Current clinical translation of microbiome medicines

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Abstract

The microbiome is experiencing increasing scrutiny for its role in disease, with new research describing microbiome-disease relationships currently being published at exponential frequency. More and more, researchers are working to translate the emerging fundamental science into microbiome medicines that will address important unmet needs in the clinic. In this piece, we summarise the types of microbiome medicines showing the most translational potential, alongside a detailed analysis of the current global microbiome medicines pipeline and challenges facing clinical translation. At present, the regulatory pipeline is dominated by probiotics intended for oral delivery to the gastrointestinal tract, however several non-living biologics and small molecules provide notable distinctions. With the first microbiome medicine set to begin the regulatory submission process in 2022, it is an exciting time to enter the field.

Keywords

Microbiota; biotechnology; drug discovery and development; postbiotics; dysbiosis; peptide therapeutics.

Microbiome medicines: the emerging therapeutic class

In recent years research describing the **microbiome's** (**see Glossary**) relationship with human disease has expanded exponentially, sparking the evolution of microbiome medicine. Microbiome medicine can be characterised as the practice of preventing or treating disease by manipulating the microbiome [1]. Humans have co-evolved alongside their microbiomes, forming a symbiotic host-microbe relationship in which humans rely on microorganisms to perform a range of essential functions, such as the synthesis of hormones and vitamins, digestion of macronutrients, and the modulation of immune pathways [2]. Changes in microbiome composition or functioning can lead to a state of **dysbiosis**, which may occur in response to numerous factors, including exposure to **antimicrobials**, a fibre-deficient diet, polypharmacy, psychological stress, and lack of physical activity [3-6]. Several diseases have strong evidence underpinning their association with dysbiosis, including infections (e.g., ***Clostridioides difficile***), cancer (e.g., colorectal), and metabolic disease (e.g., type 2 diabetes mellitus and non-alcoholic fatty liver disease) (Figure 1) [7]. Other diseases now well associated with dysbiosis include inflammatory bowel disease [8, 9], several neurological disorders (e.g., multiple sclerosis and Parkinson's disease) [10, 11], and numerous immune-related conditions (e.g., atopic dermatitis and chronic inflammation) [4, 12]. Many microbiome-associated diseases currently lack licensed preventative or curative agents, therefore targeting these diseases through the microbiome could address substantial unmet clinical need.

