

MICROBIOME FUTURES

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Translating microbiome futures

Gaspar Taroncher-Oldenburg, Susan Jones, Martin Blaser, Richard Bonneau, Peter Christey, José C Clemente, Eran Elinav, Elodie Ghedin, Curtis Huttenhower, Denise Kelly, David Kyle, Dan Littman, Arpita Maiti, Alexander Maue, Bernat Olle, Leopoldo Segal, Johan E T van Hylckama Vlieg & Jun Wang

A group of microbiome researchers discuss some of the challenges in developing a new generation of microbiome therapies.

The opportunity afforded by human microbiome research for developing therapeutic or nutritional products is matched only by the formidable task of unraveling the science behind it. Researchers are drilling down into mechanisms that underlie the associations between microbiota membership in health and disease and how they vary among individuals (Fig. 1). The breadth of associations of microbiome biology with human health has resulted in a surge of interest from the food, biotech, pharma and investor communities (Figs. 2 and 3).

As yet, no microbiome therapeutics requiring US Food and Drug Administration scrutiny have been approved for human use. Only a handful of products have entered phase 3 trials, including two donor-derived treatments for recurrent *Clostridium difficile* infection—Rebiotix's RBX2660 (a ready-to-use enema containing live microbes) and Seres' SER-109 (an oral formulations containing consortia of live bacterial spores)—and a microbiome modulator for treating lactose intolerance: Ritter Pharmaceuticals' nondigestible oligosaccharide RP-G28, which is designed to stimulate lactose metabolism by the gut microbiome.

Conference organizer Global Engage and *Nature Biotechnology* (Box 1) convened a recent roundtable to discuss the current state of the art in human microbiome research and its translation into therapies. Below we present some highlights of discussions that emerged during the roundtable and preparatory conversations for the event.

What do you see as the most important challenges for translation in the microbiome space?

Martin Blaser: We need to understand our

A full list of affiliations appears at the end of the paper.

Box 1 The translational microbiome landscape

On 23 May 2018, a panel of experts met at the New York Academy of Medicine to discuss a range of key issues in translating microbiome research into therapies. The panel was moderated by Gaspar Taroncher-Oldenburg, Consultant-in-Residence at Global Engage, and Susan Jones, Senior Editor at *Nature Biotechnology*, who had previously engaged close to 150 key opinion leaders worldwide—from researchers to investors and companies—to discuss issues including the regulatory landscape; the spectrum of modalities; the variety of potential therapeutic and preventative applications; and preclinical, clinical and manufacturing challenges.

The summit, *Microbiome Futures*, brought together leaders in the field, including CEOs and CSOs of several microbiome companies, representatives from big pharmas working in the space, and top academics from the New York area and beyond, and was produced with support from the following sponsors: Takeda Pharmaceutical, Quay Pharmaceuticals, Diversigen, Qiagen and Taconic Biosciences.

This article excerpts discussions leading up to, during and following the New York summit.



Gaspar Taroncher-Oldenburg is consultant-in-residence at Global Engage.



Susan Jones is senior editor at *Nature Biotechnology*.

interactions with our microbiota, to define what are the bases for human variation in health, and to understand the extent to which microbiome changes predispose or cause disease. Only then will we know whether and how to intervene.



Martin Blaser is a professor at New York University and director of the NYU Human Microbiome Program.

Denise Kelly: For investors, the challenge in the microbiome space is really cutting in on the science. We look at substantiation of the ideas behind a product using data from both *in vitro*

and *in vivo* studies, with the evidence coming preferably from not just one preclinical model but from multiple complementary models. This is essential to minimize the risk of making investment decisions that overlook the shortcomings of existing preclinical models, particularly mouse models.

Most animal models of disease were never developed with the microbiome in mind, and as a result there are currently no good translational models that fully mimic the complexity of the human microbiome. This has



Denise Kelly is a venture partner at Seventure Partners.

