

Modelling Holobiont Evolution
for a Better Understanding of
Symbiosis, Dysbiosis, and
Human Health

"Where There's Muck,
There's Brass" - Money
in the Microbiome

The Latest Advice on When to
Choose 16S rRNA Gene
Sequencing Over Shotgun
Metagenomics in Microbiome
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REPORTING ON THE ERA OF THE MICROBIOME

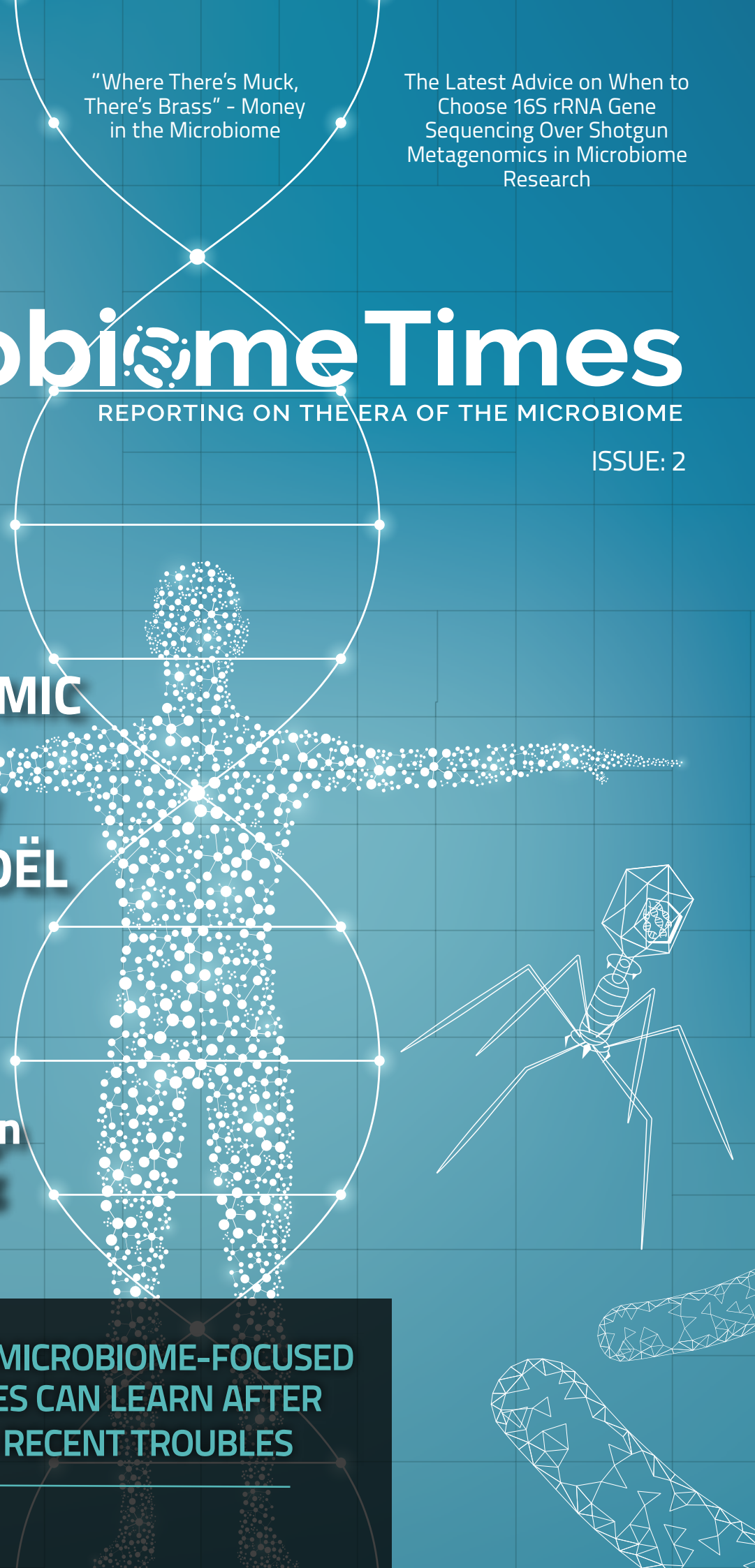
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from the editor

Dear Reader,

Major changes have occurred in the seven years I've been covering the microbiome beat. In the earlier days, the definition of 'microbiome' was being debated; scientists lacked knowledge about microbiome-related mechanisms. Furthermore, the regulatory landscape was much less defined and no one was sure what a 'microbiome therapeutic' would end up looking like.

Today, things look very different. With increasing attention from venture capitalists and with teams working hard to advance their microbiome-related R&D and clinical programs, the environment is fast-paced and competitive. There is no doubt that consumers and patients of the future will have access to an entirely new set of tools—enabled by microbiome research—for optimizing their health.

I attribute the remarkable progress in the field to a core group of pioneers in the science and business worlds: some of the best and brightest in life sciences who believed that microbiomes had the potential to benefit human, animal, and environmental health. I saw these individuals pursue the answers to difficult questions—and change their focus according to what they learned.

As the field continues to gain momentum, Microbiome Times is the trade publication that's at the forefront of the latest developments. Positioned at the intersection of science and business, the magazine is already a trusted resource for heavy-hitters in the industry and is poised to gain attention from ever broader audiences as multiple microbiome-related products move toward commercialization.

I'm delighted to be taking on the position of contributing editor at Microbiome Times. As someone who has written several hundred microbiome-related articles online and in print, and who continues to live and breathe microbiome science, I'll be bringing to these pages the best of what I'm able to do: tracking down the thought leaders; sharing diverse perspectives; highlighting the science; and challenging the dogma.

Stay tuned to contribute to the conversation—and to help shape what this field will become.

Kristina Campbell, M.Sc.
Contributing Editor

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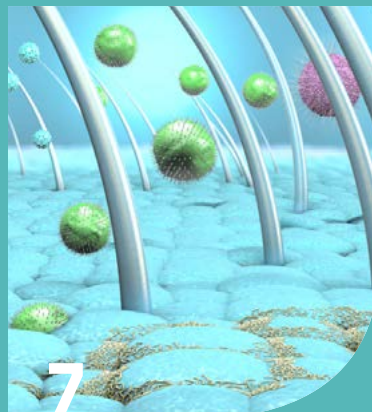
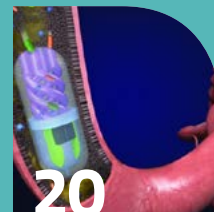
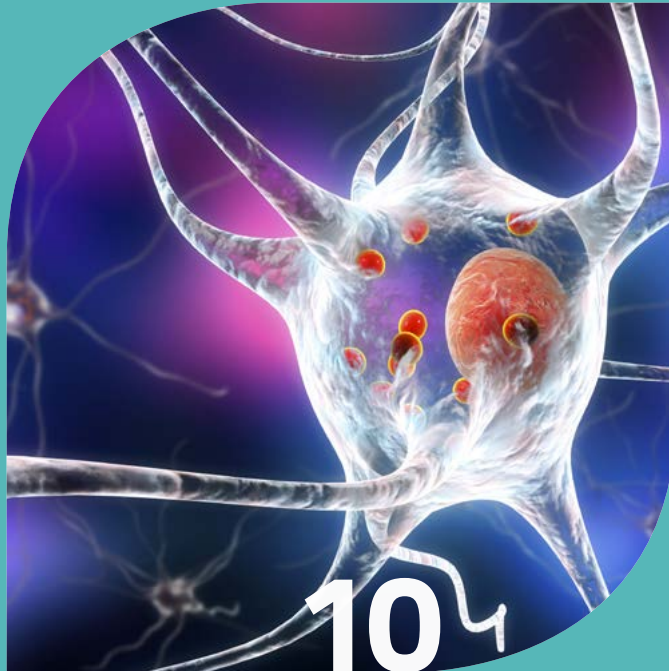
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News

YALE RESEARCHERS DESCRIBE ROLE OF BACTERIA IN DRUG RESPONSE

Yale researchers identified human gut microbes that metabolize over 150 therapeutic drugs, a finding that highlights the role bacteria play in determining how well individuals respond to medications, they report June 3 in the journal *Nature*.

Scientists in the lab of senior author Andrew Goodman of Yale's Microbial Sciences Institute and the Department of Microbial Pathogenesis also pinpointed the microbial genes responsible for many of these drug-metabolizing activities.

"It is possible that we can use genes or species of bacteria to predict the capacity of an individual's gut flora to metabolize a certain drug," said Maria Zimmermann-Kogadeeva, a postdoctoral fellow in Goodman's lab and co-lead author of the study.

"The work is a first step in identifying biomarkers that could help doctors prescribe the drugs that are the safest and most effective for individual patients."

The metabolism of drugs was once thought to be exclusively the role of human organs such as the liver, which carry out the important task of turning chemical compounds into water-soluble molecules to help their elimination from the body. This process is crucial to determining the effectiveness and safety of clinical drugs: The drugs need to stay in the body long enough to have an effect but not long enough to accumulate and potentially become toxic.

Drug metabolism studies typically do not assess the contribution of the microbiome.

"However, anecdotal examples of microbiome-metabolized drugs have emerged over past decades," Goodman said.

To map the connections between gut microbes and medical drugs, the researchers investigated whether and how each of 271 drugs are chemically modified by each of 76 different kinds of bacteria from the human gut. Almost two-thirds of these drugs were metabolized by at least one of the bacterial specimens tested. The researchers then constructed genetic libraries of the selected drug-metabolizing gut bacteria which allowed them to systematically identify many of the genes responsible for the chemical transformation of the drugs.

They found that the number of these genes varies widely across the gut microbial communities of healthy human volunteers. In some cases, these differences explain why some individuals' gut microbiomes metabolize drugs rapidly, while others modify the same drugs slowly or not at all.

"We hope this study provides a useful first step in understanding the microbiome contribution to drug metabolism," said Michael Zimmermann, postdoctoral fellow in the Goodman lab and co-lead author of the study.

"We think these approaches could shed light on how the gut microbiome also modulates our response to non-drug compounds, such as dietary nutrients and environmental agents."

The work was primarily supported by the National Institutes of Health.

News

NEW STUDY FINDS KEY FACTORS THAT SHAPE THE SKIN MICROBIOME, INCLUDING AGE AND LIFESTYLE CHOICES

Scientists are advancing our understanding of how the skin microbiome—the diverse community of bacteria, fungi, and other microbes living on the skin’s surface — is important for skin health. Knowing what shapes the skin microbiome may provide clues on how to maintain healthier skin throughout life, but so far scientists have a limited understanding of exactly which host and environmental factors contribute to the highly variable bacterial communities on the skin.

A new epidemiology study published in the scientific journal mBio found that up to 20 percent of the variability of the bacteria on people’s skin can be explained by demographic, physiologic and lifestyle factors. The study was the largest skin microbiome survey ever published, enrolling 495 individuals between the ages of 9 and 78, and collecting over 2500 skin swab samples from four skin sites and the mouth.

“While this study was a tremendous challenge to design, execute, and analyze, it takes studies with a large base like this to tease out the important factors that impact the skin microbiome and its critical role in skin health and beauty,”

says Dr. Greg Hillebrand, Senior Principal Research Scientist at Amway, who led the study.

