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Microbiome therapeutics go small molecule

Small-molecule drugs and biologics that alter the microbiome or its interaction with host tissues are poised to change the face of microbiome therapeutics.

Megan Cully

Second Genome wasn't always in the business of drug discovery. When the firm launched in 2009 as PhyloTech, its aim was to analyse microbial diversity in air, water and soil samples using DNA microchip technology. But in the subsequent years the field of human microbiomics blossomed, with researchers recognizing that bacteria are not just pathogenic villains but that they can also be health heroes. Second Genome pivoted, its business strategy tracking that of the evolving microbiome landscape.

A deluge of research now shows that bacterial species, communities, genes and metabolites are implicated in everything from *Clostridioides difficile* infection and inflammatory bowel disease (IBD) to autism, cardiovascular health and oncology. Although the majority of the companies working in the microbiome space are developing live biotherapeutics — single or multi-strain

bacterial cultures that can recolonize intestines with 'beneficial' bacteria — Second Genome and at least a dozen other firms are embracing more reductionist small-molecule and biologics approaches (TABLE 1).

Targeted drugs that act on microbial targets, that mimic or modulate microbial metabolites or that interfere with interactions between microbes and the host offer the simplest path to approval, argue advocates of this approach. "When you're doing something very new, don't take multiple risks at once," summarizes Karim Dabbagh, the CSO at Second Genome.

This is partly because the biology — and putative mechanism of action of these

therapeutics — can be more straightforward. "To me, while [live biotherapeutic] approaches seem enticing, they're not nearly as actionable as some of the other, more focused approaches," says Matt Redinbo, a chemist at the University of North Carolina, Chapel Hill.

Johan van Hylckama Vlieg, a convert to the reductionist approach and newly minted CSO at Kaleido Biosciences, adds that small molecules and biologics also offer various practical benefits. The regulatory path to approval for these agents is clear, manufacturing and distribution are well established and intellectual property considerations are understood.

Even at Johnson & Johnson, a large pharmaceutical firm with a clinical-stage live biotherapeutics product, interest in small molecules that can modulate the microbiome is on the upswing. "We continue to see opportunities for small molecules in this space," says Dirk Gevers, global head of microbiome solutions at Johnson & Johnson.

“When you're doing something very new, don't take multiple risks at once”

Table 1 | Selected microbiome-targeted small molecules in development

Drug	Company	Target/MOA	Indication	Status
Targeting microbes				
SYN-010	Synthetic Biologics	Methane production	Irritable bowel syndrome with constipation	Phase II
KB195	Kaleido Biosciences	Ammonia metabolism	Hyperammonaemia (urea cycle disorders)	Phase II planned
EB8018	Enterome Biosciences	FimH inhibitor	Crohn's disease	Phase Ib
SBX-101	Symberix	Gut microbial β -glucuronidases	Intestinal toxicities from therapies (for example, anticancer drugs)	Preclinical
KB174	Kaleido Biosciences	Ammonia metabolism; also reduces pathogenic bacteria	Hyperammonaemia (hepatic encephalopathy)	Clinical study ^a
KB109	Kaleido Biosciences	Microbiome balance	Multidrug-resistant infections	Clinical study planned ^b
CutC inhibitors	Zehna Therapeutics	Inhibit production of TMA from choline	Risk of atherosclerosis and thrombosis	Preclinical
CutC inhibitors	Emily Balskus, Harvard University	Inhibit production of TMA from choline	Risk of atherosclerosis and thrombosis	Preclinical
CVD MMT	Kaleido Biosciences	Inhibits production of CutC	Risk of atherosclerosis and thrombosis	Preclinical
Targeting microbe–host interactions				
AB-2004	Axial Biotherapeutics	4-ethylphenyl sulfate produced by intestinal bacteria	Autism spectrum disorder	Phase I planned
AB-4166	Axial Biotherapeutics	Not disclosed	Parkinson disease	Clinical study ^c
SG-2-0776	Second Genome	Promotes mucosal healing	IBD	Preclinical
SYMB-104 (polysaccharide A)	Symbiotix Biotherapies	Stimulates T _{reg} cells	IBD and MS	Preclinical
SYMB-202 (outer membrane vesicles)	Symbiotix Biotherapies	Stimulates T _{reg} cells	IBD	Preclinical
PEM compounds	Kintai Therapeutics	Gut-region-specific small molecules	UC, metabolic syndrome, PPMS and CKD	Preclinical