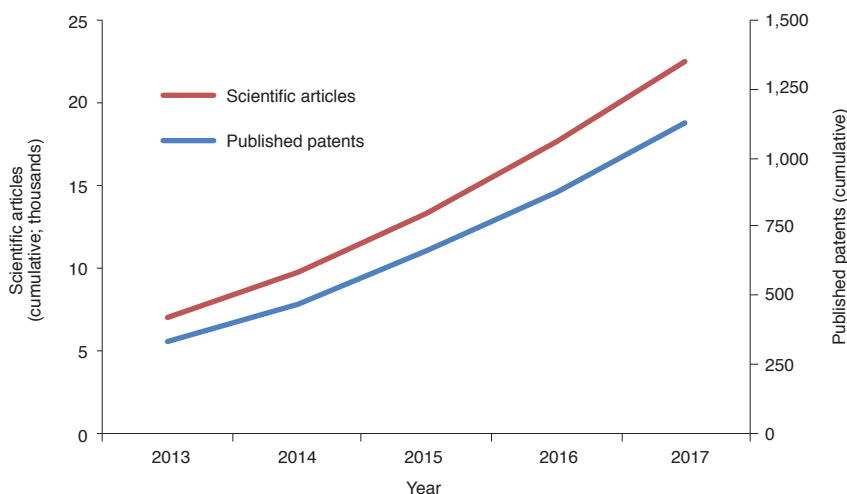


Figure 1 Over the past five years, the scientific output in the microbiome field and the number of microbiome-related published patents has been steadily rising. Sources: PubMed (search terms “human microbiome” and “human microbiota”); Fankhauser *et al.*¹; University of Chicago Technology Commercialization²; CBInsights; CrunchBase; GlobalData; the companies.

resulted in animal studies being generally less informative than desired when it comes to the microbiome component of a disease and has triggered a drive to boost early research and R&D in humans instead.

Elodie Ghedin: The first wave of microbiome research consisted of describing the microbial components of the microbiome; a second wave focused on characterizing the intra-microbiome and host-microbiome interactions within specific sites. But it is a third wave of microbiome research, zooming in on the crosstalk among sites, that is helping us truly understand how the different human microbiomes and their microbial players are interconnected to make a human human.



Elodie Ghedin is professor at New York University and director of the university's Center for Genomics and Systems Biology.



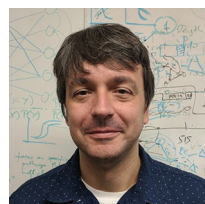
Curtis Huttenhower is an associate professor at the Harvard T.H. Chan School of Public Health and an associate member at the Broad Institute of MIT and Harvard.

and in disease. But adding population-scale epidemiology and deep clinical studies to the mix will be necessary to help refine microbiome-centric disease models. Such highly integrated models are more likely to explain disease outcomes in terms of the underlying causal molecular mechanisms, which will be necessary to target rationally in order to maximize microbiome-derived health benefits.

Curtis Huttenhower: Features, such as prevalent organisms, functional networks and metabolic pathways, have been used to describe microbiomes, both in health

Given the limitations of current preclinical models, what kinds of advances do you anticipate are needed to facilitate microbiome product development?

José C. Clemente: The key to making further progress through the use of animal models is not to reject them but to understand more precisely what their limitations are and how to make these findings translatable to humans. When we cannot reproduce a previous result, it is difficult to determine whether this is due to changes in technology or because the findings in the model were not biologically robust in the first place. If the methods are fully reproducible, we can at least remove one variable from this equation and improve the chances that findings in a single study can be reproduced and be translatable to humans.



José C. Clemente is an assistant professor and codirector of the Microbiome Translational Center at the Icahn School of Medicine at Mount Sinai in New York.

Alexander Maue: One way of addressing some of the present limitations is to use animals with humanized immune systems that have been associated with human microbiota. An additional strategy would be to perform experiments on the offspring of transplanted parental animals that would have co-developed their immune systems with the transplanted microbiome—the diversity of the transplanted microbiome has been shown to be conserved in the F₁ generation.



Alexander Maue is director of Microbiome Research Services at Taconic Biosciences.

Dan Littman: The idea of having humanized mice with human hematopoietic cells is terrific; however, this is a three-dimensional problem and it's an architectural problem. For example, in the induction of T_{reg} regulatory T cells in the colon, multi-step processes need to unfold to determine proper localization of cells in draining lymph nodes. We still don't know whether the human myeloid cells even go to the right places in mouse lamina propria or drain to lymph nodes.



Dan Littman is professor of molecular immunology at the Skirball Institute at the NYU School of Medicine.

Johan E. T. van Hylckama Vlieg: Standardization in the microbiome space is still in its infancy. For microbiome-based products to make it through the rigorous translational process, experts in a number of areas, including microbiology, ecology, epidemiology, statistics and bioinformatics, need to work together to identify sources of potential measurement variability and to develop best practices. Using these best practices in human studies will be the fastest way forward to increase our biological understanding and to develop successful microbiome intervention strategies that will make a change for consumers and patients.