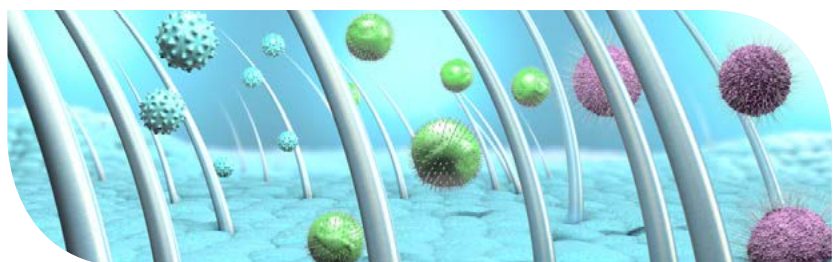
The research found the most influential factors associated with facial skin microbiome composition include levels of skin porphyrins – fluorescent molecules synthesized by *C. acnes*, the bacterium involved in the development of acne, as well as age and the use of sun protection. For the skin inside the mouth, ethnicity and smoking showed the strongest associations with its microbiome.

“The study advances our understanding of why skin microbiomes are so different from person to person,”

says first author Dr. Pedro Dimitriu, Senior Director of Bioinformatics at Microbiome Insights, Inc., which collaborated with Amway on the study.

“We know the environment outside the body influences the skin microbiome to some extent, but this study shows some of the lifestyle and host factors that shape it too.”

The study found different skin microbiome patterns that corresponded with chronological age, and others that corresponded with facial wrinkles and hyperpigmented spots. The discovery of a bacterium called *Corynebacterium kroppenstedtii* and its association with aging skin is the first step toward understanding how this microbe could be involved in the mechanisms of skin aging. A patent is pending on this potentially valuable finding.



News

GUT MICROBES EAT PARKINSON'S MEDICATION

AUTHOR: CAITLIN MCDERMOTT-MURPHY,
COMMUNICATIONS AND OUTREACH MANAGER
HARVARD UNIVERSITY

A Species of Bacteria Consumes Levodopa, the Primary Treatment for Parkinson's Disease

The first time Vayu Maini Rekdal manipulated microbes, he made a decent sourdough bread. At the time, young Maini Rekdal, and most people who head to the kitchen to whip up a salad dressing, pop popcorn, ferment vegetables, or caramelize onions, did not consider the crucial chemical reactions behind these concoctions.

Even more crucial are the reactions that happen after the plates are clean. When a slice of sourdough travels through the digestive system, the trillions of microbes that live in our gut help the body break down that bread to absorb the nutrients. Since the human body cannot digest certain substances—all-important fiber, for example—microbes step up to perform chemistry no human can.

"But this kind of microbial metabolism can also be detrimental,"

said Maini Rekdal, a graduate student in the lab of Professor Emily Balskus and first-author on their new study published in *Science*. According to Maini Rekdal, gut

microbes can chew up medications, too, often with hazardous side effects.

"Maybe the drug is not going to reach its target in the body, maybe it's going to be toxic all of a sudden, maybe it's going to be less helpful," Maini Rekdal said.

In their study, Balskus, Maini Rekdal, and their collaborators at the University of California San Francisco, describe one of the first concrete examples of how the microbiome can interfere with a drug's intended path through the body. Focusing on levodopa (L-dopa), the primary treatment for Parkinson's disease, they identified which bacteria out of the trillions of species is responsible for degrading the drug and how to stop this microbial interference.

Parkinson's disease attacks nerve cells in the brain that produce dopamine, without which the body can suffer tremors, muscle rigidity, and problems with balance and coordination. L-dopa delivers dopamine to the brain to relieve symptoms. But only about 1 to 5% of the drug actually reaches the brain.

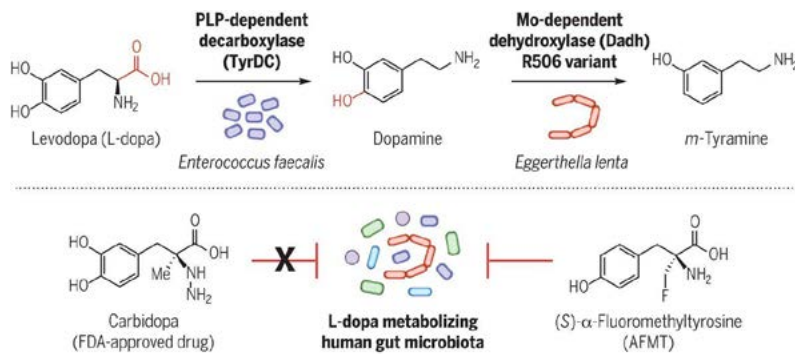
This number—and the drug's efficacy—varies widely from patient to patient. Since the introduction of L-dopa in the late 1960s, researchers have known that the body's enzymes (tools that perform necessary chemistry) can break down L-dopa in the gut, preventing the drug from reaching the brain. So, the pharmaceutical industry introduced a new drug, carbidopa, to block unwanted L-dopa metabolism. Taken together, the treatment seemed to work.

"Even so," Maini Rekdal said, **"there's a lot of metabolism that's unexplained, and it's very variable between people."**

That variance is a problem: Not only is the drug less effective for some patients, but when L-dopa is transformed into dopamine outside the brain, the compound can cause side effects, including severe gastrointestinal distress and cardiac arrhythmias. If less of the drug reaches the brain, patients are often given more to manage their symptoms, potentially exacerbating these side effects.

Maini Rekdal suspected microbes might be behind the L-dopa disappearance. Since previous research showed that antibiotics improve a patient's response to L-dopa, scientists speculated that bacteria might be to blame. Still, no one identified which bacterial species might be culpable or how and why they eat the drug.

So, the Balskus team launched an investigation. The unusual chemistry—L-dopa to dopamine—was their first clue.



When gut microbes metabolize the Parkinson's drug L-dopa, they produce dopamine; a second microbe then metabolizes dopamine, producing meta-tyramine. While L-dopa metabolism likely limits drug availability and contributes to side effects, the potential ramifications of transforming dopamine into meta-tyramine are unknown.

Few bacterial enzymes can perform this conversion. But, a good number bind to tyrosine—an amino acid similar to L-dopa. And one, from a food microbe often found in milk and pickles (*Lactobacillus brevis*), can accept both tyrosine and L-dopa.

Using the Human Microbiome Project as a reference, Maini Rekdal and his team hunted through bacterial DNA to identify which gut microbes had genes to encode a similar enzyme. Several fit their criteria; but only one strain, *Enterococcus faecalis* (*E. faecalis*), ate all the L-dopa, every time.

With this discovery, the team provided the first strong evidence connecting *E. faecalis* and the bacteria's enzyme (PLP-dependent tyrosine decarboxylase or TyrDC) to L-dopa metabolism.

And yet, a human enzyme can and does convert L-dopa to dopamine in the gut, the same reaction carbidopa is designed to stop. Then why, the team wondered, does the *E. faecalis* enzyme escape carbidopa's reach?

Even though the human and bacterial enzymes perform the exact same chemical reaction, the bacterial one looks just a little different. Maini Rekdal speculated that carbidopa may not be able to penetrate the microbial cells or the slight structural variance could prevent the drug from interacting with the bacterial enzyme. If true, other host-targeted treatments may be just as ineffective as carbidopa against similar microbial machinations.

But the cause may not matter. Balskus and her team already discovered a molecule capable of inhibiting the bacterial enzyme.

"The molecule turns off this unwanted bacterial metabolism without killing the bacteria; it's just targeting a non-essential enzyme,"

Maini Rekdal said. This and similar compounds could provide a starting place for the development of new drugs to improve L-dopa therapy for Parkinson's patients.

The team might have stopped there. But instead, they pushed further to unravel a second step in the microbial metabolism of L-dopa. After *E. faecalis* converts the drug into dopamine, a second organism converts

dopamine into another compound, meta-tyramine.

To find this second organism, Maini Rekdal left behind his mother dough's microbial masses to experiment with a fecal sample. He subjected its diverse microbial community to a Darwinian game, feeding dopamine to hordes of microbes to see which prospered.

Eggerthella lenta won. These bacteria consume dopamine, producing meta-tyramine as a by-product. This kind of reaction is challenging, even for chemists.

"There's no way to do it on the bench top," Maini Rekdal said, "and previously no enzymes were known that did this exact reaction."

The meta-tyramine by-product may contribute to some of the noxious L-dopa side effects; more research needs to be done. But, apart from the implications for Parkinson's patients, *E. lenta*'s novel chemistry raises more questions: Why would bacteria adapt to use dopamine, which is typically associated with the brain? What else can gut microbes do? And does this chemistry impact our health?

"All of this suggests that gut microbes may contribute to the dramatic variability that is observed in side effects and efficacy between different patients taking L-dopa," Balskus said.

But this microbial interference may not be limited to L-dopa and Parkinson's disease. Their study could shepherd additional work to discover exactly who is in our gut, what they can do, and how they can impact our health, for better or worse.

News

NEW RESEARCH SHOWS LINK BETWEEN PARKINSON'S DISEASE AND THE MICROBIOME

A simple saliva swab may provide an accurate way of detecting Parkinson's disease in its earliest stages, according to a study just published in the journal Plos One. In a paper titled, "The oral microbiome of early stage Parkinson's disease and its relationship with functional measures of motor and non motor function," researchers from SUNY Upstate Medical University and Quadrant Biosciences Inc., discovered that specific bacteria in the oral microbiome accurately differentiate early stage Parkinson's disease ("esPD") from healthy controls. Moreover, these changes in individuals with esPD appeared to be related to neural and brain function. The results of the study suggest that the oral microbiome may represent an easily accessible and informative microenvironment that offers new insights into Parkinson's disease.

SOURCE: QUADRANT BIOSCIENCES INC.

Changes in the function and microbiome of the upper and lower gastrointestinal (GI) tract have been documented in Parkinson's disease before, although most studies have focused on the fecal microbiome and patients with advanced disease states. In this study, the researchers sought to identify changes in the oral microbiome of patients with early stage Parkinson's disease. Using next-generation sequencing, the complete composition of microbes (including bacteria, yeast, phages and viruses), were quantified in the saliva of 48 esPD subjects and 36 age and gender-matched healthy control subjects. In addition to the collection of saliva samples, detailed assessments of motor, cognitive, balance, smell and taste functions were conducted to determine the disease stage.

According to Dr. Frank Middleton, Ph.D. at SUNY Upstate Medical University and one of the principal authors of the research, this was the first in a series of planned studies to evaluate the importance of the oral microbiome in esPD.

"The major impetus behind our efforts is the earliest possible detection of Parkinson's disease before the onset of motor symptoms. That is the Holy Grail that we all chase."

The results of this initial work were intriguing. Significant differences in the composition of the oral microbiome between the esPD and healthy control subjects were observed at an overall accuracy of 84.5%, suggesting potentially strong utility as a diagnostic tool. Most of the differences were found in bacterial species abundance, though differences in some species of yeast were also observed. The researchers also found significant changes in a set of 9 human or host mRNAs in the saliva, several of which mapped to various brain functions and showed correlations with some of the significantly changed microbial taxa. Finally, significant correlations between many of the microbiota and functional measures were also observed, including those reflecting cognition, balance, and disease duration.

The study also suggested that the oral microbiome is not only a robust and reliable source of biological information about Parkinson's disease, but likely a better source than the lower GI tract for identifying changes in microbial abundance, according to Dr. Middleton.

"To date, the vast majority of studies have focused on the fecal microbiome. However, the lower GI tract may be affected by such things as constipation and motility issues, which are common in Parkinson's disease, or the use of antacids, both of which could influence the environment of the lower GI and potentially give rise to misleading results. By focusing on the beginning of the GI tract, we are largely avoiding changes in the environmental conditions in which the bacteria grow."

Finally, the authors noted that not all bacteria found in the oral microbiome of esPD patients appear to be beneficial as they might be in non-diseased individuals. Indeed some, such as *Lactobacillus reuteri*, found in higher levels in patients with esPD, while commonly regarded as a beneficial probiotic may be pro-inflammatory and actually contribute to the progression of the disease. This may have implications for those in at-risk categories for Parkinson's disease taking probiotic supplements that contain some of these bacteria.