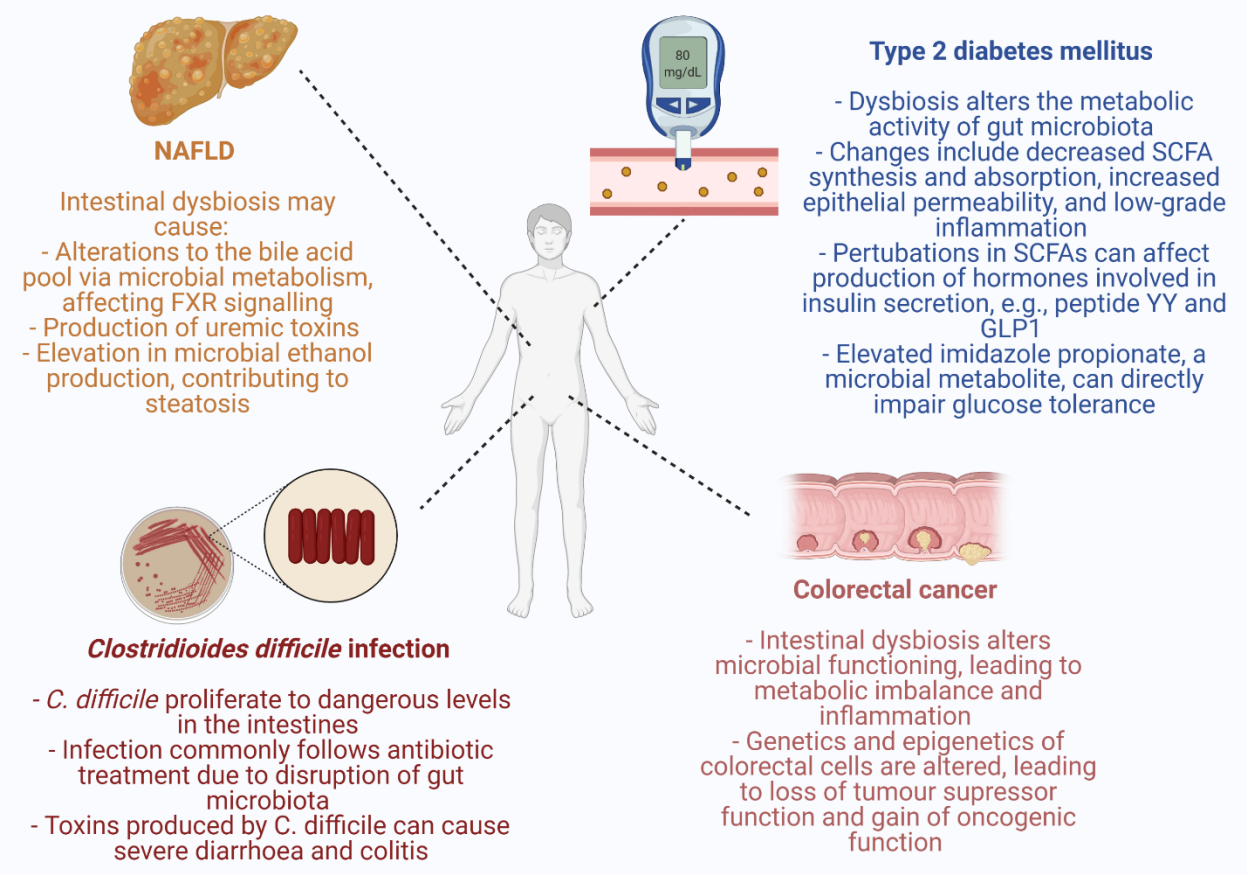

Figure 1. (see legend below 'References' section).

The identity of species forming an individual's microbiome is highly unique, with key influences including mode of birth, age, sex, lifestyle, geographical location, ethnicity, diet, medication use, and health status [3, 13-18]. Despite compositional heterogeneity, general microbiome functions (such as the gastrointestinal (GI) digestion of fibre) are well conserved between humans due to the ability of different microbial species to perform similar functions [19]. In this regard, therapeutics may seek to manipulate the microbiome in a general manner to achieve high-level benefits. For example, intake of **microbiota**-accessible carbohydrates has been shown to stimulate the microbial production of **short chain fatty acids** (SCFAs) across numerous human studies [20, 21]. On the other hand, some microbial functions can be highly strain-specific and thus cause distinct variation between individuals [22]. For example, the presence of certain strains of gut bacteria can lead to the inactivation or toxification of drugs in some patients but not others [23]. The outcomes of microbiome-targeted interventions can also be

dependent on patients' baseline microbial composition [24]. Hou et al. found that microbiome response to administration of a *Lactobacillus casei* probiotic strain was closely related with patients' basal gut microbiota [25]. This evidence signifies that microbiome medicine should account for variation in patients' microbiomes where the variation forms an important part of a condition's pathophysiology [26].

It is our opinion that the first microbiome medicines will be approved by regulatory agencies in the next few years, and will ignite significant innovation in the field, increasing the development of precision therapeutics with highly engineered mechanisms of action. It is our aim in this piece to present the types of microbiome medicines currently receiving the most research attention alongside an overview and analysis of the current global microbiome medicines pipeline. The current barriers facing the translation of microbiome medicines will also be discussed alongside suggestions to overcoming them. This information will hopefully provide inspiration for researchers looking to translate microbiome science into the clinic.

The two classes of microbiome medicines

Probiotics

Strategies for therapeutically manipulating the microbiome can be broadly organised into two groups: those that utilise living microorganisms and those that utilise non-living components. Products containing viable microorganisms are commonly referred to as **probiotics**, and can be administered by any route, though fermented foods are not classed as probiotics because microbial benefits cannot be easily disaggregated from the food matrix itself [27]. Probiotics typically aim to increase beneficial functional pathways in the microbiome by introducing defined microbial strains that may colonise the host [28]. At present, no probiotic products have been licensed as medicines and therefore cannot claim to prevent or treat disease. **Probiotic supplements** must be safe for human consumption, though can vary widely in efficacy [29]. In contrast, numerous probiotic strains are now under investigation as investigational drug products (Table 2). These formulations will have defined indications and mechanisms, and are commonly referred to as precision probiotics, next generation probiotics, or live biotherapeutic products [30].

Interest surrounding probiotics is steadily increasing: in 2020 the global probiotics market was valued at $54.7 billion and is expected to grow by 7.2% from 2021 to 2028 [31]. The design and selection of probiotics may increasingly utilise genetic engineering to impart selected functionalities to the live microorganisms. Bacteria and other microbiota could be synthetically programmed to bind to targets, sense and respond to pathogens, and secrete beneficial peptides, nucleic acids, or metabolites for defined therapeutic purposes [32]. For example, Verma et al. developed a strain of *Lactobacillus paracasei* that expressed a gene encoding human angiotensin converting enzyme 2. When delivered by gavage, the engineered probiotic was demonstrated to significantly reduce the severity of diabetic retinopathy in two murine models compared to a wildtype bacterial strain [33]. Elsewhere, engineered probiotics have exhibited promise for treatment of tumours [34], inflammation [35, 36], and metabolic disorders [37], to name just a few applications. In addition to increasing therapeutic precision, genetic engineering could be used to ensure the safety of probiotics. This could involve imparting **auxotrophism** or preventing horizontal gene transfer and/or self-replication, to avoid the replication of probiotics beyond their therapeutic abundance or acquisition of pathogenic genes [24, 38].