Johan E. T. van Hylckama Vlieg is VP of Microbiome and Human Health Innovation at Chr. Hansen Holding.

To what extent is microbiome research moving away from correlations to identifying causal relationships?

Eran Elinav: We spent the first decade finding associations of microbiota with different clinical indications, but now we are discovering that many of these are not phenotypes that are caused by alterations in microbiota. Large efforts are underway to move the needle from



Eran Elinav is professor at the Weizmann Institute of Science and a scientific consultant for DayTwo and BiomX.

correlation to causation, but acquiring the necessary in-depth understanding of microbiome-determined phenotypes, microbe–microbe and microbe–host interactions, and the pleiotropic molecular mechanisms underlying these complex interactions is nontrivial.

David Kyle: When we're thinking about causality versus correlation, I feel causality is a concept that is overemphasized because of the regulatory process—to get a drug approved you have to establish clinical efficacy on a disease endpoint, or direct causality, turning the process into a binary decision. Correlation is not binary. We can generate compelling data that put us somewhere in the continuum of possibility, plausibility or probability that there is a link between an action and an effect.



David Kyle is chairman and CSO of Evolve BioSystems.

Bernat Olle: I also think this is undervalued. The conversation in the field of correlation and causation really undervalues the importance of triangulating with different pieces of evidence. If you have interesting associations from human studies, evidence from bacteria playing a pharmacological role, and maybe some mechanistic evidence of causation in animals, then I feel a lot better about going forward with a program than if I were just relying on causative evidence from an animal model, or only on associative evidence from human studies.



Bernat Olle is CEO of Vedanta Biosciences.

Richard Bonneau:

When we talk about causation in the microbiome field, we sometimes mix different concepts. When I think of causation, I think of establishing models that capture durable mechanistic underpinnings of the system, either from a priori knowledge or through *de novo* inference. Uncovering causality thus requires experimental accuracy and reproducibility but also an appropriate mechanistic context.



Richard Bonneau is a professor at New York University and Group Leader for Systems Biology at the Flatiron Institute Center for Computational Biology.

What about looking more broadly at microbiome composition beyond bacteria?

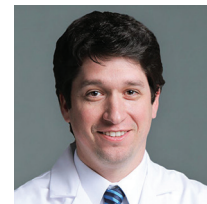
E.G.: Microbiome research was bacteriocentric in origin due to a 16S analytical constraint carried over from the microbial ecology field. With the development of next-generation sequencing metagenomic approaches, microbiome research has now become more inclusive of other microbial groups. The caveat is that work on microbiome components, such as the virome or the mycobiome, is still playing catch-up with the bacterial component in terms of basic identification and characterization of the players.

For example, the virome is an important component of the microbiome, but it's much more difficult to do very high-throughput profiles of the viral diversity because there are no marker genes like you see in fungi or in bacteria. As a result, you have to use metagenomic approaches to try to capture viral particle diversity, which is a very difficult thing to do. The discovery of crAssphage, the most abundant virus associated with humans, is a case in point. Identifying it and generating its complete sequence required combining datasets from lots of humans and lots of gut microbiomes.

B.O.: Are there potentially harmful viruses, parasites or bacteria that could be transmitted from one donor to many different recipients? With fecal microbiota transplantation, a procedure of undefined composition, we have a few thousand patients' worth of experiences, and we believe it to be a safe procedure. But once we apply it to hundreds of thousands of people, we're going to start learning a lot of things that we could not learn from thousands of people. If there is one potential safety concern that keeps me awake at night, it is the potential for a public healthcare scenario reminiscent of the HIV blood bank situation in the 1990s.

Why has so much work focused on the gut, and what other microbiomes should the field be considering?

Leopoldo Segal: Microbiome research beyond the gut faces two hurdles: accessibility and density. The gut microbiome can be accessed relatively easily, either directly or through fecal matter analysis, and the amount of bio-material available is in the tens to hundreds of grams and billions to trillions of cells. Once you leave the gut, accessibility and/or biomass diminish exponentially.



Leopoldo Segal is an assistant professor at the New York University School of Medicine.

A. Maue: There is an intrinsic bias when speaking of 'microbiome' that naturally pivots the discussion toward gut-centric models. It is maybe not a challenge *per se*, but it has resulted in a paucity of models, if any, that capture different microbiome sites (for example, lung, gut, skin), let alone integrate them into one singular translational animal model.

