic microbial consortia.** In a top-down approach, a complex community, or multiple communities, with the desired function is fractionated or enriched for specific microbiota members and tested for the desired function, such as specific metabolite production, before individual components are then identified before preparation for clinical use. In a bottom-up approach, constituents of healthy microbial communities can be isolated and screened for desired functional characteristics. Ideally, selected isolates can then be recombined in an iterative process to produce simple consortia that cooperatively drive the desired function. These two consortium approaches are not mutually exclusive and can be complementary, in that top-down approaches can guide the reassembly of isolated components in bottom-up approaches. Similarly, bottom-up approaches could help direct further optimizations of consortia identified through top-down approaches. Identified consortia can then be prepared for clinical use through optimization of the growth conditions, delivery form (for example, frozen cells, spores or lyophilized cells) and delivery method or route.

them in settings of autoimmunity. With these potential goals in mind, several studies have identified populations of symbiotic bacteria that enhance T_{reg} cells, T_H1 cells or T_H17 cells. T_{reg} cells could be induced in germ-free mice by colonization with 17 distinct bacterial strains that belong to the order Clostridiales, leading to attenuated bowel inflammation[47]. Subsequent studies that did not select for spore-forming bacteria identified a variety of bacterial strains belonging to the phylum Bacteroidetes that also, upon monocolonization of germ-free mice, induce T_{reg} cells[132]. Induction of T_H1 cells in the mouse colon can be mediated by *K. pneumoniae*[133]. A consortium composed of 11 different symbionts belonging to the phyla Bacteroidetes (7 strains), Firmicutes (3 strains) and Fusobacterium (1 strain) was found to enhance $CD8^+$ T cell populations in the intestine and responses to checkpoint inhibitor cancer immunotherapy[134].

The goal of developing effective bacterial consortia will also be aided by recent efforts to establish, publish and share large collections of diverse whole-genome-sequenced isolates from healthy human donors[12,13,135–138]. Together, these strain collections represent drastic increases in the size of the isolate toolbox investigators can exploit to assemble consortia.

Computational approaches. Although the approaches that have been used to assemble consortia largely consisted of digging through correlations between different microbiota compositions or microbiome-encoded metabolic pathways and experimental or clinical phenotypes, various attempts have been made to use computational platforms to predict which microbiota compositions are likely to produce a desired phenotype. With use of a group of 12 commensal bacterial species in co-cultures, interesting interdependencies were detected, with a mix of competitive (negative) and cooperative (positive) interactions. The results of these types of experiment were used to model and distinguish pairwise and higher-order interactions between intestinal symbionts in a complex microbiota[139]. Another approach was to use different combinations of the 17 bacterial species that were demonstrated to induce T_{reg} cells in the colon[47]. The investigators used ecological modelling with different combinations of 12 bacterial strains to successfully predict smaller combinations of symbionts that induce T_{reg} cells[140]. The computational platforms that are being developed are at early stages and are limited by a paucity of longitudinal and quantitative data on bacterial densities, metabolite production and consumption, and a wide range of environmental factors. Nevertheless, given the impossibly large number of potential combinations of different symbiotic species, exploitation of machine learning and artificial intelligence platforms will undoubtedly move this field forward.

Engineered symbiotic bacteria

Although intestinal symbiotic bacterial species are diverse and, in many circumstances, can provide health benefits, the ability to engineer bacterial strains that reside in the gastrointestinal tract has potential utility. Bacterial strains such as *E. coli* and *Lactococcus lactis* have been used as vehicles for the delivery of recombinant proteins; however, these species generally do not persist in the gut. Genetic manipulation of obligate anaerobic bacteria residing in the gut is a relatively new area of investigation, and great progress has been made over the past decade. In particular, a number of platforms have been developed to alter the genomes of the Bacteroidales. The complexity of developing tools for the Bacteroidales is substantial, but progress has been made in identifying inducible and constitutive promoters, and ribosome binding site sequences that provide a dynamic expression range of several orders of magnitude[141]. More recently, with use of the 16S ribosomal RNA gene promoter and the repressor TetR, it has been possible to modulate the expression of recombinant proteins up to 9,000-fold in the Bacteroidales[142]. The breadth of Bacteroidales strains that can be genetically manipulated has been increased by construction of gain-of-function vectors that enable utilization of specific polysaccharides to enable allelic replacement[143]. *Bacteroides thetaiotaomicron* has been used to heterologously express tryptophan decarboxylase from *R. gnavus* to promote tryptamine expression in the lower intestinal tract[144]. Subsequent studies demonstrated that colonization of mice with this recombinant *B. thetaiotaomicron* strain increased mucus release from goblet cells and rendered mice more resistant to dextran sulfate sodium-induced colitis[145]. An interesting approach to facilitate colonization of the gut with symbiotic bacterial strains is to engineer them to express PUL that provide them with a selective advantage over other resident bacterial populations. This was accomplished by expressing the PUL for porphyran, a polysaccharide derived from *Porphyra yezoensis*, an edible seaweed, in *B. thetaiotaomicron* and *Bacteroides stercoris* and demonstrating that feeding mice a diet containing porphyran enabled recombinant strains to gain a competitive edge over resident Bacteroidales members[146]. In contrast to genetic manipulation of the Bacteroidales, progress with the Clostridiales has been slower. However, in recent years several new platforms using CRISPR–Cas9 have been introduced that enable the deletion of *Clostridium sporogenes* genes encoding proteins that generate ten different metabolites, including SCFAs and branched-chain fatty acids[14]. Genes derived from *C. scindens* involved in the conversion of primary bile acids to secondary bile acids have been heterologously expressed in *C. sporogenes*, enabling the recombinant bacteria to synthesize deoxycholic acid and lithocholic acid[15]. These recent advances in the genetic manipulation of the Clostridiales raise hope that further tools will soon be available to engineer these major members of the gastrointestinal tract to provide health benefits.