Non-living microbiome therapeutics

The second class of microbiome therapeutics encompasses any non-living component that exerts a beneficial effect on the microbiome. Possible examples within this group vary from **prebiotics**, to **postbiotics**, peptides, inactivated microorganisms, small molecules, and **bacteriophages** (Table 1) [39]. Common types of prebiotics include fructans, galacto-oligosaccharides, resistant starches, and non-carbohydrate oligosaccharides [40]. A key health-promoting action of prebiotics is their digestibility to lactate and SCFAs by colonic bacteria. SCFAs are known to suppress cancer cell proliferation and modulate intestinal motility, wound healing, inflammation, and intestinal integrity among many other processes [8, 41]. Prebiotics may also be combined with other microbiome therapeutics. Combinations of prebiotics and probiotics are known as synbiotics and may achieve synergistic therapeutic effects [42]. Whilst prebiotics have traditionally been regarded as dietary supplements, the investment and

positive results in prebiotic clinical trials demonstrates that they could play an important role in microbiome medicine [43-45].

Table 1. Promising types of non-living microbiome therapeutics.

Therapeutic type	Mechanism of action	Evidence to date
Bacteriophages	Repair of dysbiosis by selectively killing pathogenic bacterial species via lysis.	A patented *Escherichia coli*-targeted bacteriophage cocktail, sold as a supplement (PreforPro®), has been shown to reduce human faecal *E. coli* load without disrupting wider bacterial communities [46]. Other human studies with bacteriophages have largely failed clinical endpoints, potentially due to insufficient phage titres [47]. Bacteriophages targeted to *E. faecalis* showed therapeutic benefit for alcoholic liver disease in human microbiota-colonised mice [7].
Inactivated microorganisms	Strain-dependent immunomodulatory effects.	When administered orally to neonatal mice, a heat-inactivated strain of *Lactobacillus paracasei* alleviated antibiotic-induced intestinal dysbiosis and abnormal expression of BDNF, $GABA_{A\alpha1}$, $GABA_{b1}$, and $5\text{-}HT_{1A}$ in the hippocampus [48].

		Administration of pasteurised *Akkermansia muciniphila* raised intestinal and hepatic concentrations of SCFAs, bile acids, and polyamines to a greater extent than live *A. muciniphila* when administered to mice [49].
Inhibitors of microbial TMA synthesis	Inhibition of TMA synthesis could occur at the transcriptional or enzyme level. TMA is produced by intestinal bacteria and is a precursor to TMAO. TMAO is produced in the liver and is likely a key influence in the formation of atherosclerotic plaques and cardiovascular disease [50].	An inhibitor of microbial TMA lyases has been shown to reduce TMA production in polymicrobial communities and lower TMAO levels in mice. Mice treated with the TMA lyase inhibitor had reduced atherosclerotic development [7].
Liraglutide	A marketed GLP-1 agonist for type 2 diabetes mellitus, liraglutide may aid weight loss in obesity by increasing Bacteroidetes-to-Firmicutes ratio in the gut. This signature is associated with healthy body weight [7].	Liraglutide has been shown to increase the Bacteroidetes-to-Firmicutes ratio in the gut microbiomes of rats, changes which were associated with rodent weight loss [7].
Metformin	Reduction of metabolic dysfunction and systemic inflammation by altering gut microbiota composition and gene expression. May also alter	Several animal and human studies have demonstrated associations between metformin's microbiome effects and its impact on systemic

	bile acid production and associated intestinal FXR signalling.	glucose and inflammatory regulation [51].
n-3 PUFAs	Alteration of gut microbiome composition with possible prebiotic-like effects, leading to rectification of dysbiosis, mediation of the intestinal bile acid pool, and modulation of nuclear receptor activation [52, 53].	Daily supplementation with n-3 PUFAs has been shown to decrease the abundance of *Collinsella* species in the human gut, which are associated with NAFLD [52]. A meta-analysis has also found n-3 PUFAs to significantly improve biomarkers of NAFLD in patients [53].
SCFAs	SCFAs may be delivered as **postbiotics**, i.e., they exert their beneficial effects without the need for upstream elements, such as live bacteria or prebiotics. SCFAs can inhibit inflammatory cytokine production, e.g., through GPR43 activation, and promote epithelial barrier function, e.g., through HIF-1 stabilisation, among other beneficial actions [8].	Propionate has the strongest clinical evidence of the SCFAs to date. When bound to the prebiotic inulin, propionate has been shown to prevent an increase in intrahepatocellular lipids in patients with NAFLD. It has also reduced weight gain, deterioration in insulin sensitivity, appetite, and intraabdominal adipose tissue distribution in overweight adults [54].
Statins (e.g., simvastatin, atorvastatin)	Protective against the *Bacteroides*2 (Bact2) **enterotype**, a gut microbiome composition	Associations between protection against Bact2 and statin therapy