^aEvaluation in patients with cirrhosis. ^bEvaluation in patients with multidrug-resistant infections. ^cEvaluation for safety and tolerability in a subpopulation of subjects with Parkinson disease. CKD, chronic kidney disease; CVD, cardiovascular disease; FimH, fimbrial adhesin; IBD, inflammatory bowel disease; MOA, mechanism of action; MMT, microbiome metabolic therapy; MS, multiple sclerosis; NASH, nonalcoholic steatohepatitis; P2X7, P2X purinoceptor 7; PPMS, primary progressive MS; TMA, trimethyl amine; T_{reg} cells, regulatory T cells; UC, ulcerative colitis.

Five million genes

At the heart of every drug discovery programme lies a target. Although the human genome encodes nearly 21,000 genes, only 3,000 or so of these are currently considered druggable and fewer than 700 are targeted by FDA-approved drugs. Part of the appeal of the microbiome, consequently, is the opportunity to expand target space to include an estimated 5 million bacterial genes that are expressed in the gut.

For Paul Peter Tak, CEO of Kintai Therapeutics, ignoring these genes and their protein products is akin to ignoring a major organ. And an orally accessible organ, at that.

It is no surprise, therefore, that many of the biotech firms that are exploring small-molecule candidates are going directly after bacterial genes and their products.

As a case in point, Enterome Bioscience is developing a fimbrial adhesin (FimH) inhibitor for the treatment of IBD. Patients with IBD often have atypical gut microbiomes, likely both a cause and a consequence of intestinal inflammation. Whereas current IBD therapies such as TNF-lowering drugs

can reduce this inflammation, they don't act on its underlying cause. Building on the observation that some IBD patients have increased numbers of adherent-invasive *Escherichia coli* in their gut, Enterome set out to block the ability of these bacteria to bind to the intestinal epithelium through the surface adhesion molecule FimH.

The firm thinks that this approach will decrease the ability of these bacteria to colonize the gut, thereby reducing inflammation. A phase Ib trial of its EB8018 is underway.

The targeted approach can be used not just to control colonization, but also to more subtly modify the activity of the bugs that live in the gut. Synthetic Biologics' SYN-010, for example, is a modified-release prodrug of lovastatin for the reduction of painful bloating in patients with irritable bowel syndrome with constipation. Whereas lovastatin targets the human enzyme HMG-CoA reductase to reduce cholesterol production in the liver, it also binds and inhibits the archaeal enzyme F420-dependent Mtd, a methane-producing enzyme expressed by gut-resident microbes.

SYN-010 is formulated to release lovastatin only in the intestinal lumen, delivering the drug to Mtd-expressing archaea to decrease methane production.

Phase II trials are underway, with results expected in early 2020.

Drug hunters are also using small and mid-sized molecules to modulate the activity of microbiome constituents even when the exact target isn't clear. Kaleido ran a phenotypic screen on human microbiome samples to identify glycans that could alter bacterial ammonia production, for example. The mechanism of action of their lead candidate, KB195, is not known: it could cause bacteria to use ammonia as a food source, or it might alter the balance between ammonia-consuming and ammonia-producing bacteria, for example. But clinical data show that it reduces levels of nitrogen metabolism, a marker of ammonia production, in healthy people fed a high-protein diet.

A phase II trial for the treatment of hyperammonaemia, a metabolic disturbance caused by urea cycle disorders, is set to start soon.

Box 1 | Stop metabolizing my drugs!