Microbiota-derived proteins and metabolites

The intestinal microbiota impacts the host by releasing metabolic products, cellular components and secreted proteins that can activate a wide range of host receptors mediating effects that range from inflammatory to immunosuppressive. Comparison of metabolites from 96 sample sites across 24 organs from germ-free and conventionally raised mice revealed that the presence of the microbiota alters metabolite presence across

all organs[147]. A vast array of biosynthetic and metabolic capabilities are encoded within the microbiome, including the production of tryptophan metabolites that can impact immune cell differentiation by acting as AhR ligands[51,53]. Increasing numbers of microbiota-derived mediators are being identified and, for those that induce potentially beneficial responses in the host, developed as potential therapeutic drugs. For example, gene clusters that encode peptide synthetases that produce peptide aldehydes with protease inhibitory activity have been identified[148]. The production of some classes of metabolites, including SCFAs and secondary bile acids, is reduced or ablated in the aftermath of broad-spectrum antibiotic treatment. These prevalent and often concentrated metabolites impact both the microbiota and the host, and therapeutic approaches have been directed at restoring 'healthy' levels of these metabolites following microbiota disruption.

There are clinical circumstances, particularly in highly immunocompromised individuals, where the administration of live biotherapeutics would be risky. In these circumstances, the administration of the products derived from the microbiota can provide the benefits of the microbiota without the risk of inducing systemic infection. An important challenge with this approach is their production, their volatility and adequate drug delivery to the appropriate site in the gastrointestinal tract. A further consideration is that microbiota-derived drugs can potentially impact the microbiota or the host. Potential mediators that can impact bacterial taxa that constitute the gut microbiota includes a wide range of bacteriocins that have recently been reviewed[149]. In terms of proteins or metabolites, their impact on the host will be influenced by absorption and systemic dissemination versus function only in the gut lumen. Recent screening studies have identified a wide range of microbiota-derived metabolites that activate G-protein-coupled receptors[150].

SCFAs represent a major category of potential therapeutics. In the gut, acetate, propionate and butyrate, the most abundant SCFAs, are produced in large quantities through the anaerobic fermentation of dietary fibres (reviewed in REF.[151]). Acetate production is widespread across different taxonomic groups. Notably, acetogenic bacteria, such as *Blautia hydrogenotrophica*, are able to use CO_2 and H_2 to produce acetate through the Wood–Ljungdahl pathway[58]. Members of the phylum Firmicutes, particularly within the family Lachnospiraceae or the family Ruminococcaceae, are major contributors to butyrate production, although not all species in these families ferment carbohydrates to butyrate[13,152]. The Bacteroidetes significantly contribute to propionate production[153]. The pathways immediately upstream of acetate, propionate and butyrate production have been elucidated[151]. However, the quantities and balances of different SCFAs produced by individual members of a microbiota are less clear and can vary on the basis of the carbohydrates available. The impact of this flexibility is not yet well understood. Given the important roles of SCFAs in host metabolism[45,154], immunoregulation[155–157], contributions to colonization resistance[97–99] and even connections with protection from viral infection at distant sites in the lower respiratory tract[158], administration of SCFAs in individuals following broad-spectrum antibiotic treatment might be a potential therapeutic intervention.