	that has been positively associated with obesity and systemic inflammation.	have been demonstrated in 3 large human cross-sectional studies [55].
Synthetic self-assembling peptides	Peptides partition through the membranes of select bacterial species responsible for dysbiosis, leading to interrupted transmembrane potential and impaired cell growth.	Orally administered cyclic d,l-α-peptides were found to reverse changes to gut microbiome composition induced by a Western diet; plasma total cholesterol; atherosclerotic plaques; and production of pro-inflammatory cytokines in mice. Animals' gut barrier integrity and intestinal immune markers were also improved [56].
Traditional prebiotics (e.g., fructans, GOS, RS, and non-carbohydrate oligosaccharides)	Colonic microbiota digest prebiotics to lactic acid and SCFAs, which exert a number of beneficial local and systemic effects on the host [8].	Human studies have demonstrated benefits of prebiotics in numerous disease states, such as atopic dermatitis, cardiovascular disease, cognitive-impairment, constipation, HIV, and type 2 diabetes mellitus [40, 57]. However, high doses of prebiotics are typically required to confer a therapeutic effect.

BDNF: brain-derived neurotrophic factor; FXR: Farnesoid X Receptor; GABAAα1: γ-aminobutyric acid type A receptor α1; GABAb1: γ-aminobutyric acid type B receptor 1; GOS: galacto-oligosaccharides; GPR43: G protein-coupled receptor 43; HIF-1: hypoxia-inducible factor 1; NAFLD: non-alcoholic fatty liver disease; n-3 PUFAs: n-3 polyunsaturated fatty acids; RS: resistant starch; SCFAs: short chain fatty acids; TMA: trimethylamine; 5-HT1A: 5-hydroxytryptamine receptor 1A.

Typically required at much lower doses than prebiotics are microbiome targeted small molecules, peptides, and bacteriophages. Due to the global antimicrobial resistance crisis there is great need for selective antimicrobial compounds with novel mechanisms of action. Such therapeutics would have use in fighting infections and could also be used to target defined strains of microbiota implicated in dysbiosis. Chen et al. have demonstrated selective microbial killing with their synthetic self-assembling cyclic d,l-α-peptides (Table 1) [56]. The specificity of bacteriophages also holds great potential, as they can selectively kill strains of bacteria whilst posing no threat to human cells [46]. If safely administered to the site of action, bacteriophages could allow direct remodelling of dysbiotic communities by targeting pathogenic microbiota and facilitating expansion of symbionts.

Distinct to other examples are drugs that have had their positive microbiome effects discovered after being licensed [23]. A prominent case is metformin, licensed for type 2 diabetes mellitus, which was found to alter gut bacterial composition and gene expression decades after it was approved for market use [51]. The drug is now hypothesised to promote the growth of beneficial microbiota over pathogenic species in the GI tract, subsequently influencing inflammatory pathways and improving glucose control. These findings have supported initiation of the TAME (Targeting Aging by MEtformin) trial, which aims to examine whether metformin could be repurposed to support healthy ageing [51]. It is likely that numerous other marketed drugs could show potential for repurposing based on their interactions with microbiota. To identify promising candidates, developers could screen large libraries of licensed drugs for pre-specified interactions with the microbiome [18].

The global microbiome medicines pipeline

Despite the therapeutic potential of the microbiome, to date no products have been specifically developed and licensed as microbiome medicines. To achieve licensing in the US and EU for the prevention or treatment of disease, therapeutics must demonstrate a positive benefit to risk balance by meeting the same standards as other classes of medicines seeking regulatory approval [58]. To accomplish this, investigatory products are expected to fulfil criteria outlined by the **International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use** (ICH) [I]. Table 2 presents a number of investigational

146 microbiome medicines currently undergoing the regulatory process. The findings demonstrate
147 that most novel microbiome therapeutics undergoing translation contain live bacteria, with the
148 small molecule sibofimloc, inactivated bacterium EDP1815, and Enterome's multi-peptide
149 vaccines as notable non-living distinctions [II-III] [59].

150 Table 2. Summary of the global microbiome medicines pipeline as of Q1 2022 featuring
151 products with at least clinical trial phase I progression.