Richard Uhlig, Founder and CEO of Quadrant Biosciences, explained why this research on esPD nicely dovetails with other work the company has been conducting collaboratively on the microbiome and epigenetics.

"It is exciting and extremely gratifying to be on the forefront of these exciting discoveries in epigenetics and the microbiome, and we are optimistic that, like our autism research, this work will eventually translate into a viable and widely available test to help millions of people afflicted with this terrible disease."

Dr. Middleton summarized the overarching significance of this research.

"I think we've entered a new era in terms of neurodegenerative disease research and are now starting to consider the oral microbiome and the bacteria and other biota that are there as a ripe place for discovery of potential risk factors."

News

JUST A PHAGE? HOW BACTERIA'S PREDATORS CAN SHAPE THE GUT MICROBIOME



SOURCE: BRIGHAM AND WOMEN'S HOSPITAL

Study finds that bacteriophages can have a cascade of effects on the microbiome and change metabolite levels, with implications for therapeutic use.

The gut microbiome is a complex, interconnected ecosystem of species. And, like any ecosystem, some organisms are predators and some are prey. A new study led by investigators at Brigham and Women's Hospital and the Wyss Institute investigates the impact of bacteriophage, viruses that infect and kill bacteria. They find that phage can have a profound impact on the dynamics of the gut microbiome, not only affecting certain species directly but also having a cascading effect on others. Phage may also be impacting their human host by modulating metabolites, including chemical substances found in the brain. The team, which includes first author Bryan Hsu, PhD, and co-corresponding senior author Pamela Silver, PhD, at the Wyss Institute, and Lynn Bry, MD, PhD, at the Brigham and director of the Massachusetts Host-Microbiome Center, has published its results in *Cell Host & Microbe*.

"One of the major interests in my lab is understanding the changes in the dynamics of the gut microbiome. Bacteriophage are a huge component of the microbiome but haven't been studied much yet,"

said co-corresponding senior author Georg Gerber, MD, PhD, MPH, co-director of the Massachusetts Host-Microbiome Center and chief of the Division of Computational Pathology in the Department of Pathology at the Brigham.

"Some people are exploring phage therapy, using phage to kill off microbes, but phage are also found naturally in the gut, co-existing with the rest of the ecosystem. We wanted to find out what they are doing in there."

To address this question, the team colonized the guts of mice with a defined set of human bacterial species and then added phages, tracking the growth of each microbe. Using high-throughput sequencing and computational analyses, the team found that the phage caused attritions of the species they preyed upon as expected, but with a rippling effect on the rest of the ecosystem including blooms of non-targeted species.

In addition to looking at the effects on microbes, the team also looked for effects on the metabolome — chemical substances that can come from both the host and the bacteria present. They found that when they modulated the microbiome with phage, they could see targeted changes in the metabolome, including changes in neurotransmitter levels and bile acids.

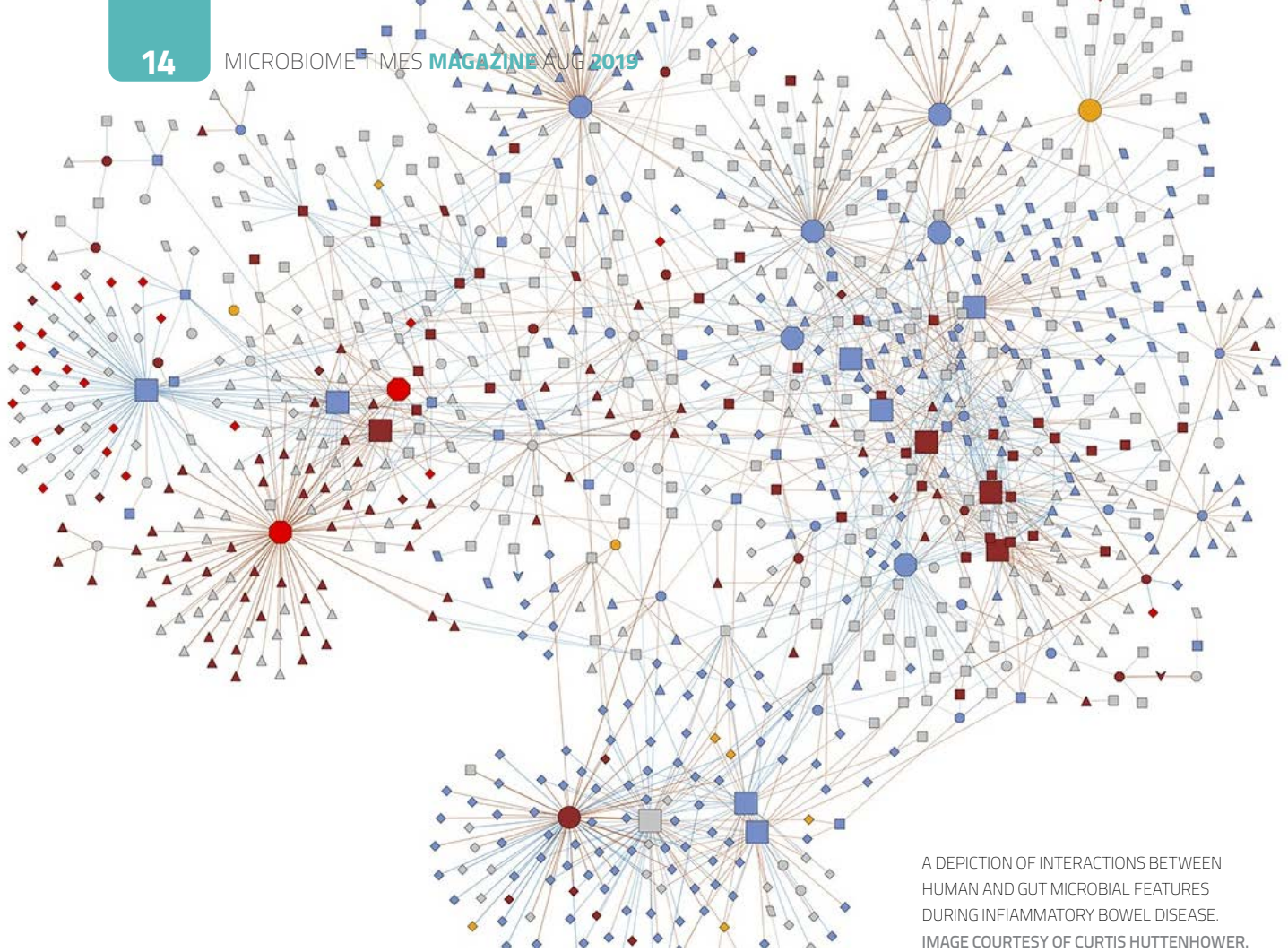
"This finding fascinates me for followup and raises significant questions: Could we use phage to modulate these activities? Could this be an intervention for conditions, such as depression, where you'd want to change neurotransmitter levels?" said Gerber.

"Even if they aren't used as a direct therapeutic, our study suggests that phage may be a good tool for understanding the potential effects of other therapeutics that alter the microbiome."

Gerber and colleagues are especially interested in looking at the intersection of phage and malnutrition in the developing world, given the profound effects on the metabolome and microbiome that malnutrition can have.

"We hope that our work will provide a framework to guide future investigations to elucidate the interplay between phage, the microbiota, and host health and disease," said Gerber.





NEW FINDINGS FROM HUMAN MICROBIOME PROJECT REVEAL HOW MICROBIOME IS DISRUPTED DURING IBD

SOURCE: HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

A new study led by researchers from Harvard T.H. Chan School of Public Health and the Broad Institute of MIT and Harvard is the first to have observed the complex set of chemical and molecular events that disrupt the microbiome and trigger immune responses during flare-ups of inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis.

While previous studies have cataloged microbial changes during IBD, the researchers in this study developed a unique biotechnology toolbox to understand why microbiomes change during IBD and how this provokes an unhealthy inflammatory reaction. These tools allowed them to measure microbial chemical changes and human gene regulatory shifts, potentially allowing for new therapies in the future.

The study, which included dozens of collaborators, was part of the second phase of the Human Microbiome Project (HMP). The project, the first phase of which was launched in 2007 by the National Institutes of Health (NIH) Common Fund, aimed to characterize the microbiome in healthy adults and in people with specific microbiome-associated diseases. The most recent phase of work began in 2013 with support from across the NIH, and with the mandate to tease apart the molecular mechanisms underlying the microbiome's roles in disease.

"The Human Microbiome Project overall has been a flagship effort in understanding the microbiome's contributions to health, and in creating a community of researchers who can study the microbiome to discover new diagnostics and therapies for disease,"

said Curtis Huttenhower, professor of computational biology and bioinformatics at Harvard Chan School and associate member at the Broad Institute and senior author of the study.

"Our results from this study pave the way for early detection of upcoming flares in disease activity — which can then be aggressively treated — or potentially for new biochemical therapeutic opportunities to encourage complete remission of IBD."

The gut microbiome is a community of trillions of microbes, including bacteria, viruses, and fungi. Each person has a distinct microbiome, and research indicates that the microbiome plays an important role in numerous diseases, including IBD, which affects more than 3.5 million people worldwide and is growing in prevalence. IBD is a chronic disease that is marked by periods of remission followed by flare-ups in which the disease becomes active.

For this study, the most comprehensive analysis to date of human microbiome interactions during IBD, researchers followed 132 participants for one year and compared Crohn's disease and ulcerative colitis patients to a control group of participants that did not have IBD. Participants provided stool samples every two weeks, blood samples approximately once a quarter, and a set of colon biopsies at the start of the study for analysis. In total, 2,965 stool, biopsy, and blood samples were analyzed with an unprecedented suite of molecular, cellular, and clinical tools to understand the detailed biochemistry of the disease.

First, these detailed measurements made it easy to observe and confirm findings from previous studies, such as a reduction in overall gut ecological diversity and the gain and loss of specific "pro-" and "anti-inflammatory" microbes during disease.

More importantly, the suite of tools deployed for this study allowed researchers to determine the reasons for the changes. The results showed that during periods of disease activity, people with IBD had fewer microbially-derived chemicals, which they speculated could be due to a combination of factors, including less beneficial microbial metabolism, poorer nutrient absorption, greater water or blood levels in the bowels, and more urgent bowel movements. These factors decreased the overall stability of the gut microbial ecosystem, leading to more episodes of

improper immune responses and overreaction to the normal gut microbiome among IBD patients.

Specifically, during periods of disease activity people with IBD had higher levels of polyunsaturated fatty acids, including adrenate and arachidonate. The researchers also discovered that nicotinuric acid was found almost exclusively in the stool of patients with IBD and that levels of vitamins B5 and B3 were particularly depleted in the gut of people with IBD.

The team also found that bile acids — a set of compounds made by humans but chemically modified by gut microbes — were disrupted during IBD as well, in tandem with molecular regulation in groups of microbes. These included a group of bacteria related to the genus *Subdoligranulum* that are carried by almost everyone, but are depleted during inflammation, and which have not been previously isolated or characterized.

Overall, the findings provide the most detailed snapshot to date of the microbiome in people with IBD, during active and non-active disease states. The findings showed that different forms of IBD—Crohn's disease compared with ulcerative colitis, for instance—had different effects on the activity and composition of the microbiome. The researchers said the findings provide promising new targets for potential IBD treatments, including polyunsaturated fatty acids, bile acid derivatives, and human immune response pathways, as well as new data, tools, and protocols that will enable future research on IBD and the microbiome.