A second category of microbiota-impacted metabolites that affect host immune defences is bile acids. Primary bile acids are produced in the liver and are delivered to the intestinal lumen to facilitate solubilization and absorption of dietary lipids through their detergent properties. Whereas most bile acids are absorbed from the ileum and transported via the bloodstream back to the liver, ~5% of bile acids enter the large intestine, where they are modified by members of the microbiota. The first step in microbial metabolism of primary bile acids is the removal of glycine or taurine residues by bile salt hydrolases (BSHs) to yield cholate or chenodeoxycholate. Variations in BSH activity can have important impacts on host health. For example, decreased BSH activity has been associated with susceptibility to *Vibrio cholerae* infection[159]. Surprisingly, some members of the microbiota, including *Clostridium bolteae*, are also able to conjugate cholic acid to phenylalanine, tyrosine or leucine, producing novel compounds that signal through the farnesoid X receptor[147]. Unconjugated bile acids can be transformed in a large number of ways by different members of the microbiota. 7α dehydroxylation by a limited subset of gut residents, including *C. scindens*, results in the production of the secondary bile acids deoxycholic acid and lithocholic acid, from cholate and chenodeoxycholate, respectively[160]. These secondary bile acids are more hydrophobic, which impacts toxicity towards *C. difficile*[44,161]. Deoxycholic acid can be further converted by bacterial species expressing 3α-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase into isodeoxycholic acid, a bile acid with reduced toxicity towards mammalian cells and commensal bacterial species[162]. Isodeoxycholic acid downregulates immunostimulatory properties of dendritic cells, thereby enhancing generation of peripherally induced T_{reg} cells[51]. Some bacterial strains belonging to the order Bacteroidales express 5α-reductase and 3β-hydroxysteroid dehydrogenase, enabling them to generate isoallolithocholic acid from bile acid intermediates along the Bai pathway from chenodeoxycholate to lithocholic acid. Several Gram-positive pathogens are inhibited by isoallolithocholic acid[62], which can also contribute to the development of T_{reg} cells[63,163]. Other gut residents oxidize or epimerize bile acids at the 3, 7 and 12 positions. These transformations can have important consequences; for example, the production of isodeoxycholic acid, isolithocholic acid or 3-oxo-lithocholic acid regulates T_{reg} cell differentiation[51,164]. Alternatively, one study demonstrated that isoallolithocholic acid promotes T_{reg} cell development, whereas 3-oxolithocholic acid decreased T_H17 cell differentiation[163]. These studies, and others, show that restoration of both widespread (BSH) and more specialized (7α dehydroxylation) activities are important factors to be considered in the design of microbiome-based therapeutics and also suggest that direct delivery of these bile acid variants may have therapeutic potential.

Challenges and bottlenecks

Most new therapeutics follow a long and treacherous path to market that begins with compound discovery, laboratory testing and patenting. Patent protection of intellectual property reassures investors and venture capitalists that their investments will yield profits and limit competitors' abilities to produce and deliver similar products without having to shoulder research and development costs. A corollary is that non-patentable but potentially effective therapies will be challenging to develop for lack of capital investment. The patentability of microbiome components and their combinations remains unclear. Patenting of natural products is controversial, in part because it may adversely impact their clinical development. For example, during the early days following the sequencing of the human genome, genes could be patented, but this restricted the ability of other investigators to study these genes and held back research groups and potential competitors from developing diagnostic tests[165]. The US Supreme Court ruled against the ability to patent genes in 2013. Bacterial strains that have been genetically manipulated to modify or enhance specific functions have been patented since 1981. Whether unmanipulated bacterial strains isolated from human donors can be patented and protected as intellectual property is unclear. Recently, however, patents have been granted for consortium compositions consisting of genetically unmanipulated bacterial strains isolated from human faeces.

Translation of laboratory discoveries to the clinic requires clinical trials to demonstrate safety and effectiveness, safe manufacturing, distribution and, ultimately, delivery to the patient. Development and safe delivery of a medicine consisting of live bacteria poses special challenges. Unlike chemical drugs, the composition and purity of which can be determined with precision, live bacteria must be cultured, often in complex media, and, even if maintained in pure form, can mutate and either gain or lose functions upon extended culturing. Thus, guaranteeing uniformity, purity and effectiveness becomes a major challenge that requires a high level of vigilance. Because health claims are made for next-generation probiotics (that is, to treat or prevent a disease), the US Food and Drug Administration and the European Directorate for the Quality of Medicines and Health Care will regulate their progression to the clinic in the United States and Europe, respectively. Both authorities have released guidelines for the development of live biotherapeutic products[166,167], and companies have reported on the nuances of navigating both regulatory environments[168]. Probiotic bacteria that treat or prevent *C. difficile* infection or that reduce intestinal colonization by antibiotic-resistant pathogens would be classified as drugs and will therefore be rigorously tested for safety and effectiveness.