Company	Product	Indication	Developmental stage
4D Pharma (Aberdeen, UK)	Blautix®: single-strain human gut bacteria (*B. hydrogenotrophica*) capable of consuming H_2 gases [IV]	IBS (both constipation and diarrhoea predominant)	Phase IIb complete. Expected market launch 2025 (US) and 2026 (EU)
4D Pharma (Aberdeen, UK)	Thetanix®: GR capsule containing live *B. thetaiotaomicron,* a human gut bacterium that stimulates colonic mucus production and attenuates inflammation [60]	Crohn's disease	Phase Ib complete
4D Pharma (Aberdeen, UK)	MRx0518: capsule containing a live biotherapeutic in combination with pembrolizumab (Keytruda)® [V]	Numerous solid-tumour cancers in patients with secondary resistance to immune checkpoint inhibitors	Phase I/II underway

Enterome/Takeda (Paris, France)	Sibofimloc: orally administered gut restricted, small molecule type 1 fimbrial FimH adhesin antagonist [59]	Crohn's disease	Phase II underway, expected market launch 2025
Enterome (Paris, France)	EO2401 and EO2463: multi-peptide vaccines based on homologies between tumour-associated antigens and microbiome-derived peptides, coadministered with or without nivolumab (administration route unclear) [VI-VII]	Glioblastoma and adrenal cancer (EO2401) B-cell malignancies (EO2463)	Phase I/II
Ferring Pharmaceuticals (Saint-Prex, Switzerland)	RBX2660: liquid suspension containing live microbiota sourced from the stools of healthy donors [61]. Administered via enema.	Recurrent *C. difficile* infection	Phase III underway, FDA has granted Orphan Drug status, Fast Track status and Breakthrough Therapy Designation
Ferring Pharmaceuticals (Saint-Prex, Switzerland)	RBX7455: capsules containing lyophilised live microbiota sourced from the stools of healthy donors [62]	Recurrent *C. difficile* infection	Phase I complete

Finch Therapeutics (Massachusetts, US)	CP101: hypromellose capsules containing lyophilised live microbiota sourced from healthy human donors [VIII]	Recurrent *C. difficile* infection	Phase III underway
NuBiyota (New Jersey, US)	Microbial Ecosystem Therapeutic-2 (MET-2): capsule containing 40 live gut bacteria from a healthy donor [IX]	Major depression, ulcerative colitis, Recurrent *C. difficile* infection	Phase I (ulcerative colitis and recurrent *C. difficile* infection) Phase II (major depression)
NuBiyota (New Jersey, US)	Microbial Ecosystem Therapeutic-3 and 5 (MET-3, MET-5): capsules containing live gut bacteria from healthy donor [X]	Hypertriglyceridemia	Phase II
KoBioLabs (Seoul, South Korea)	KBL697: capsule containing live bacteria with IL-10 inducing (anti-inflammatory) properties [XI]	Psoriasis and IBD	Phase II
KoBioLabs (Seoul, South Korea)	KBL693: oral dosage form comprising *Lactobacillus Crispatus* KBL693 showing attenuation of histamine secretion properties [63]	Asthma and atopic dermatitis	Phase I complete

AOBiome (Massachusetts, US)	B244: a live single strain of *Nitrosomonas eutropha* (D23), an ammonia-oxidising bacterium (topical administration) [XII]	Acne, pruritis/atopic dermatitis, and rosacea	Phase IIb completed (acne) Phase IIb recruited (pruritis/atopic dermatitis) Phase IIa complete (rosacea)
Evelo Biosciences (Massachusetts, US)	EDP1815: capsules containing single inactivated strain of *Prevotella histicola*, an anti-inflammatory bacterium isolated from the duodenum of a healthy donor [XIII] [64]	COVID-19, psoriasis, atopic dermatitis	COVID-19 (phase II/III), psoriasis (phase II), atopic dermatitis (phase Ib)
Vedanta Biosciences (Massachusetts, US)	VE303: capsules containing 8 human gut bacterial strains in powdered form, sourced from clonal cell banks [XIV]	Recurrent *C. difficile* infection	Phase II complete, FDA has granted Orphan Drug Status. Phase III to begin in 2022.
Seres Therapeutics (Massachusetts, US)	SER-109: capsules containing Firmicutes spores [XV]	Recurrent *C. difficile* infection	Phase III, FDA has granted Breakthrough Therapy Designation and Orphan Drug Status

152 EU: European Union; GR: gastro-resistant; IBD: inflammatory bowel disease; IBS: irritable bowel syndrome; UK: United Kingdom; US: United
153 States of America.

154 Whilst no microbiome therapeutics have been formally licensed, there is an accepted
155 treatment used in clinical practice that does not require formal approval as a drug under certain

156 restrictions, known as **faecal microbial transplant** (FMT) [27]. Because FMTs contain undefined
157 populations of microbiota they are not formally classed as probiotics but have been suggested
158 as tissue or biological treatments [27]. FMT has been researched for a range of indications,
159 including IBD, Parkinson's disease, and obesity. However, the only disease permitted to be
160 treated with FMT in the US and many other countries without a drug licence is recurrent *C.*
161 *difficile* infection (rCDI). Frequently, first line antibiotics metronidazole and vancomycin are
162 ineffective at treating rCDI with reported recurrence rates of 27.1% and 24.0%, respectively
163 [65]. Systemic antibiotic treatment can also have long lasting negative effects on gut
164 microbiome diversity. In comparison, FMT has a reported 92% resolution rate for rCDI and as
165 such is recognised as a potentially life-saving treatment [66].