Bacteria can metabolize a vast and diverse array of molecules, including therapeutics. In a recent paper in *Nature*, researchers showed that as many as two-thirds of drugs that are designed to act on human targets are metabolized by at least one strain of gut bacteria. Because these metabolic pathways can impact drug efficacy in at least some cases, this too provides a means of tapping the growing understanding of the microbiome to improve the efficacy of drugs against established human targets.

Matt Redinbo, a chemist at the University of North Carolina, for example is working on small molecules that can overcome the dose-limiting diarrhoea associated with the cancer chemotherapy irinotecan. Irinotecan is an intravenously administered prodrug that is converted into its active form in vivo, and then inactivated in the liver and excreted through the bile duct. But β -glucuronidases that are produced by bacteria in the gut can reactivate the drug as it is being passed, causing intestinal damage and diarrhoea.

In 2010, Redinbo showed that β -glucuronidase inhibitors can prevent the reactivation of the drug and the associated side effects in mice. Based on these results he founded Syerberix, which has since progressed its β -glucuronidase inhibitor SBX-101 into investigational new drug-enabling studies.

Parkinson disease patients, too, might benefit from tweaking microbial metabolic pathways to improve outcomes with standard-of-care treatment. The primary treatment for Parkinson disease is L-dopa, a prodrug that crosses the blood–brain barrier before decarboxylases metabolize it into dopamine. But decarboxylases in the periphery and gut also metabolize L-dopa outside of the brain, reducing the amount of drug that can drive the desired effect and inducing unwanted adverse events. Inhibitors of peripheral decarboxylases, such as carbidopa, can alleviate some of these effects, but patient responses to these agents are highly variable and much of the L-dopa is still metabolized before it reaches the brain.

“That variability potentially pointed to a factor like the microbiome,” say Emily Balskus, a chemist from Harvard University. In a recent *study in Science*, she showed that tyrosine decarboxylase (TyrDC) from *Enterococcus faecalis* metabolizes L-dopa in the gut, and that it is insensitive to the peripheral host decarboxylase inhibitor carbidopa. Her lab’s TyrDC inhibitor increased the peak serum L-dopa concentration in mice colonized with *E. faecalis* and given L-dopa and carbidopa.

Balskus plans to develop gut microbial L-dopa decarboxylase inhibitors for therapeutic use. “I hope [our inhibitor] is a promising starting place,” she says.

Meddling with metabolites

Microbial metabolism — of everything from food to drugs (BOX 1) — provides another low-hanging opportunity for microbiome modulation.

“Think of the gut as a command centre,” says Tak. Products of microbial metabolism can cross the intestinal wall, enter the circulation and affect distal organs, he explains. There are also hundreds of millions of neurons in the intestine with connections to the brain, especially via the vagus nerve, and metabolites that interact with these neurons may offer a means of bypassing the blood–brain barrier.

“We’re not going to try to get drugs across the blood–brain barrier, we’re going to try to get drugs into the gut, which is far easier,” says Sarkis Mazmanian, a microbiologist at the California Institute of Technology.

Mazmanian’s approach stems from seminal discoveries that have showed that patients with autism spectrum disorder (ASD), depression, schizophrenia and Parkinson disease (PD) have different microbial communities to neurotypical controls. Faecal transplantation from PD patients into a transgenic mouse model of disease can induce gastrointestinal symptoms and motor impairment associated with the disease. And, critically, these effects are mediated by microbial metabolites that interact with host tissues in the gut and distal organs.

“We were able to transfer the core symptoms of PD from humans into animals simply by transplanting microbiomes,” says Mazmanian, who carried out some of this work.

Axial Biotherapeutics was born out of these observations, and is developing small-molecule approaches to alter the production and the absorption of specific bacterial metabolites. Their lead therapeutic,

AB-2004, reduces levels of the bacterial metabolite 4-ethylphenylsulfate (4-EPS) in mice; high 4-EPS levels in the blood are found in children with ASD. Axial is currently screening subjects for a phase Ib/Ia clinical trial of AB-2004 in adolescents with ASD.