News

MORE THAN 100 NEW GUT BACTERIA DISCOVERED IN HUMAN MICROBIOME

**Study will help
understand role of
microbiome in health
and disease**

Scientists working on the gut microbiome have discovered and isolated more than 100 completely new species of bacteria from healthy people's intestines. The study from the Wellcome Sanger Institute, Hudson Institute of Medical Research, Australia, and EMBL's European Bioinformatics Institute, has created the most comprehensive collection of human intestinal bacteria to date.

SOURCE: WELLCOME TRUST SANGER INSTITUTE

This will help researchers worldwide to investigate how our microbiome keeps us healthy, and its role in disease. Reported 4th February in Nature Biotechnology, the new resource will allow scientists to detect which bacteria are present in the human gut, more accurately and faster than ever before. This will also provide the foundation to develop new ways of treating diseases such as gastrointestinal disorders, infections and immune conditions.

About 2 per cent of a person's body weight is due to bacteria and the intestinal microbiome is a major bacterial site and an essential contributor to human health. Imbalances in our gut microbiome can contribute to diseases and complex conditions such as Inflammatory Bowel Disease, Irritable Bowel Syndrome allergies and obesity. However, as many species of gut bacteria are extremely difficult to grow in the laboratory, there is a huge gap in our knowledge of them.

In this study, researchers studied faecal samples from 20 people from the UK and Canada, and successfully grew and DNA sequenced 737 individual bacterial strains from these. Analysis of these isolates revealed 273 separate bacterial species, including 173 that had never previously been sequenced. Of these, 105 species had never even been isolated before.

Dr Samuel Forster, first author on the paper from the Wellcome Sanger Institute and Hudson Institute of Medical Research, Australia, said:

"This study has led to the creation of the largest and most comprehensive public database of human health-associated intestinal bacteria. The gut microbiome plays a major in health and disease. This important resource will fundamentally change the way researchers study the microbiome."

Standard methods to understand how the gut microbiome impacts on human health involves sequencing the DNA from mixed samples of gut bacteria to try to understand each component. However, these studies have been severely hampered by the lack of individually isolated bacteria and reference genomes from them.

The new culture collection and reference genomes will make it much cheaper and easier for researchers to determine which bacteria are present within communities of people and research their role in disease.

Dr Rob Finn, an author from EMBL's European Bioinformatics Institute, said:

"For researchers trying to find out which species of bacteria are present in a person's microbiome, the database of reference genomes from pure isolates of gut bacteria is crucial. Then if they want to test a hypothesis, for example that a particular species is enriched in a certain disease, they can get the isolate itself from the collection and physically test in the laboratory if this species seems to be important."

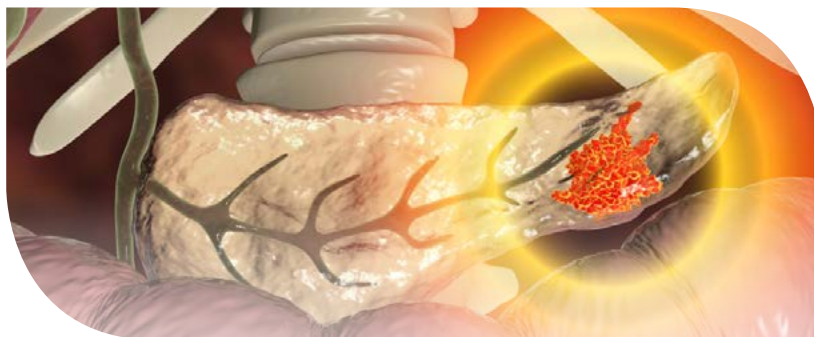
Dr Trevor Lawley, Senior author from the Wellcome Sanger Institute, said:

"This culture collection of individual bacteria will be a game-changer for basic and translational microbiome research. By culturing the unculturable, we have created a resource that will make microbiome analysis faster, cheaper and more accurate and will allow further study of their biology and functions. Ultimately, this will lead us towards developing new diagnostics and treatments for diseases such as gastrointestinal disorders, infections and immune conditions."

News

BACTERIA ON TUMORS INFLUENCES IMMUNE RESPONSE AND SURVIVAL OF PATIENTS WITH PANCREATIC CANCER

SOURCE: UNIVERSITY OF TEXAS M. D. ANDERSON CANCER CENTER



Study Finds Specific Microbiome Tied to Long-Term Survival; Points to Fecal Transplant Treatment

A key difference between the few pancreatic cancer patients who survive long-term and the many whose disease overcomes all treatments is the bacterial signatures on their tumors that either stimulate or suppress immune response, a team led by researchers from The University of Texas MD Anderson Cancer Center reports in the journal *Cell*.

The researchers also showed that fecal microbiota transplants (FMT) from long-term survivors prompted immune response and stifled tumors in a mouse model of the disease by altering the bacteria on the tumor – its microbiome.

“Results of the FMT experiments represent a significant therapeutic opportunity to improve pancreatic cancer treatment by altering the tumor immune microenvironment,”

said senior author Florencia McAllister, M.D., assistant professor of Clinical Cancer Prevention at MD Anderson.

“There is promise here but we have a lot of work ahead.”

MD Anderson’s Pancreatic Cancer Moon Shot™ has provided McAllister with funding to develop a clinical trial of fecal transplants for pancreatic cancer. The Moon Shots Program™ is a collaborative effort to accelerate the development of scientific discoveries into clinical advances that save patients’ lives.

Most patients with pancreatic ductal adenocarcinoma – the most common form of pancreatic cancer – have late-stage disease when diagnosed. Just 9% survive to five years. Those with earlier stage cancer that can be surgically removed have a high recurrence rate and median survival of 24-30 months.

No genomic biomarkers have been identified that shed light on the reasons for long-term survival in that fraction of patients, McAllister said.

Long-term survivors have diverse tumor microbiome

While recent research has shown that the composition and diversity of microbes living in the digestive tract – the gut microbiome – can affect how cancer immunotherapy works, little research has focused on the bacteria in the tumor and how it might affect prognosis and survival, McAllister said.

“We’ve known there are bacteria on pancreatic tumors, so we asked ‘do these bacteria have a role in cancer?’”

To launch the first such study in pancreatic cancer, McAllister and colleagues analyzed the bacterial DNA in tumors of long-term survivors matched to short-term survivors from two independent cohorts at MD Anderson and Johns Hopkins Hospital. In the MD Anderson cohort, median survival

was 10 years for the long-term survivors (22 patients) and 1.6 years for the short-term survivors (21 patients). In the validation cohort from Johns Hopkins, 15 patients had overall survival greater than 10 years, and 10 survived fewer than five years.

Using 16S rRNA gene sequencing, the team found the long-term survivors had much greater diversity of bacterial species than the short-term survivors. Stratifying the MD Anderson patients only by this diversity measure showed those with high diversity had median survival of 9.66 years and those with low diversity had median survival of 1.66 years.

The diversity results were independent of other factors, such as previous therapies, body mass index, and antibiotics use, making it a predictor of survival for surgical patients and indicating the potential importance of the tumor microbiome in cancer progression.

Researchers also found marked differences in the bacterial communities found in each survivor group. The long-term survivors showed a relative abundance of *Pseudoxanthomonas*, *Saccharopolyspora* and *Streptomyces*. The presence of all three taxa, as well as the species *Bacillus Clausii*, predicted better outcomes for patients in both MD Anderson and Johns Hopkins cohorts.

Specific microbiome boosts immune attack on tumors

Immunohistochemistry showed a greater density of T cells, including the CD8-positive cell-killing variety, in the tumors of long-term survivors in both the MD Anderson and Johns Hopkins cohorts, consistent with previous research that showed more active immune response in long-term survivors.

McAllister and colleagues found a strong correlation between immune cell infiltration and the microbiome diversity of the tumors. Additional analysis showed immune infiltration and activation of T cells was associated with the three enriched bacterial types discovered on long-term survivors' tumors.

With an apparent connection between the tumor microbiome and immune response, the team set out to find a way to change the tumor microbiome.

Using the gut microbiome to alter tumor microbiome

"You cannot modulate the tumor microbiome directly, but you can modulate the gut microbiome, and if there's cross-talk between the gut and the tumor microbiomes, you could change the tumor microbiome indirectly," McAllister said.

The team compared the bacteria in the gut, in the tumor and in adjacent tissue in three surgery patients. They found the gut microbiome represents about 25% of the tumor microbiome, but is absent from the normal, adjacent tissue, suggesting bacteria in the gut can colonize pancreatic tumors.

The researchers transplanted fecal microbiota from patients with

advanced cancer into mice and found that the donor microbiome represented about 5% of the resultant tumor microbiome but that 70% of the overall tumor microbiome had been otherwise altered by the transplant.

"Now we know you can completely change the bacterial composition of the tumor microbiome by doing FMT," McAllister said.

Reversing immune suppression with fecal transplants

Next, they performed stool transplants into mice from patients who had advanced pancreatic cancer, patients who had survived more than five years and had no evidence of disease, and healthy controls.

Five weeks after tumor implementation, mice that had received FMT from patients with advanced disease had much larger tumors than those that received FMT from long-term survivors (70% smaller average size) or healthy controls (50% smaller average size).

Immune profiling showed the mice that received the FMT from long-term survivors had significantly higher numbers and greater activation of CD8-positive T cells compared to the other two groups. Those who received FMT from advanced-stage patients had increased regulatory T cells and myeloid-derived suppressor cells, both of which suppress immune response.

To evaluate whether the effect of FMT relies on the immune system, the team depleted T cells in a group of mice treated with the long-term survivor FMT, which completely blocked the anti-tumor effect of the transplant.

News

3D PRINTED PILL SAMPLES GUT MICROBIOME TO AID DIAGNOSIS AND TREATMENT

The pill is the first known working device capable of non-invasively and accurately assessing the profile of bacterial species inhabiting any stage of the gastrointestinal tract

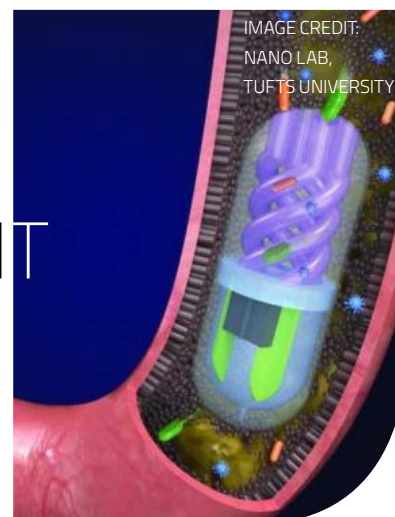
A research team led by Tufts University engineers has developed a 3D printed pill that samples the microbiome as it passes through the gastrointestinal tract (GI). The ability to profile bacterial species inhabiting the gut could have important implications for the diagnosis and treatment of conditions that are affected by the microbiome, according to the researchers.

The 3D printed pill described in the journal *Advanced Intelligent Systems* represents the first non-invasive diagnostic tool capable of providing a profile of microbiome populations throughout the entire GI tract, according to the researchers. Current methods of sampling the microbiome involve analysis of fecal DNA and metabolites, but that approach provides little information of the environment upstream of the distal colon, where bacterial species can vary significantly.

The pill has been studied and found to provide accurate identification of bacterial populations and their relative abundance in both in vitro and in vivo applications, the paper says. It has been tested in pigs and primates, yet clinical trials will be needed to determine if the pill can be used routinely in humans for clinical care.

More than 1,000 species of bacteria can inhabit the gut. The vast majority of these bacteria have a beneficial, supportive role in digestion and protection against disease. When the natural balance of the microbiome is skewed, a condition called "dysbiosis" occurs, which can be associated with inflammation, susceptibility to infections, and even the exacerbation of other diseases such as cancer. Research is increasingly unveiling specific microbiome metabolites that have beneficial or protective effects for the host against disease.