E.G.: After the gut, there has been a lot of work on the skin microbiome, also for obvious reasons. But niches such as the respiratory tract turn out to be incredibly important, and we've had some oral microbiome projects that have shown there are associations between microbes or infections and cardiovascular disease. The lung, for example, once considered a sterile environment, has now been shown to contain a diverse, low-density microbiome that, in turn, has been implicated in the local and systemic T helper 17 (T_H17)-driven inflammatory response to acute and chronic lung disease. We have also compared skin microbiome and lung microbiome data for fungi and bacteria, and we see that the fungi play a completely different role on the skin than in the lungs in stabilizing the community—we don't know, however, if the fungi are as important in the gut.

L.S.: The complex role the microbiome plays in modulating drug activity in lung, specifically of macrolides in emphysema patients, and in regulating the inflammatory response, is an example of how complex and relevant the interactions between the microbiome and its specific host niche, and between the different microbial groups within the microbiome, can be.

What kinds of challenges does integration of microbiome data with other data pose?

D.L.: I think we're going to have to develop much more sophisticated types of algorithms

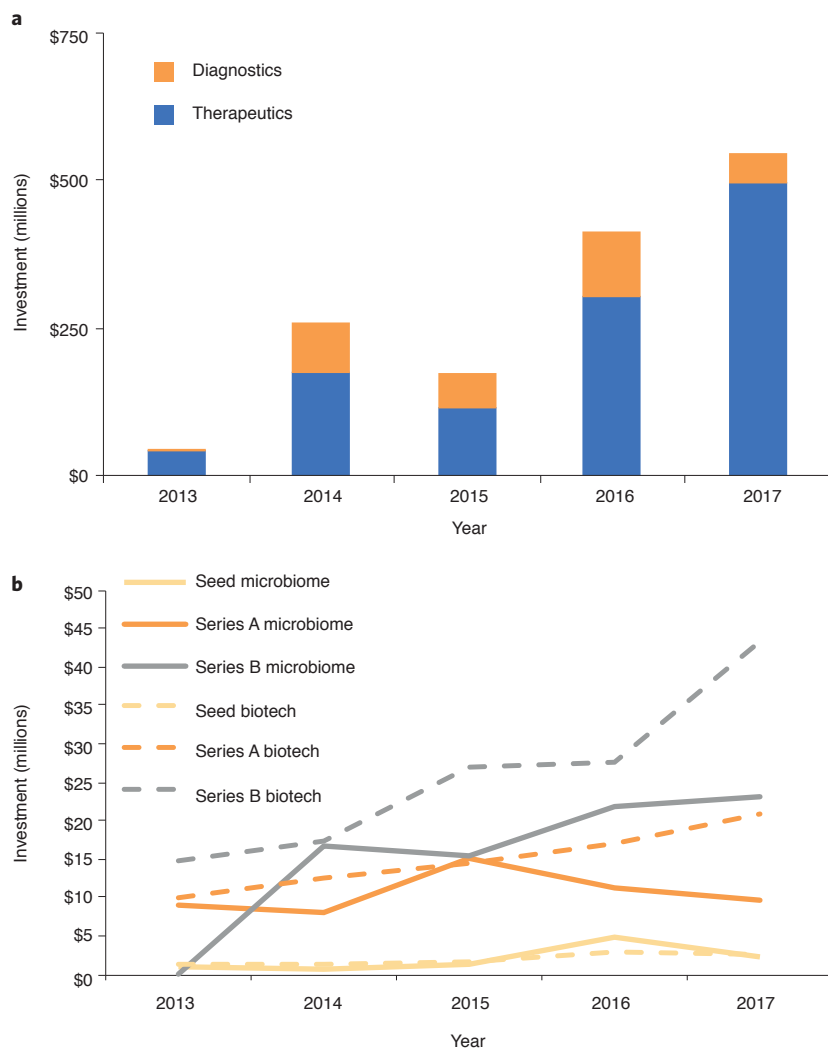


Figure 2 The microbiome investment landscape. (a) Venture capital investment in the microbiome space, categorized as therapeutics or diagnostics. (b) Average venture capital investment per round (seed or venture; series A; series B) in the microbiome space (solid lines) has been growing at similar rates to per round venture capital investment in the biotechnology field overall (dotted lines). Average seed funding (yellow) over the past five years is equivalent, whereas series A (orange) and series B (gray) funding in the microbiome space have been about 30% below those of the whole biotech field. Sources: Fankhauser *et al.*¹; University of Chicago Technology Commercialization²; CBIInsights; CrunchBase; GlobalData; SEC; Pitchbook; the companies.

and experimental systems to be able to understand the myriad complex multibody interactions modulating microbiome dynamics. I often fall back on empirical data, but I think they are going to have to be looked at in ever more integrated ways to try to derive some useful information that can be applied clinically.