But microbial metabolites might also be useful as drugs in their own right. In a recent *study in Cell*, Mazmanian showed that two metabolites — taurine and 5-aminovaleate — are found at lower abundance in the stool from people with autism, and that when administered to a mouse model of ASD they improve behavioural symptoms.

“The reason why we chose to study [taurine and 5-aminovaleate], even though other molecules are depleted... is that these two molecules were reported to have weak GABA agonist activity,” says Mazmanian. According to the hyperactivity hypotheses of ASD, the disorder could be caused by an imbalance in the excitatory and inhibitory components of the brain. Because GABA activates inhibitory neurons, reduced levels of taurine and 5-aminovaleate in ASD could tip the balance towards hyperactivity. His hypothesis is that these molecules travel from the gut to the brain, where they exert their effects. “When you dig deeper you can actually start to unravel how microbiomes are communicating with the brain through these small molecules,” says Mazmanian.

“We continue to see opportunities for small molecules in this space”

Microbial metabolic processes can also impact cardiovascular function, with therapeutic implications.

The effects of diet on cardiovascular health are well-known, and dietary interventions still form the backbone of therapies for hypercholesterolaemia, hypertriglyceridaemia and other conditions. One common dietary recommendation is for patients to reduce their intake of meat, egg yolks and high-fat dairy products. Emerging evidence suggests that one problem with these foods may be that they have high levels of choline, an essential nutrient that is metabolized by gut bacteria into trimethylamine (TMA), which is further metabolized into trimethylamine N-oxide (TMAO) in the liver. High circulating levels of TMAO are associated with an increased risk of cardiovascular events.

Last year, Stanley Hazen at the Cleveland Clinic and colleagues reported in *Nature Medicine* that small-molecule inhibitors of the bacterial enzyme choline TMA lyase (CutC) can reduce circulating TMAO levels in mice, with a corresponding reduction in thrombosis potential. Hazen and the Cleveland Clinic recently founded Zehna Therapeutics to develop these and other compounds. He foresees a future in which clinicians will measure circulating TMAO levels in patients and then prescribe a CutC inhibitor, in the same way that they currently measure LDL levels before prescribing statins.

“The gut microbiome is a perfect target for marrying the concept of precision medicine with a therapeutic,” Hazen says.

Kaleido Biosciences is also working on CutC. And Emily Balskus, a chemist from Harvard University, is optimizing a small-molecule CutC inhibitor, and has

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performed a high-throughput phenotypic screen to identify additional compounds that inhibit choline metabolism.

"TMA production is nearly entirely in the microbiome," says Katharine Knobil, Kaleido's CMO and head of R&D, which makes it attractive from a drug development perspective. "If we can succeed in reducing the production of TMA in conditions where it has been linked to disease, I think there is potential to have a huge impact on patients."

To immunity and beyond

Just as microbial metabolites from the gut can have effects in distal organs, interactions between immune cells and gut bacteria play out in biological consequences throughout the body. "70–80% of the immune cells are found in the gut at any time point," says Tak. For diseases such as IBD that involve gut inflammation, the effects of therapeutics that modulate these interactions don't even need to travel very far.

Second Genome started working on IBD in part because biopsies from these patients could be readily obtained, analysed for microbial composition and assessed using multi-omics and in silico-enabled approaches. This quickly revealed that microbes from patients with IBD were secreting molecules that activated the innate immune system, and that the host responded in kind with intestinal inflammation. Similar effects were also taking place in the intestines of people with metabolic conditions and nonalcoholic steatohepatitis (NASH), they found.

Armed with this knowledge, Second Genome in-licensed an inhibitor of P2X purinoceptor 7 (P2X7), a cell surface ion channel that triggers the innate immune system in response to bacterial threats by activating the **inflammasome**. By dampening this pathway, the firm hoped to get overactive immune systems under control.

Earlier this year Second Genome terminated a phase II clinical trial of this molecule, SGM-1019, in NASH because of a compound-related toxicity signal that would preclude chronic use of the drug.