The pill is more sophisticated than just a sponge. It is manufactured in a 3D printer with microfluidic channels that can sample different stages of the GI tract. The surface of the pill is covered with a pH sensitive coating, so that it does not absorb any samples until it enters the small intestine (bypassing the stomach) where the coating dissolves. A semi-permeable membrane separates two chambers in the pill – one containing helical



channels that take up the bacteria and the other containing a calcium salt-filled chamber. The salt chamber helps create an osmotic flow across the membrane which pulls the bacteria into the helical channels. A small magnet in the pill enables one to hold it at certain locations in the gut for more spatially targeted sampling using a magnet outside the body. A fluorescent dye in the salt chamber helps locate the pill after it exits the GI tract.

The researchers see this technology as bridging an important gap in gastrointestinal diagnosis.

"We have incredible technology to analyze bacterial populations using DNA sequencing techniques, but until now have not had a way to sample bacteria throughout the GI tract in a way that was not invasive," said Hojatollah Rezaei Nejad, a post-doctoral fellow studying novel applications of 3D printing in Sonkusale's laboratory at Tufts and lead author of the study.

"By sampling non-invasively, this pill could help us better identify and understand the role of different bacterial species in health and disease."

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1 4D Pharma & MD Anderson Cancer Center to Evaluate LBPs in Solid Tumours

4D pharma plc and The University of Texas MD Anderson Cancer Center announced a strategic collaboration to evaluate 4D's Live Biotherapeutic oncology pipeline across a range of cancer settings. The alliance brings together MD Anderson's translational medicine and clinical research capabilities with 4D's expertise in the discovery and development of Live Biotherapeutics. The collaboration will initially assess 4D's lead oncology candidate, MRx0518, as a potential treatment for solid tumours. The first clinical study, an open label Phase I study of MRx0518 in combination with Keytruda® and conducted in collaboration with Merck & Co., Inc., Kenilworth, NJ., USA, has been initiated. The study enrolls up to 132 patients with metastatic cancer across multiple histologies (non-small cell lung cancer, renal cell carcinoma, bladder cancer and melanoma) who have failed prior anti-PD-1 therapy. Subsequent studies are also being planned under the collaboration, including using MRx0518 in combination with stereotactic body radiotherapy (SBRT) for the treatment of pancreatic cancer.

About MRx0518: The microbiome has been implicated in cancer treatment and response in a range of clinical settings. The microbiome profile of patients has been demonstrated to drive response to anti-PD-1 therapy in

both melanoma and non-small cell lung cancer. MRx0518 has demonstrated robust efficacy as an immuno-stimulant and anti-tumour agent in multiple tumour models such as breast cancer, renal cell carcinoma and lung cancer. About Phase I study: This study is pursuant to the clinical collaboration agreement entered into in June of this year with a subsidiary of Merck Sharpe & Dohme (tradename of Merck & Co., Inc., Kenilworth, N.J., USA).

The open label Phase I study will evaluate the safety, tolerability and preliminary clinical benefit of the combination of MRx0518 and Keytruda® in up to 132 participants who have progressed on prior PD-1 inhibitor therapy and will take place at the MD Anderson Cancer Center in Texas, US. Participants will receive an intravenous infusion of Keytruda® every three weeks in combination with two capsules of MRx0518 daily for up to two years. The primary endpoints are safety, tolerability, and anti-tumour effect. Other outcome measures will also be assessed, including overall survival and biomarkers.

2 Large Meta-Analysis Backs Probiotic Benefits in Gut Health

A systematic review and meta-analysis was recently published in the World Journal of Clinical Cases¹ that assesses the effects of probiotic preparation Medilac-S® (*Enterococcus faecium* Rosell®-26 and *Bacillus subtilis* Rosell®-179), as adjunctive therapy in ulcerative

colitis patients.

Involving 53 randomized clinical trials and close to 4,000 patients altogether, this robust meta-analysis concluded that, when used with conventional drugs, this probiotic significantly improved chances of clinical remission by 21%, improved symptoms of the gastrointestinal tract and reduced the risk of the anti-inflammatory drugs side-effects, as compared to the drug alone.

Ulcerative colitis is an inflammatory bowel disease of the colonic gastrointestinal tract that is steadily increasing across the globe. Conventional treatments include anti-inflammatory drugs. In China, where the disease is growing rapidly, probiotics, and in particular Medilac-S formula have been used as adjuvant therapy for many years. The 53 clinical trials included in the meta-analysis span from 2007 to 2017.

Potential mechanisms of action of the probiotic formula to explain the adjuvant effect include improvement of the gut microbiota balance, immune-modulatory effects, protection against undesired microorganisms that may trigger a recurrence, also, as recently suggested, a role of certain probiotics in drug pharmacokinetics.



Research

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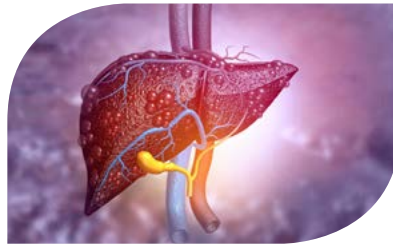
Second Genome Doses First Patient In Phase 2 Clinical Study Of SGM-1019 For NASH

Second Genome, Inc., a clinical-stage company focused on the development of novel therapeutics identified through microbiome science, announced that the first patient has been dosed in a Phase 2 clinical trial evaluating SGM-1019 for the treatment of nonalcoholic steatohepatitis (NASH). SGM-1019 is a first-in-class, oral, small molecule inhibitor of P2X7, a receptor that is involved in inflammasome activation and mediates inflammation and fibrosis.

The Phase 2a randomized, double-blind, placebo-controlled study of SGM-1019 will enroll 100 patients with NASH at leading treatment centers across the United States. The trial (NCT03676231) is designed to evaluate the preliminary safety, tolerability, pharmacokinetics and efficacy (measured by ALT and MRI) of oral twice-daily dosing of SGM-1019 at two different dose levels. Top line data are expected in the first half of 2020.

4

Evelo Announces Dosing of First Patient in Phase 2a Clinical Trial in Patients with Metastatic Melanoma



Evelo Biosciences, Inc. (Nasdaq: EVLO) ("Evelo"), a biotechnology company developing monoclonal microbials, a new modality of oral biologic medicines, announced that the University of Chicago has dosed the first patient in an investigator-sponsored Phase 2a clinical trial evaluating EDP1503 in combination with KEYTRUDA® (pembrolizumab), Merck's anti-PD-1 therapy. EDP1503 is an orally delivered monoclonal microbial product candidate being developed for the treatment of cancer.

This open-label clinical trial, led by Dr. Jason J. Luke, M.D., FACP, will evaluate the safety, tolerability, and efficacy of EDP1503 in combination with KEYTRUDA® in up to 70 patients with metastatic melanoma who are previously untreated or who have relapsed following treatment with an anti-PD-1 inhibitor. Evelo will provide and retain all rights to EDP1503.

Patients will receive daily EDP1503 monotherapy for two weeks followed by treatment with daily EDP1503 in combination with KEYTRUDA®. The study will evaluate biomarkers identified from paired biopsies taken before and after the two-week run-in, as well as clinical outcomes observed over the course of the trial, and potential alterations to the intestinal microbiome. Evelo anticipates initial clinical data from the trial in the second half of 2020.

In preclinical studies orally delivered EDP1503 shows activation of multiple clinically validated systemic immune pathways which are complementary to and potentially synergistic with checkpoint inhibitors. Effects include increased CXCL9 and CXCL10 production in the tumor microenvironment, augmentation of NK and T cell infiltration to the tumor site as well as upregulation of MHC Class I expression in tumors.

5

Clinical Study Results Demonstrate Benefits of High-Potency, Multi-Strain Probiotic Formulations

Results from a clinical trial performed by Taverniti and colleagues from the University of Milan (IT) suggest that ingesting higher doses of multispecies probiotic formulations may permit higher, earlier and longer recovery of probiotics in feces of healthy adults.

The aim of the study, which was published in the journal *Nutrients* (Taverniti *et al.* *Nutrients* 11(2):285, 2019), was to understand the effect of bacterial count on the transient colonization in the human intestinal tract of four different DuPont probiotic strains administered in a single, commercially available, formulation. The four DuPont strains under investigation were (1) *Bifidobacterium lactis* BI-04®, (2) *Lactobacillus acidophilus* La-14®, (3) *Lactobacillus plantarum* SDZ-

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11 and (4) *Lactobacillus paracasei* SDZ-22. The study compared the formulation at two different doses; 7 billion and 70 billion colony forming units (CFU), with the goal of measuring cell recovery in feces after oral administration.

In the study, 40 healthy adults of both genders aged between 18 and 60 were randomly divided into two equal groups. A single-blind, two-arm parallel microbiological pilot study was then conducted in which the volunteers, depending on which group they were assigned to, consumed either the 7 billion or 70 billion CFU formulation daily for two weeks. They were then monitored for a follow-up period of an additional two weeks. For the duration of the study, the volunteers were instructed to follow their usual diet (without the intake of any other probiotic products) and to collect 19 fecal samples in total, in accordance with the study design. These samples were then tested for probiotic recovery.

The study found the first day of detection of the four probiotic strains was earlier in the high dose group when compared to that of the low dose group. Furthermore, on the last day of probiotic consumption, viable cells of all four probiotic strains were recovered from those consuming the 70 billion CFU dose, whereas recovery was not successful for five volunteers who consumed the 7 billion CFU dose.

During the follow-up period of two weeks after consumption stopped, viable recovery was significantly higher and detectable longer in

those who consumed the higher dose formulation than those who consumed the lower dose one. This demonstrates that higher doses of bacterial cells in probiotic formulations may allow for a higher, earlier and longer recovery time suggesting that higher doses may lead to an earlier and more stable transient colonization. In addition, the study shows that strains belonging to diverse taxa may be combined in a single formulation and be selectively quantified upon digestion.

6 New Study Identifies Potential Treatment For Higher Rate Of Preterm Birth Among African American Women

It may be possible to lower the high risk of preterm birth among African American women with certain immune and bacterial factors in the microbiome of the cervix and vagina that appear to modulate this risk, according to research published in *Nature Communications*. March of Dimes, the leading nonprofit for the health of moms and babies, sponsored research at their Prematurity Research Center at the University of Pennsylvania Perelman School of Medicine that helped lay the foundation for these recently reported findings.

The new study from the Perelman School of Medicine at the University of Pennsylvania and the University of Maryland School of

Medicine that found seven types of bacteria and certain immune factors in a woman's vagina and cervix may be responsible for increasing the risk of spontaneous preterm birth or protect against it. The authors suggest this information could help physicians better predict preterm birth, especially for African-American women early in pregnancy.

The presence or absence of certain bacteria in a woman's cervix and vagina microbiome – the community of organisms such as bacteria and viruses that normally inhabit the body – are known to affect the risk of preterm birth. Some bacteria are associated with the significantly higher preterm birth rate among African American women in the United States.

The rate of preterm birth (defined as birth before 37 weeks of pregnancy) among black women in the United States is 49 percent higher than the rate among all other women. More than 20 percent of premature babies are born to black women — that's 1 in 5 U.S. babies. Preterm birth and its complications are the largest contributor to death in the first year of life in the United States, and the leading cause of death of children under age 5 worldwide.