166 To standardise FMT and remove the risk of disease transfer, several companies are exploring
167 the oral delivery of microbiota for rCDI, which unlike traditional FMT will require regulatory
168 approval. Table 3 expands on the information provided in Table 2 by comparing the oral FMT
169 therapeutics' doses and most recent clinical trial results.

170 Table 3. A comparison of the oral faecal microbial transplant (FMT) therapies currently
171 undergoing clinical trials.

Company	**Product**	**Storage conditions**	**Dose**	**Stage at which results are available**	**Most recent results**
Ferring Pharmaceuticals	RBX7455: capsules containing lyophilised live microbiota sourced from the stools of healthy donors [62]	Room temperature for up to 12 months	*Cohort 1:* 4 capsules BD for 4 days *Cohort 2:* 4	Phase I	Overall, 90% of patients were rCDI-free at 8 weeks (cohort 1: 9/10, cohort

			capsules BD for 2 days *Cohort 3:* 2 capsules BD for 2 days		2: 8/10, cohort 3: 10/10 patients rCDI-free). No serious adverse events observed.
Finch Therapeutics	CP101: hypromellose capsules containing lyophilised live microbiota sourced from healthy human donors [VIII]	Room temperature stable for at least 4 days (extended stability studies ongoing)	1-2 isolated doses. Each dose likely 2-3 capsules, based on past literature [67].	Phase II extension	80.3 % sustained clinical cure rate for rCDI at 8 weeks. 88.2% cure rate in patients who received 2 doses. No serious adverse events observed.
Vedanta Biosciences	VE303: capsules containing 8 human gut bacterial strains in powdered form,	Unpublished	*Cohort 1:* high dose (10 capsules OD for 14 days)	Phase II	86.2% patients on high dose treatment were rCDI-free at 8

	sourced from clonal cell banks [XIV]		*Cohort 2:* low dose (2 capsules OD for 14 days)		weeks. Low dose treatment did not outperform placebo. No serious treatment-related adverse events observed [XVII].
Seres Therapeutics	SER-109: capsules each containing 1 x 10^8 Firmicutes spores [XV]	Unpublished	4 capsules OD for 3 days.	Phase III	88.9% of patients were rCDI-free at 8 weeks. No serious treatment-related adverse events observed [XVIII].
NuBiyota	Microbial Ecosystem Therapeutic-2 (MET-2): capsule containing 40 live gut bacteria from a healthy donor [IX]	Room temperature stable for 9 months	Initially 10 capsules OD for 2 days then 3 capsules	Phase I	79% patients had no rCDI after the initial dose. After 130 days, 84% patients were

		OD for 8 days. If rCDI reoccurred, then 20 capsules for 2 days followed by 3 capsules for 8 days was administered.	rCDI-free. No serious treatment-related adverse events observed [68].

172 BD: twice daily; OD: once daily; rCDI: recurrent *Clostridioides difficile* infection.

173 Oral delivery of faecal microbiota has strong commercial potential, as it has been shown as
174 equally effective as FMT delivered via colonoscopy, better accepted by patients, and to result in
175 fewer adverse effects [69]. Whilst similar, the oral FMT interventions under commercial
176 development have significant differences. For one, their administration regimens are distinct,
177 which could be a key determining factor in product success, as the latest evidence suggests that
178 the therapies have similar efficacies and safety profiles (Table 3). At present, VE303 from
179 Verdanta Biosciences comprises the highest capsule burden for patients, and CP101 from Finch
180 Therapeutics the least. A shorter treatment duration involving fewer capsules will likely be
181 more popular with prescribers and patients. Further, the cost of therapeutics will be a pivotal
182 consideration. The cost of a single intra-colonic FMT administration has been estimated at
183 around £3,000 (~$4,100 USD), thus new oral FMT dosage forms should not be priced well above
184 this as to prohibit their use [70]. The necessary storage conditions will play a role in
185 therapeutics' cost and accessibility; currently RBX7455 from Ferring Pharmaceuticals has

published the longest room temperature stability, showing sustained microbial viability over 12 months [62]. RBX7455 is a reformulated version of RBX2660 (Table 2), whereby the RBX2660 liquid suspension for rectal administration was redesigned into a capsule containing lyophilised microbiota to enhance storage stability and ease of administration. The newer RBX7455 also seems to increase treatment efficacy, as the RBX2660 formulation achieved a 60% response rate in patients receiving ≥ 1 dose during phase II trials [61]. Specific colonic delivery of microbiota may also improve treatment efficacy, as demonstrated using a targeted coating technology, Phloral® [71]. In a 2019 study, Phloral® coated capsules accomplished an 80.6% rCDI cure rate compared to 75% for untargeted capsules; the coated capsules were proven safe and produced superior microbial engraftment patterns in patients [72]. Of all the oral FMT agents under investigation, SER-109 appears to be closest to entering the market, as Seres Therapeutics have announced that they will begin the FDA regulatory submission process in early 2022 [XIX].