Jun Wang is CEO of iCarbonX.

Jun Wang: The time has come to launch truly collaborative efforts that delve deeper into the

biology of the microbiome—its functional modules and their interplay with the human genome. Vertical, multi-omic association analyses are the key to unlocking this deeper understanding and eventually developing targeted and efficient microbiome-based probiotics and antibiotics.

R.B.: From the chromosome structure up to RNA, we have extremely efficient and scalable analytical methods. As soon as we deviate from nucleotides, though, we're in a very dark place in terms of scale, costs and the ability to structure experimental designs. The challenge comes from measuring something at one informational level but then not having a clean way

to align it with the informational level above it. As we make our way up the different informational levels—nucleotide to protein, protein to enzymatic activity, enzymatic activity to metabolite, and so on—each level becomes more complex and more expensive to elucidate. The only way around this challenge is to treat every approach to the microbiome the same way we treat model systems in biology: we need to identify the key components of the system and design very detailed experiments.

Peter Christey:

Real progress will be achieved when the focus moves from identifying and characterizing the physical presence of known organisms to deeper analysis of the functions of individual microbes, microbial communities and their host interactions.



Peter Christey is CEO of General Automation Lab Technologies.

At present, the field lacks the tools to understand how microbiome communities function, and this deficiency has become a roadblock to progress. Priority should be given to the development of scalable microbial cultivation and screening tools in order to model the function of the whole microbiota, looking beyond the limitations of the petri dish.

R.B.: The key to using all these multiple data types is to be much more diligent about engaging the analysts, the modelers, the biomathematicians, from the inception of a study, to determine what can be done—can you put these numbers side by side? Can you get enough power to actually look at 20,000 things interacting with a nearly infinite number of small molecules if you only have 100 samples? Sometimes you get a hard no, but it's better than wasting the money to just have an analyst rank a list of correlations with no statistical significance and thus no biological or clinical relevance.

In my opinion, one of the things that we should try to do is think big. The technologies in ten years are going to be a thousand times cheaper, and their penetration into the clinic and their ability to get uniform measurements will be considerably higher. When you combine that with all the advances that we've been seeing in statistics, in machine learning, I think we are going to be able to get a lot more out of our models and we're going to be able to model things much more broadly. But to achieve this potential, we will actually need to have reliable models, and I think we can deliver them a lot sooner than we think.

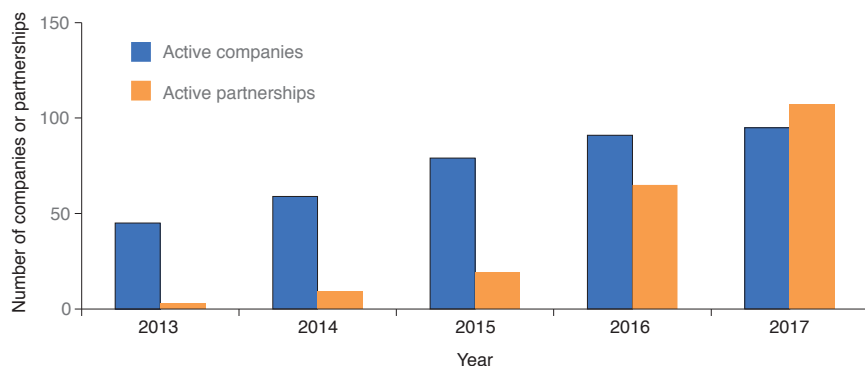


Figure 3 New microbiome-related companies have been steadily founded, and the number of partnerships in the field has grown exponentially. Sources: Fankhauser *et al.*¹; University of Chicago Technology Commercialization²; CBInsights; CrunchBase; GlobalData; SEC; Pitchbook; the companies.

Compared with other interventions, what do researchers need to consider when moving a microbiome therapy into the clinic?

Arpita Maiti: One of the reasons pharma does not invest in live biotherapeutics right now is the lack of work around pharmacokinetics and pharmacodynamics, two bits of information that are crucial to understanding what went wrong when a trial fails. Microbiome pharmacokinetics can be defined by analogy to traditional small molecule drugs as the kinetics of bacterial engraftment in an individual over time. And similarly, microbiome pharmacodynamics can be defined as the changes observed in the individual's microbiome over time, including shifts in microbial metabolites and host biomarkers.