Research

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7 SkinBiotix® Shows Efficacy in First Human Study

SkinBioTherapeutics plc (AIM: SBTX – ‘SkinBioTherapeutics’ or the ‘Company’), a life science company focused on skin health, provides the results of its first human study. The study was primarily undertaken to show the effects of SkinBiotix® on the barrier of healthy skin. An important secondary endpoint was to show that the technology is safe and well tolerated in a large group of people (129) using it twice a day for an extended period of time (29 days).

The results of this independent study demonstrate that SkinBiotix® is safe, well tolerated and has efficacy in certain age groups and time points.

The study design involved three groups:

- Group 1 applied the active (cream containing SkinBiotix®) to one leg and nothing to the other
- Group 2 applied the vehicle (cream containing no SkinBiotix®) to one leg and nothing to the other
- Group 3 applied the vehicle to one leg and active to the other

Measures of the barrier were then performed in all groups at 15 days and 29 days. The primary measures were Corneometry – a measure of how hydrated the skin is, and Transepidermal water loss (‘TEWL’) – a measure of water loss from the skin. A change to the barrier might be expected to be reflected in an increase in skin hydration or a reduction in TEWL.

Some additional measurements of skin elasticity were also taken. Data showed:

1. None of the 129 volunteers experienced any adverse skin reactions to the active.
2. A statistically significant increase in skin hydration at day 15 with the active, which was better than that produced by the vehicle. This effect was seen in the group under 50 years old. At day 29 there was no difference in skin hydration between any of the groups.
3. A small but statistically significant decrease in TEWL with the active at day 29 in the group over 60 years old.

4. In other age groups and also in measures of skin elasticity there was no difference between the vehicle and the active.

The results of this independent study demonstrate that SkinBiotix® is safe, well tolerated and has efficacy in certain age groups and time points. The change in skin hydration and water loss are in line with expectations from laboratory studies which have shown that SkinBiotix® increases the levels of proteins within the skin that are crucial for a healthy barrier. The increase in skin hydration in the younger group at day 15 may reflect the ability of younger skin to respond to the SkinBiotix® faster than older skin.



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8

Locus

Biosciences Enters Collaboration and License Agreement with J&J to Develop CRISPR-Cas3 Bacteriophage Therapeutics

Locus Biosciences Inc., a biotechnology company developing precision antibacterial therapies, announced that it has entered into an exclusive collaboration and license agreement with Janssen Pharmaceuticals, Inc. (Janssen), one of the Janssen Pharmaceutical Companies of Johnson & Johnson, to develop, manufacture and commercialize CRISPR-Cas3-enhanced bacteriophage ('crPhage™') products targeting two key bacterial pathogens for the potential treatment of infections of the respiratory tract and other organ systems. Johnson & Johnson Innovation LLC facilitated the transaction. Under the terms of the agreement, Locus will receive \$20 million in initial payments, and is eligible to receive up to a total of \$798 million in potential future development and commercial milestone payments, and royalties on any product sales. Locus is the world leader in CRISPR-engineered phage therapeutics, uniquely leveraging the powerful Type I CRISPR-Cas3 system to specifically degrade the DNA of target bacteria cells, quickly destroying them. This patented DNA-shredding technology precisely and selectively removes unwanted bacteria from the human body while leaving the many

other species of good bacteria unaffected. If proven safe and effective in clinical trials, these products could provide a turning point in the global battle against antibiotic resistant infections and other microbiome dysbiosis-related

9

Bioharmony Therapeutics and Boehringer Ingelheim to Advance Bacteriophage Lysin Therapeutics

Bioharmony Therapeutics, Inc. ('Bioharmony'), a biopharmaceutical company focusing on the development of novel therapeutics for hard to treat bacterial infections, announced that it has entered into a Collaborative Research and Licensing Agreement with Boehringer Ingelheim to develop bacteriophage lysins for the treatment of multidrug resistant (MDR) *Acinetobacter* infections, a frequent cause of hospital-acquired pneumonia and life-threatening blood or wound infections. Bioharmony licensed this technology from the Rockefeller University. The discoveries are from the laboratory of Vincent A. Fischetti, Ph.D., a faculty member at The Rockefeller University. Recently, the World Health Organization listed drug-resistant *Acinetobacter baumannii* to be a critical priority pathogen, causing around 9% of all bacterial infections in intensive care worldwide against which there are few treatment options available. Financial terms

are not disclosed.

Infectious diseases are amongst the leading causes of morbidity and mortality worldwide and continue to be a major health challenge for medicine. Increasing levels of resistance threaten the effectiveness of many existing antimicrobial drugs. Lysins are bacteriophage-derived enzymes that cleave the essential bonds in the bacterial cell wall leading to rapid killing of the target bacteria. As a new antimicrobial class, they could enable the development of urgently needed treatment options for MDR infections.

Rockefeller has an equity stake in Bioharmony. Rockefeller and Dr. Fischetti may benefit financially as a result of successful commercialization of the licensed technology.



Industry

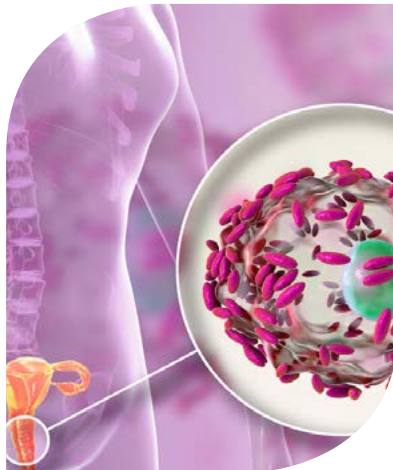
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The partnership is part of Boehringer Ingelheim's Research Beyond Borders function (RBB), which explores emerging science and technology beyond the company's core areas of focus. RBB also focuses on gene therapy, regenerative medicine, immune-infection, and the role of the microbiome in human health and disease.

10 Ferring, Rebiotix and Karolinska Institutet Extend Collaboration to Research Next Generation of Microbiome Treatments

Ferring Pharmaceuticals and Karolinska Institutet announced a five-year extension of their collaboration to explore the potential of the human microbiome in reproductive medicine and women's health and gastroenterology. The collaboration brings together specialist expertise from Karolinska Institutet in early stage research, Rebiotix Inc. (acquired by Ferring in 2018), a late-stage clinical microbiome company, and Ferring's therapeutic area and commercialisation capabilities. The extension includes six reproductive health clinical studies of approximately 6,000 women and babies and four gastroenterology studies of approximately 3,000 adults and children, to further investigate the role of the microbiome in areas of high unmet need including recurrent

pregnancy loss, preterm birth and inflammatory bowel disease. Up to 5% of couples face the heartache of recurrent pregnancy loss and around 15 million babies are born preterm every year around the world, with approximately 1 million children dying each year due to related complications. Over 10 million people worldwide live with the pain and discomfort of inflammatory bowel disease. With reproductive health and inflammatory bowel concerns on the rise there is a need to increase understanding of their causes and develop new solutions.



11 LUCA Biologics Launches to Develop Live Biotherapeutics for Women's Health Starting with UTI

Emerging from 15 years of dr. Jacques Ravel's vaginal microbiome research, LUCA's pipeline targets UTI, Bacterial Vaginosis (bv), and Preterm Birth (ptb) LUCA Biologics, a biotechnology company co-founded by Dr. Jacques Ravel,

announced it will develop live biotherapeutics for widespread, unmet medical needs in women's health. Ravel will serve as LUCA's Chief Scientist.

The company's first therapeutic targets urinary tract infection (UTI), estimated by the World Health Organization to impact half of women globally, and is the most common bacterial infection in the United States. FDA trials are anticipated to be led by Harvard Medical School faculty and conducted at Massachusetts General Hospital. Patient recruitment will begin this fall.

Antibiotics are currently the only frontline treatment despite patient side effects, failure to prevent recurrence, and the alarming rise in antibiotic resistance. Persistent, prophylactic antibiotic use has both contributed to the increase in causative pathogen resistance and been linked to collateral damage in the gut and vaginal microbiome. The FDA has also actively advised against the use of several antibiotics for community-acquired UTI, leaving patients with few viable options.

Ravel's research is foundational to the current understanding of the vaginal microbiome and how microbes may prevent or treat pervasive conditions in gynecology, urology, infectious disease, and reproductive medicine.

With a strain bank and gene catalogue isolated over 15 years from longitudinal, Gates Foundation and NIH-funded research, the company has built a metagenomic and metatranscriptomic platform

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to identify and validate strains that modulate the vaginal and urogenital microbiome. LUCA's pipeline includes therapeutics for UTI, preterm birth (PTB), and bacterial vaginosis (BV).

The company's Board of Advisors is comprised of a consortium of experts across vaginal health, microbiology, reproductive medicine, microbial genomics, and mucosal immunology, including Dr. Marina Walther-Antonio (Mayo Clinic), Dr. Douglas Kwon (Harvard Medical School, MIT), Dr. Maria Gloria Dominguez-Bello (Rutgers), Dr. Anne Dunlop (Emory School of Medicine), Dr. Gregor Reid (Western) and Dr. Indira Mysorekar (Washington University). Dr. Greg Sieczkiewicz (MPM Capital) and Raja Dhir (Seed Health) will serve as board members.

12 Seres Therapeutics Announces Microbiome Immuno-Oncology Focused Collaboration with AstraZeneca

Seres Therapeutics, Inc. (Nasdaq: MCRB) announced a three-year research collaboration with AstraZeneca. The collaboration will focus on advancing mechanistic understanding of the microbiome in augmenting the efficacy of cancer immunotherapy, including potential synergy with AstraZeneca compounds.

Preclinical and early clinical evidence suggests that the composition of the gastrointestinal microbiome impacts clinical response to checkpoint inhibitor immunotherapy and supports the hypothesis that modification of the microbiome may improve outcomes. These data provide strong support for continued research to further understand the microbiome as a predictor of response to checkpoint inhibitors and to elucidate the potential of microbiome therapeutics to augment immunotherapy.

Under the collaboration, research will evaluate microbiome-based approaches as a predictor for which patients may respond best to certain cancer immunotherapies. Additionally, SER-401, an investigational microbiome therapeutic, may be studied in combination with AstraZeneca compounds targeting various cancers. The collaboration will apply Seres' microbiome drug discovery and manufacturing expertise with AstraZeneca's extensive oncology experience to evaluate the potential for microbiome therapy to improve clinical response when used in conjunction with adjunctive pharmaceutical approaches. Under the terms of the exclusive collaboration, AstraZeneca will provide Seres with \$20 million in three equal installments over two years, with the first payment due at the start of the agreement. In addition, AstraZeneca will also reimburse Seres for research activity related to the collaboration. Seres will maintain rights to oncology targeted microbiome therapeutic candidates, and

AstraZeneca will obtain the option to negotiate for rights to those programs and other inventions arising out of the collaboration.



13 Enterome Announces Research Collaboration with Dana-Farber Cancer Institute Focused on New Microbiome-derived Cancer Immunotherapy

ENTEROME SA, a clinical-stage biopharmaceutical company leveraging its unique knowledge of the key functional and molecular interactions between the gut microbiome and the human body to develop targeted therapeutics, has entered into a research and development collaboration with Dana-Farber Cancer Institute (Boston, MA) to evaluate and develop gut microbiome-derived antigens as potential cancer immunotherapies.