Of the other microbiome therapeutics presented in Table 2, the work underway by AOBiome is striking in the sense it is the sole treatment intended for topical administration. AOBiome has patented a strain of *Nitrosomonas eutropha* (D23), an ammonia-oxidising bacterium (AOB), as a treatment for acne, pruritis/atopic dermatitis, and rosacea [XII]. AOB are thought to be natural skin microbiota that have been depleted by modern hygiene practices in recent decades. There is evidence that *Nitrosomonas eutropha* D23 has immunomodulatory actions through its suppression of overactive helper T cell (type 2 CD4+) polarisation and production of inflammatory cytokines (IL-5, IL-13, IL-4) [73]. This is the expected mechanism for the probiotic's benefits in treatment of pruritis and eczema, the indication that AOBiome appear to be prioritising in their clinical pipeline. Whilst *Nitrosomonas eutropha* D23 achieved positive results in phase IIb trials in 2017, statistically reducing acne severity compared to a control, AOBiome cites that its more recent work involving pruritis/eczema is currently being prioritised over phase III trials for acne [XVI]. If successful in clinical trials, *Nitrosomonas eutropha* D23 will become the first licensed probiotic for topical administration, marking a milestone for dermatology.

Similarly striking, for its ability to produce a therapeutic effect without living microorganisms, is the EDP1815 formulation from Evelo Biosciences (Table 2). EDP1815 is comprised of a single inactivated strain of *Prevotella histicola*, a bacterium isolated from the duodenum of a healthy donor [64]. Inactivated *P. histicola* has been found to modulate systemic inflammation through the enteric immune system by interacting with antigen presenting cells at the small intestinal epithelium. These interactions stimulate passage of intestinal immune cells to the mesenteric lymph nodes and eventual modulation of systemic T cells. In this manner, EDP1815 can reduce inflammation throughout the body without entering systemic circulation or colonising the gut [64]. A 2021 press release from Evelo Biosciences has confirmed positive results for EDP1815 in its phase II trials for psoriasis [XX]. Based on a Bayesian analysis, the probability that EDP1815 was superior to placebo was estimated as 80 - 90%. Interestingly, the 3 investigated doses found no dose-response relationship, signifying that one capsule containing 0.8 x 10^{11} cells taken daily for 16 weeks was sufficient to elicit a clinically significant immune response. This cell count per dose is similar to that in effective probiotic formulations, demonstrating that preserving the viability of microorganisms may not always confer additional efficacy [28, 49]. EDP1815's mechanism of action could provide inspiration for the development of other medicines based on inactivated microbiota, which seek to instigate a systemic response via the microbiome without altering microbial composition.

The challenges facing microbiome medicine

As highlighted, there is clearly substantial opportunity to prevent or treat human disease via the microbiome. At present the number of disease-microbiome relationships described in the literature far outnumbers the successful attempts to harness such relationships. One contributory factor to the low translation rate of microbiome science to microbiome medicine could be the substantial heterogeneity found between studies. Whereas a certain enterotype or microbial species may be positively associated with a disease state in one study, in others it could be negatively associated with the same pathology [7]. This uncertainty underlying the basic science could dissuade drug developers from entering the field. Instead of focusing on taxonomic signatures, it may be more effective to mechanistically explore disease-microbiome relationships using functional analyses. Here, **omics** technologies could be leveraged to identify

causative relationships between the microbiome and pathophysiology [2, 9, 74, 75]. An example of this is the discovery that a four-gene cluster within bacteria is responsible for the synthesis of TMA; the cluster's abundance was subsequently correlated with plasma TMAO and cardiovascular risk in humans [50].