Arpita Maiti is senior director for External Science & Innovation, Inflammation & Immunology and Microbiome at Pfizer.

I think it's an underappreciated fact in the early stages of discovery that placebo effect, for example, is really large, especially in some of the key diseases that you want to go into for microbiome. You're looking at a placebo effect of 40% for ulcerative colitis trials, and even for small molecules, trying to find a 20% delta is a challenge. Even the best-designed trials sometimes fail because of this piece.

B.O.: Instead of just putting a bacterial formulation in humans and rushing to determine its efficacy—and see if we can be first in reporting it—we should be taking a step back and asking whether we understand the pharmacokinetics and the pharmacodynamics. Based on

phase 1 pharmacokinetics and pharmacodynamics data, we want to be able to optimize dosing for a phase 2 trial to increase the probability of success. If, on the contrary, the phase 1 data are inconclusive or negative, we want to be able to do a proper root cause analysis and figure out what didn't work.

A. Maiti: Traditionally, clinical outcomes have been defined around the notion of whether or not a drug provides treatment benefit. In the context of the microbiome, however, clinical outcome may have to be redefined as an outcome whose clinical effect is not the treatment of a condition but rather the prevention of a condition.

D. Kyle: Taking the example of our work on the infant microbiome, we have seen a significant reduction in the antibiotic-resistant gene load and virulence factors following the return of the natural microbiome to dysbiotic breastfed and vaginally delivered babies. This reestablishment of the natural *Bifidobacterium*-dominant microbiome also significantly lowers the overall pathogen load, restores fecal pH and increases the microbiome stability. This is not treating, curing, mitigating or preventing a disease, but it is clearly a preferable situation to the dysbiotic microbiome of most babies in the United States today.

B.O.: I'd also like to see the field arriving at agreed upon biomarkers that we believe are a proper surrogate endpoint for recovery of a microbial community. Related to that, tests to determine how resilient the microbiome is to perturbations would provide an added measure of health.

A. Maiti: I'm going to go somewhere that maybe most pharma companies tend to get very nervous because it relates to their clinical

data: can we come up with microbial signatures that indicate somebody is going to be a responder versus a nonresponder regardless of treatment? Can we build an infrastructure for sharing deidentified clinical data devoid of specific drug and intervention information but capturing overall response in a way that sheds light on the underlying microbiome dynamics? I don't have a solution yet, but I'm suggesting this is a hurdle we could think of overcoming, and it's going to require a lot of thought and internal championship within companies to recognize the value it would add to what we do.

COMPETING INTERESTS

G.T.-O. is consultant-in-residence at Global Engage and became an advisor to the Janssen Human Microbiome Institute after completion of *Microbiome Futures*; M.B. serves on the scientific advisory boards of Elysium, Inc, Ubiome, Inc., Commense, Inc. and Seed, Inc.; R.B. has served as an expert witness for Kirkland and Ellis LLC, in advisory and consulting roles to the Simons Foundation, Novartis, Eli Lilly and Merus, and is currently collaborating with Novartis and Merus; P.C. is CEO and Co-Founder of General Automation Lab Technologies, Inc.; E.E. is a scientific consultant to DayTwo & BiomX; C.H. serves on the scientific advisory boards of Seres Therapeutics and Microbiome Insights; D. Kyle is an employee of, and owns shares in, Evolve BioSystems Inc.; D.L. is founder of and advisor to Vedanta, Director at Pfizer, Inc., and a recipient of research funding for microbiota projects from Boehringer Ingelheim; A. Maiti is an employee of Pfizer Inc.; A. Maue is an employee of Taconic Biosciences; B.O. is an employee of Vedanta Biosciences, a company developing drugs in the human microbiome field, and holds stock options in the company; J.E.T.v.H.V. is an employee of Chr. Hansen A/S; J.W. is founder & CEO of iCarbonX.

1. Fankhauser, M., Moser, C. & Nyfeler, T. Patents as early indicators of technology and investment trends: analyzing the microbiome space as a case study. *Front. Bioeng. Biotechnol.* **6**, 84 (2018).
2. University of Chicago Technology Commercialization. Polsky Microbiome – Venture Funding Data. <https://polsky.uchicago.edu/microbiome-research-at-uchicago/> (2018).

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FEATURE

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