Conclusions and outlook

The notion that administration of live bacteria can improve health continues to sustain the multibillion-dollar probiotics industry. Pharmacy shelves are stacked with a wide range of live bacterial preparations advertised as enhancing general well-being while avoiding specific health claims that would invite regulatory scrutiny and necessitate controlled clinical trials and US Food and Drug Administration approval. Advances in our understanding of the intestinal microbiota,

Table 1 | **Examples of microbiome-based therapeutics in development and clinical trials**

Type	Delivery	Product	Current phase	Recent outcomes	Clinical trial ID
Faecal microbiota transplantation or fractionated, partially undefined communities	Enema	Rebiotix RBX2660 for recurrent CDI	III	29.4% relative risk reduction of CDI recurrence compared with placebo (week 8)[a]	NCT03244644 (PUNCHCD3)[169–173]
	Oral capsule	Finch Therapeutics CP101 for recurrent CDI	II	21% relative risk reduction of CDI recurrence compared with placebo (week 8)[b]	NCT03110133 (PRISM3)
	Oral capsule (Firmicutes spores)	Seres Therapeutics SER-109 for recurrent CDI	III	73% relative risk reduction (week 8) 54% relative risk reduction of CDI recurrence compared with placebo (week 24)[a]	NCT03183128 (ECOSPORIII)
	Oral capsule (lyophilized stool suspension)	Rebiotix RBX7455 for recurrent CDI	I	Microbiota shifts after treatment, no CDI recurrence in 80–100% of participants depending on treatment (8 weeks)	NCT02981316 (REF.[174])
Prebiotics	Dietary supplement (with chickpea, peanut, soybean flours and green banana)	Microbiota-directed complementary food prototype (MDCF-2) for moderate acute malnutrition	II	MDCF-2 increased measures of weight-for-length and weight-for-age compared with ready-to-use supplementary food	NCT04015999 (REF.[111])
Symbiotic microbial consortia	Oral capsule (40 lyophilized isolates)	NuBiyota MET-2 for CDI	I	Increased microbiota alpha diversity CDI absent in 79% of participants (day 40)	NCT02865616 (REF.[175])
	Oral capsule (8 lyophilized isolates)	Vedanta Biosciences VE303 for CDI	II	Promoted microbiota recovery in healthy volunteers after antibiotics	NCT03788434 (CONSORTIUM)[176]

CDI, *Clostridioides difficile* infection. [a]Results released at Digestive Disease Week 2021. [b]Results released at American College of Gastroenterology Meeting 2020.

including interactions between bacterial species residing in the gut and production or modification of metabolites that impact host immune defences and physiology, have brought to light an array of opportunities for the development of novel therapeutics. Microbiota deficiencies associated with adverse clinical outcomes or chronic diseases are providing a picture of how human health might be improved by microbiota optimization. In agreement, clinical trials are currently testing different classes of microbiome-based therapeutics for a range of conditions (TABLE 1).

Whereas preclinical studies can be conducted in standard laboratories approved for work with microbial pathogens, clinical trials with live bacteria require facilities to adhere to good manufacturing practice guidelines to ensure the quality, purity and stability of live biotherapeutic agents. The expense and complexity of developing good manufacturing practice facilities has restricted the development of new, live therapeutic agents largely to commercial entities that are testing bacterial consortia in narrowly focused studies of a small number of diseases. Given the marked genomic variation in bacterial strains that constitute the microbiota and our incomplete understanding of their metabolism and cooperativity in complex populations, advances in this field will require the type of curiosity-driven research that occurs in academic laboratories and that focuses on the microbiology, metagenomics, biochemistry, metabolomics and ecology of symbiotic bacterial populations. Although preclinical studies using animal models can provide valuable information on some functions of symbiotic bacterial consortia in the gut, it is well appreciated that the intestines and the microbiota of each mammalian species are distinct. To move the needle on microbiome optimization in humans, studies of different live biotherapeutic agents will require extensive and iterative studies in well-designed clinical trials that are more likely to produce results in academic settings supported by government, foundation and/or philanthropic funding than by ongoing, narrowly focused commercial efforts.

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Competing interests
E.G.P. serves on the advisory board of Diversigen, has received speaker honoraria from Bristol Myers Squibb, Celgene, Seres Therapeutics, MedImmune, Novartis and Ferring Pharmaceuticals, is an inventor on patent applications WPO2015179437A1, entitled "Methods and compositions for reducing *Clostridium difficile* infection", and WO2017091753A1, entitled "Methods and compositions for reducing vancomycin-resistant enterococci infection or colonization", and holds patents that receive royalties from Seres Therapeutics Inc. M.T.S. declares no competing interests.

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