Another barrier facing the approval of microbiome medicines is inappropriate study design. The microbiome shows substantial inter-individual variability, therefore microbiome-targeted therapeutics could be variably effective across populations [25]. Due to this, trial results could show large variation unless patients can be screened for inclusion suitability prior to initiation. This could involve identifying a target patient population based on the presence of a specific microbiome signature, and only offering the treatment to this target population. This methodology is used in other fields, for example in oncology trastuzumab (Herceptin) is only used for tumours that overexpress human epidermal growth factor receptor-2 (HER2) [76]. In addition, the microbiome is susceptible to alteration by many external factors, which could confound the results of clinical trials [7]. Researchers should attempt to account for common confounding variables relating to the microbiome in their studies, such as diet, medication use, and lifestyle [77]. Doing so will provide more accurate representations of treatments' outcomes. The effective dose of investigational treatments should also be thoroughly investigated before and within human studies; insufficient dosing has been hypothesised as contributing to the failure of three high profile bacteriophage studies (Table 1) [47]. Other pertinent factors to consider before trials include the probability of food interactions and production of anti-treatment antibodies, where the latter have been documented as occurring in response to bacteriophage therapy [47, 78].

Concluding remarks and future perspectives

Considerable research activity over the last 20 years has defined modern understanding of disease-microbiome relationships and illuminated the therapeutic potential of the microbiome. Many types of therapeutics have been investigated to prevent or treat dysbiosis, however they can be broadly classified into two groups: living microorganisms and non-living agents. Living microorganisms are commonly known as probiotics, and non-living agents could include prebiotics, postbiotics, peptides, inactivated microorganisms, small molecules, and

bacteriophages. The current global microbiome medicines pipeline demonstrates a major focus on oral administration of probiotics for action in the GI tract. That said, instances of topical formulations and non-living therapeutics are also presenting promising results. Whilst no microbiome-targeted medicines have yet been approved as formal medicinal products, the entry of several candidates into phase III clinical trials suggests that the first will soon enter the market (see **Outstanding Questions**). The closest candidate to approval is likely Seres Therapeutics' SER-109, an oral capsule containing Firmicutes spores for the treatment of rCDI, as its FDA submission process is expected to begin in early 2022.

Looking towards the future, it is advisable that researchers focus on functional aspects of the microbiome when designing new treatments. Therapeutics that target functional pathways within the microbiome with clear links to pathophysiology are more likely to be successful than those aimed at achieving a general shift in microbiome composition. Moreover, more attention should be given to identifying target patient populations for new therapeutics, and potential confounding variables in microbiome clinical studies. It is our prediction that the approval of the first microbiome medicines will spark heightened innovation within the field. This movement will be apparent by an increase in translational studies entering the literature and an upswing in global investment into microbiome medicine. At present, the microbiome likely holds many unexploited therapeutic opportunities. If targeted effectively, these opportunities could be translated into commercially successful medicines that improve patients' lives by addressing unmet clinical needs.

Glossary

- **Antimicrobials:** an agent that results in microorganism death or prevention of growth.
- **Auxotrophism:** inability to synthesise own key nutrients.
- **Bacteriophages:** naturally occurring viruses composed of a nucleic acid molecule surrounded by a protein casing that can infect bacterial cells.
- ***Clostridioides difficile* infection:** a life-threatening form of dysbiosis in which intestinal *C. difficile* proliferate to dangerous levels, producing toxins and causing severe diarrhoea with intestinal damage.
- **Dysbiosis:** a state of imbalance in microbiome composition or functioning leading to promotion of host disease.
- **Enterotype:** a type of microbiome composition that can be identified across human populations and may be associated with specific phenotypes.
- **Faecal microbial transplant:** the transfer of faecal material from a healthy donor to the GI tract of a patient with dysbiosis.
- **International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use:** a body recognised by the North American and European regulatory agencies that provides guidelines on proving medicines' quality, safety, and efficacy.
- **The microbiome:** The trillions of microorganisms that inhabit the human body and their theatre of activity, which encompasses environmental conditions, microbial metabolites, and microbial structural elements.
- **Microbiota:** living microorganisms within the microbiome, for example bacteria, protists, fungi, and archaea.
- **Omics:** technologies with the suffix -omics, such as transcriptomics, metabolomics, genomics, and metagenomics.
- **Postbiotics:** Preparations of inanimate microorganisms and/or their components that confer a health benefit on the host.
- **Prebiotic:** a substrate that is selectively utilised by host microorganisms conferring a health benefit.

- **Probiotic:** live microorganisms which when administered in adequate amounts confer a health benefit on the host.
- **Probiotic supplements:** products containing live microorganisms that are freely available to purchase by the public without professional advice.
- **Short chain fatty acids:** fatty acids with less than 6 carbon atoms; the main metabolites from bacterial fermentation of polysaccharides in the colon.
- **Untargeted oral dosage forms:** orally administered formulations that do not deliver drugs to a specific region of the gastrointestinal tract, typically releasing drug in the stomach or small intestine.

Resources

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Figure legends

Figure 1. The microbiome's pathophysiological role in the development of four diseases, in which evidence underpinning the microbiome-disease relationship has been classed as strong [7]. FXR: Farnesoid X receptor; GLP-1: Glucagon-like peptide-1; SCFAs: short chain fatty acids.