This first foray into immunity is disappointing for Dabbagh, not just because the trial didn't pan out. Despite setting out with state-of-the-art tools and massive databases to mine the microbiome for drug leads, "all we

found were biological processes that we already knew about," says Dabbagh. P2X7 had already been implicated in inflammation, for instance. Dabbagh wanted more, and so has since built a platform to mine bacterial genomes for secreted proteins that alter microbe–host interactions that are entirely novel.

By searching for proteins that are differentially secreted by bacteria in healthy people relative to those in people with IBD, with a focus on molecules that alter the mucosal barrier, the team has hit on its preclinical candidate SG-2-0776. SG-2-0776, a 'secreted-protein-replacement therapy' of sorts, replaces the missing protein in IBD patients. Although the team has yet to fully work out SG-2-0776's mechanism of action, this molecule binds to proteins at the interface of the cell membrane and the extracellular matrix. It may bridge anti-inflammatory signalling from the extracellular matrix to the cytoskeleton and to proteins that control the circadian rhythm, speculates Dabbagh.

Second Genome's platform continues to evolve, and now incorporates metagenomic bacterial data as well. "More and more groups are starting to do this," says Dabbagh.

Symbiotix Biotherapies is also taking an immune-centric approach. Their lead candidate is SYMB-104, a zwitterionic capsular polysaccharide that is naturally produced by a gut commensal organism and taken up by dendritic cells, which can traffic to distal sites of inflammation to convert naive T cells into regulatory T (T_{reg}) cells. T_{reg} cells dampen inflammation locally, and Symbiotix is investigating SYMB-104 in inflammatory bowel disease and multiple sclerosis.

This class of molecule is "well-known to industry because bacterial capsular polysaccharides form the foundations of existing therapeutics such as pneumococcal vaccines," adds Nader Yaghoubi, Symbiotix's president and CEO.

Sorting out sampling

Although more and more microbiome-modulating small-molecule and biologic candidates are approaching the clinic, the field is still in its nascency. And the reductionist approach — for all its rationale and simplicity — has not yet proved its ability to harness the health benefits of the

microbiome. "The game-changer is going to be having therapeutics that actually make it to clinical practice," says Hazen.

This leaves plenty of opportunity for different ways forward. "I don't think that there will be only one approach, or one company or group that will unearth this and make it a success," says Dabbagh.

But a few things could help to increase the field's overall odds of success, he adds. Sequencing and metabolomics technologies and computational biology approaches are already in a good place to enable research, and are going in the right direction, fast. But a dearth of longitudinal microbiome samples from clinical cohorts that are well-characterized in the disease areas of interest, with associated metadata on genetics, diet, disease history and more, is holding things up, says Dabbagh.

Bigger sample sizes are also needed to ensure that potential leads are generalizable, and not restricted to narrow patient populations. "You don't want to develop something that only works in people who eat seaweed," Hazen points out.

Microbial sampling and analysis could also be improved. At the moment, researchers primarily rely on faecal samples to assess the composition of the microbiome, so the abundances of the bacterial residents of the colon are overrepresented. But when Eran Elinav, a microbiologist at the Weizmann Institute, and colleagues recently assessed intestinal biopsies, they saw that different strains of bacteria **colonize different parts of the intestine**. These differences cannot be observed in faecal samples.

Kintai Therapeutics has also probed for region-specific differences. The interconnected biology of the enteric signalling network — which is made up of gut immune cells, the enteric nervous system and the gut microbiome — is "very different when you compare, say, the upper part of the jejunum with a more distal part, or with the ileum or the colon," says Tak. Kintai's compounds modulate the enteric signalling network in a region-specific way to improve efficacy and outcomes.

Researchers are nevertheless optimistic that once these kinks are worked out, and the unfettered hype of this space fades, molecular and biologics drug hunters will have plenty of microbial medicine leads to work with. "The microbiome right now is sort of like what sequencing the human genome was 20 years ago — 'when we figure this out it's going to tell us everything,'" says Redinbo. "But one of the fundamental things I'm sure of is that the gut microbes and the immune system are talking all the time, and that level of communication is critical."

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