Enterome is developing an innovative approach to cancer

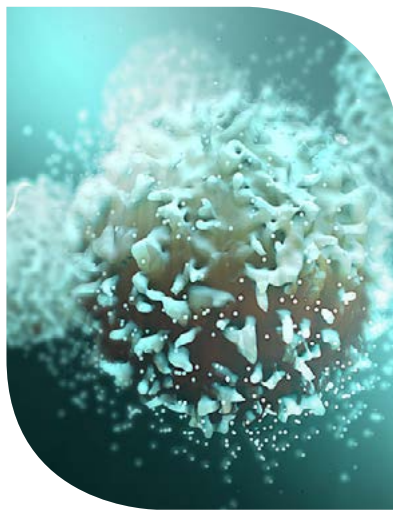
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immunotherapy, based on the concept of 'molecular mimicry,' whereby microbiome-derived bacterial antigens show molecular similarity with Tumor-associated Antigens (TAAs) and Tumor-specific Neoantigens (TSNAs). Based on this similarity, bacterial antigens ('onco-mimics') mimic key tumor antigens that are highly expressed by tumors to trigger tumor-specific cytotoxic T cell immune responses. Enterome has developed a discovery platform to identify such onco-mimics from the human gut microbiome and has advanced EO2401 as its first clinical candidate. EO2401 comprises several microbiome-derived antigens that mimic antigens highly expressed by brain tumors and is targeting first clinical trials in 2019 as a potential new immunotherapy for recurrent glioblastoma multiforme (GBM) for which no curative treatments exist. The collaboration brings together Enterome's ability to identify potential TAAs and TSNAs as well as to generate bacterial onco-mimics for the selected TAAs and TSNAs with the complementary translational expertise from the research groups at Dana-Farber Cancer Institute led by Dr. David Reardon at the Center for Neuro-Oncology and Dr. Paul Kirschmeier at the Belfer Center for Applied Cancer Science. Drs. Reardon's and Kirschner's groups are focused on driving innovative research programs and clinical trials to improve cure rates in patients with brain and spinal cancers, with a particular focus on immunotherapies.

Through this collaboration Enterome will have access to

knowledge, scientific expertise and preclinical models at Dana-Farber Cancer Institute that are intended to support the in vivo validation and further development of Enterome's immunotherapy approach for the treatment of cancer.



14 Lonza and Chr. Hansen in Joint Venture to Accelerate Momentum in Microbiome

Danish bioscience company, Chr. Hansen, joins forces with Lonza AG to pioneer the emerging market for live biotherapeutic products. Chr. Hansen Holding A/S, a leading global bioscience company, and Lonza AG, a leading pharma contract manufacturing company, have signed an agreement to establish a 50/50 joint venture to pioneer the live biotherapeutic products (LBP) industry and position themselves as the leading contract development and manufacturing partner (CDMO) for biotech and pharma customers.

The joint venture will be a 50/50 controlled legal entity which will operate from its headquarters in Basel, Switzerland and have production facilities in Denmark and Switzerland.

The joint venture brings together best-in-class, complementary capabilities and will be the first CDMO globally to provide a full supply chain offering manufacturing of bacteria strains for therapeutic use. Whilst Chr. Hansen contributes its extensive know-how in developing, upscaling and manufacturing bacteria strains, Lonza brings strong capabilities in pharma contract manufacturing and outstanding formulation and drug delivery technologies.

Furthermore, the joint venture will possess leading competences in handling, characterizing, formulating, manufacturing and encapsulating strict anaerobe bacteria. These competences under one roof, with seamless exchanges between drug substance and drug product activities will decrease development timelines and increase chance of 'right-first-time'.

15 Flagship Pioneering Unveils Kintai Therapeutics

Flagship Pioneering, a unique life science innovation enterprise, unveiled Kintai Therapeutics and named Paul-Peter Tak, M.D., Ph.D., as president, chief executive officer, and member of the board of directors. Kintai is focused on leveraging the body's diverse

Industry

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signaling networks to pioneer a new class of Precision Enteric Medicine™ therapeutics that restore health and fight disease.

Kintai was founded in 2016 by Flagship Labs, the innovation foundry of Flagship Pioneering. Flagship's team of entrepreneurial scientists asked: What if the gut offered a more impactful means to drive physiology based on the confluence of the enteric nervous system, immune system, and microbiome? The team began investigating the ways in which the gut adversely transforms drugs, and how the same biologies, as well as the nuanced interconnections of a wide variety of systems, could be harnessed as a new way to develop drugs. These early explorations led to fruitful insights and data that inspired a foundational view that the gut could serve as a way to effectively drive systemic therapeutic effects.

Since its founding, Kintai has made substantial discoveries through its mapping capabilities, including the identification of over 44,000 new genes and hundreds of new metabolites that had not been previously known to exist. Based on these research efforts and through its proprietary Precision Enteric Medicine™ (PEM™) discovery platform, Kintai is today unveiling a portfolio of more than ten programs focused on multiple therapeutic areas, including oncology, neurology, and immunology.

Kintai's PEM™ discovery and therapeutic development platform is being applied to identify and develop orally available small

molecules based on underlying biology to selectively target the enteric signaling network. In addition, PEM™ compounds are designed with a biogeographic attribute, targeting the drug precisely to its optimal location to maximize therapeutic activity. The platform relies on a diverse and multidimensional scientific approach involving life science and technology disciplines, namely in chemistry, human biology, immunology, microbiology, and artificial intelligence. The company is building on emerging science in microbiome research, deploying some of the brightest minds in academia and industry and applying cutting edge experimental medicine approaches to expedite development and increase the probability of success.

The company's initial pipeline includes an array of programs across autoimmune, metabolic, neurologic, and cancer indications, including initiatives in ulcerative colitis, chronic kidney disease, NASH, metabolic syndrome, and multiple sclerosis, to name a few.



16 C3J

Therapeutics Announces Issuance of U.S. Patent Covering Synthetic Bacteriophage

C3J Therapeutics, Inc. (C3J) a private clinical stage biotechnology company focused on the development of novel targeted antimicrobials based on bacteriophage, announced that the United States Patent and Trademark Office (USPTO) has issued patent No. 10,221,398, entitled "Compositions of and Methods for In Vitro Viral Genome Engineering."

The patent, which covers the composition of a synthetically engineered *Pseudomonas aeruginosa* phage, is an important addition to C3J's expanding patent estate, and the Company's first issued patent covering its proprietary technology platform that enhances natural bacteriophage through genetic engineering. The patent includes claims intended to improve host range and increase the antimicrobial activity of wild type (natural) phage, including activity against biofilm. C3J possesses a significant library of naturally occurring phage and other biological materials that are critical for the creation of synthetic phage via the C3J phage engineering platform.

C3J is committed to protecting its novel therapeutics in key jurisdictions. Related international patent applications are currently

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under examination in several countries. These applications support C3J's work in building a strong intellectual property portfolio around its technology and strengthening its leadership position in the field of synthetic phage-based therapies.

On January 4, 2019, C3J and AmpliPhi Biosciences Corporation ("AmpliPhi") (NYSE American: APHB), a clinical-stage biotechnology company focused on the development of precisely targeted bacteriophage therapeutics for antibiotic-resistant infections, announced that the companies have agreed to merge. The consummation of the merger transaction will result in a combined company that has a diverse clinical-stage pipeline, including a Phase 1/2-ready natural phage candidate targeting *Staphylococcus aureus*, as well as a synthetic phage candidate targeting *Pseudomonas aeruginosa* respiratory infections poised to enter Phase 1 development later this year. In addition, the combined company will have an extensive natural phage library and the capability to develop synthetic phage against a wide range of microbial agents.

17 OraSure Technologies, Inc. Announces Acquisition of CoreBiome

OraSure Technologies, Inc. (NASDAQ: OSUR), a leader in point of care diagnostic tests and specimen collection and stabilization devices, announced that it has entered into definitive agreements to acquire CoreBiome. CoreBiome is a privately-held, Minnesota-based early-stage microbiome services provider that accelerates discovery for customers in the pharmaceutical, agricultural, and research communities. CoreBiome's technology provides information-rich characterization of microbial diversity and function, paired with machine learning and expert analytics. CoreBiome's proprietary genomics pipeline and optimal algorithms deliver speed and scalability in the lab as well as highly precise analytics. CoreBiome was co-founded in 2016 by Dr. Dan Knights, a globally recognized expert in microbiome informatics who has developed leading methods for analyzing microbiome data, along with Dr. Daryl Gohl

and Dr. Kenny Beckman, domain experts in genomics methods and clinical lab operations.

18 Eagle Genomics Closes \$3.5M Investment

Microbiome discovery platform company Eagle Genomics announces funding of \$3.5M led by the Environmental Technologies Fund (ETF Partners), a European growth fund specialising in promoting sustainability through innovation. The raise will accelerate the development of Eagle Genomics' award-winning software platform to meet rapidly growing demand from enterprise companies in the consumer goods, agritech and healthcare industries. The investment will also support global expansion, with plans to open international offices in Paris and New York to service the European and American markets. The microbiome, the ecosystem of bacteria, fungi and viruses present in virtually all living organisms, is directly linked with health and is proven to be affected by the products we use and consume. Eagle Genomics' ground-breaking knowledge discovery platform, the e[automateddata]scientist, utilizes Artificial Intelligence (AI) to analyze complex genomic and microbiomic data at scale, delivering new insight and allowing enterprise brands to assess the viability, efficacy and safety of products.

The funding round follows the recently announced partnership between Eagle Genomics and



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Microsoft Genomics, which marked Microsoft's first venture into the microbiome. With momentum from this partnership and a significant market opportunity to address, Eagle Genomics is well positioned to revolutionise life sciences R&D.

19 S-Biomedic Completes Series A Round With DSM Venturing

S-Biomedic, a pioneer in patented human microbiome skin applications, announced the successful completion of their second Series A financing round. The second closing welcomed a new investor, DSM Venturing and was also supported by existing shareholder investiere.ch, the leading Swiss VC platform. In total, S-Biomedic raised €4 million in this Series A. The first tranche was led by skin care global player Beiersdorf. S-Biomedic also secured an investment from investiere.ch, as well as previous contributions from Johnson & Johnson Innovation – JJDC, Inc. The total sum raised will enable S-Biomedic to rapidly progress their portfolio of products and enter into development and commercialisation agreements with leading dermatological and cosmetic brands.

20 BiomX Raises \$32 Million in Series B Financing

BiomX Ltd., a microbiome company

developing customized phage therapies, announced the closing of a \$32 million series B equity financing.

The financing was led by existing investors OrbiMed, Johnson & Johnson Innovation – JJDC, Inc., Takeda Ventures, Inc., 8VC, MiraeAsset, Seventure Partners' Health for Life Capital I, SBI Japan-Israel Innovation Fund and additional European investors and included new investors led by RM Global Partners (RMGP) BioPharma Investment Fund, with participation from Chong Kun Dang Pharmaceutical Corp., Handok, Inc., KB Investment Co., Ltd. and Consensus Business Group. Proceeds from the financing will be used primarily to advance the Company's leading drug candidates for the treatment of acne and Inflammatory Bowel Disease (IBD) to the clinic.

In conjunction with the investment, Yaron Breski, Managing Director at RM Global Partners, and Eric de la Fortelle, Ph.D., MBA, Venture Partner at Seventure Partners, join BiomX's Board of Directors.

21 Axial Biotherapeutics Raises \$25 Million in Series B Financing

Axial Biotherapeutics, a biotechnology company dedicated to building a unique class of gut-targeted programs for neurodegenerative and neuropsychiatric diseases, today announced the completion of a \$25

million series B equity financing.

New investor Seventure Partners, via its microbiome-focused fund Health for Life Capital™, led the financing along with existing investors Longwood Fund, Domain Associates, Heritage Medical Systems and a group of high net worth individuals based in Southern California. The new funding will be used to advance Axial's programs in Parkinson's disease (PD) and Autism spectrum disorder (ASD). Both programs include small molecules targeting the gut-brain axis.

22 SNIPR BIOME Raises \$50 Million Series A financing to Advance CRISPR-based Microbiome Drugs to Human Clinical Trials

SNIPR BIOME, a leading CRISPR and microbiome private biotech company incorporated in Copenhagen, Denmark, announced a \$50 million series A financing led by existing investor Lundbeckfonden



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Emerge (Copenhagen) and LSP (Amsterdam), together with North-East Family Office (Copenhagen) and Wellington Partners (Munich).

SNIPR BIOME is pioneering a novel use of CRISPR/Cas technology to selectively and precisely eradicate target bacteria, while leaving the rest of the patient's microbial community intact.

The proceeds of the \$50 million (€43 million, DKK320 million) financing will fund the further development of the company's pioneering CRISPR technology platform and the company's first clinical programs.

23 Microeos Raises €30 Million for Endolysin Technology Set to Replace Antibiotics

Dutch biotechnology company Microeos announced it has secured €30 million in funding to accelerate the development of its endolysin technology, set to replace antibiotics. Proceeds are earmarked for the clinical development program of its endolysin XZ.700 and the USA launch of its breakthrough OTC Gladskin product for eczema. According to the World Health Organization, antibiotic resistance is one of the world's biggest health threats. The call for true alternatives is more pressing than ever. Antibiotics do not distinguish between bad and good bacteria, and their use induces resistance. Endolysins have three distinct

features: (1) ability to target only unwanted bacteria while preserving the microbiome, which is essential for our health. (2) ability to kill antibiotic resistant strains of bacteria (3) emergence of resistance against endolysins is not expected.

Endolysin technology opens a new therapeutic window for treatment of chronic inflammatory conditions, such as atopic dermatitis and persistent wound infections, including those caused by MRSA. Microeos' Staphfek™ SA.100, is the world's first endolysin approved for human use. It selectively targets *Staphylococcus aureus* – often referred to as 'Staph' – which is a major trigger of eczema and other inflammatory skin conditions.

24 Vedanta Biosciences Closes Extended \$45.5 Million Series C Financing

Vedanta Biosciences, a clinical-stage company developing a

new category of therapies for immune-mediated diseases based on rationally-defined consortia of human microbiome-derived bacteria, has raised an additional \$18.5 million as an extension of its Series C financing, bringing the total for the round to \$45.5 million. The new investment comes from JSR Corporation, Shumway Capital, SymBiosis LLC, and Partners Investment Co., Ltd., who join previously disclosed investors the Bill & Melinda Gates Foundation, Bristol-Myers Squibb, Rock Springs Capital, Invesco Asset Management, Health for Life (Seventure Partners), and founder PureTech Health.

Proceeds from the financing will be used to advance Vedanta's clinical portfolio including a Phase 1/2 study of VE416 in food allergy, a Phase 1b/2 study of VE800 and OPDIVO® (nivolumab) in advanced or metastatic cancers, and the ongoing Phase 2 study of VE303 in recurrent *Clostridium difficile* infection (rCDI). A Phase 1 clinical study of VE202 in healthy volunteers is being advanced with Janssen as part of an ongoing collaboration in inflammatory bowel disease (IBD).



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consortia of bacterial strains designed to effect robust and durable therapeutic changes in a patient's gut microbiota. In contrast to fecal transplants or administration of fecal fractions, Vedanta Biosciences' consortia are defined compositions of bacteria manufactured from pure, clonal cell banks, without the need to rely on direct sourcing of fecal donor material of inconsistent composition.

25 Kaleido Biosciences Announces Closing of Initial Public Offering

Kaleido Biosciences, Inc. (Nasdaq: KLDO), a clinical-stage healthcare company with a differentiated, chemistry-driven approach to leveraging the potential of the microbiome organ to treat disease and improve human health, announced the closing of its initial public offering of 5,000,000 shares of common stock at a public offering price of \$15.00 per share. The gross proceeds from the offering were \$75 million before deducting underwriting discounts and commissions and estimated offering expenses. All of the shares in the offering were offered by Kaleido. The shares commenced trading on The Nasdaq Global Select Market on February 28, 2019, under the ticker symbol 'KLDO.'

Goldman Sachs, J.P. Morgan and Morgan Stanley acted as joint book-running managers for the offering. Canaccord Genuity acted as lead manager for the offering.

26 Finch Therapeutics Raises \$53 Million to Advance Microbiome-Based Therapies

Finch will use the Series C proceeds to advance its pipeline of novel microbial therapies, including CP101, a Full-Spectrum Microbiota® (FSM®) therapy delivered in an oral capsule that is designed to contain a diverse community of microbiota and restore a balanced microbiome. CP101 is currently being evaluated for the prevention of recurrent *C. difficile* infections (CDI) in Finch's PRISM3 trial, a potentially pivotal clinical study. Compelling results from the PRISM3 trial may be sufficient to support FDA approval, based on recent communications with the agency.

The Series C proceeds will also enable Finch to accelerate the development of its FSM therapy for Autism Spectrum Disorder (ASD). Finch is supporting an actively enrolling, Phase II, investigator-initiated clinical study evaluating the safety and efficacy of its FSM therapy in adults with ASD. Finch has also received FDA Fast Track designation for its pediatric ASD program.

Beyond CDI and ASD, Finch is continuing to expand its pipeline of microbiome-based therapeutics, including a pre-clinical program in IBD in partnership with Takeda Pharmaceuticals.



27 Chardan Healthcare Acquisition Corp. Announces Merger Agreement with BiomX Ltd.

Chardan Healthcare Acquisition Corp. (NYSE: CHAC, "CHAC"), a special purpose acquisition company ("SPAC") sponsored by affiliates of Chardan Capital Markets LLC ("Chardan"), announced that it has entered into a definitive agreement for a business combination with BiomX Ltd. ("BiomX"), a microbiome company developing both natural and engineered phage therapies. Assuming no redemption of CHAC shareholders, the combined company will have an initial market capitalization of approximately \$254 million. Upon closing of the transaction, it is expected that CHAC will be renamed BiomX and remain on the NYSE American Stock Exchange, listed under a new ticker symbol.

CHAC has entered into a mix of commitments including purchase and sale, backstop, and voting agreements with BiomX and with

Microbiome

Frozen Aliquotting of Fecal Samples

“Our fecal samples can’t be tested after freeze-thaw cycling.

Is there a way to test samples now and preserve them for additional testing in the future?”

“I’d like to perform both DNA and RNA analysis.

Can I process a single sample for multiple types of analyses?”

“We currently process fecal samples manually.

Is there a way to automate fecal sample processing to limit contact with samples and improve consistency?”



You can with Frozen Aliquotting.

Fecal Sample Processing

Meaningful analysis of the microbiome depends heavily on maintaining the in vivo profile of the microbiome in an ex vivo state. Timely freezing of raw or stabilized fecal samples is the most common method used for preserving sample integrity. Frozen aliquotting enables re-access to frozen samples without freeze-thaw cycling that can bias data in downstream analyses.

Automated frozen aliquotting reduces processing time and handling of frozen feces by enabling researchers to aliquot and distribute frozen fecal samples in a controlled and efficient manner. Automated Frozen Sample Aliquotting generates uniform aliquots that thaw faster than a full sample, improving sample consistency.



1 CORE SAMPLE
Automate
Aliquotting



2 FREEZE CORES &
PARENT SAMPLE
Store for future testing



3 PREP CORE FOR ANALYSIS
Test individual aliquots



Improve Consistency of Fecal Aliquots

Packing raw-homogenized feces in a specimen container can lead to a large degree of variability in the geometry of the sample. Freezing feces following homogenization in a stabilization buffer and employing the level-sensing capability of the CXT 750 Automated Frozen Sample Aliquotter enables cores of a particular size to be generated with a high degree of reproducibility.

Figure 1: Core Size

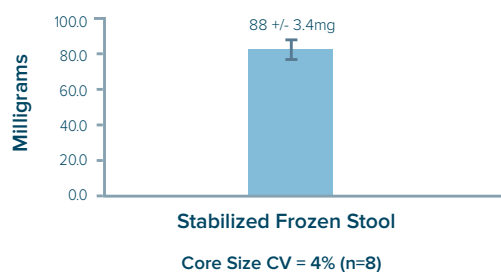


FIGURE 1. FROZEN FECAL SAMPLES STABILIZED IN RNALATER® WERE CORED ON THE CXT 750 AUTOMATED FROZEN SAMPLE ALIQUOTTER. A CORE MASS OF 90MG WAS TARGETED. AVERAGE CORE MASS OF 88 +/- 3.4MG WAS OBSERVED.

Extract RNA or DNA from a Single Core

Frozen Sample Aliquotting yields frozen cores (aliquots) that can be used to extract RNA or DNA for genomic analysis. A sufficient quantity of DNA was successfully extracted from a single core from both raw and stabilized feces. Stabilized feces consistently yielded high-purity DNA.

Figure 2: Concentrations and Purity

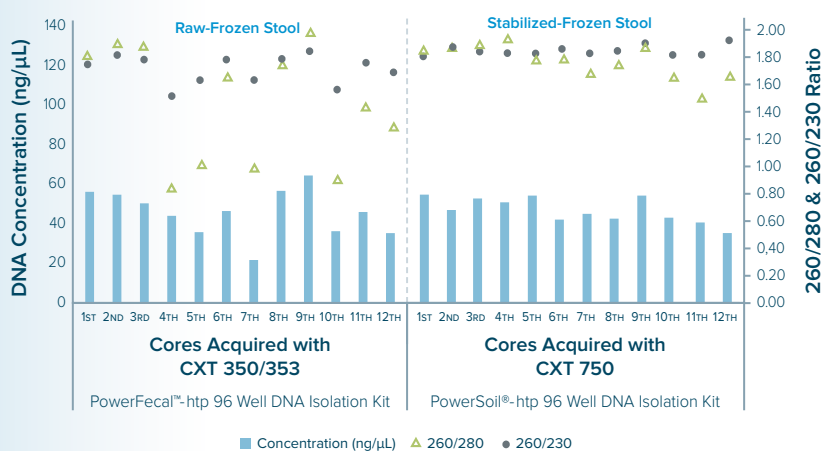


FIGURE 2. CORES WERE ACQUIRED FROM WIDE-MOUTH TUBES OF RAW FROZEN FECES AND 2ML TUBES OF FROZEN STABILIZED FECES. DNA ISOLATION WAS PERFORMED USING A POWERFECAL™ OR POWERSOIL®-HTP 96 WELL DNA ISOLATION KIT (MO BIO LABORATORIES). DNA CONCENTRATIONS (22 - 67.5NG/ML) WERE MEASURED BY FLUOROMETRIC QUANTITATION USING A QUBIT™ (THERMOFISHER). DNA PURITY WAS ASSESSED BY NANODROP™ (THERMOFISHER) 260/280 (AVG. 1.80) AND 260/230 (AVG. 1.63) RATIOS.

Top Five Benefits of Frozen Aliquotting

- PRESERVE MICROBIAL PROFILES**
Avoid freeze-thaw cycles
- PREP FOR RNA, DNA, AND PROTEIN**
Use individual cores for extraction
- INCREASE SAMPLE UTILIZATION**
Distribute frozen aliquots
- REDUCE FECES HANDLING**
Automate aliquotting
- INCREASE CONSISTENCY OF ALIQUOTS**
Eliminate operator variability

Seeing is Believing

Schedule an on-site demonstration of the CXT 353 Frozen Sample Aliquotter or the CXT 750 Automated Frozen Sample Aliquotter.



Call Today
617.962.0309 or email
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investors — including OrbiMed, RTW Investment, Johnson & Johnson Innovation – JJDC, Inc. (JJDC), Takeda Ventures, Inc., Seventure Partners' Health for Life Capital I, SBI Japan-Israel Innovation Fund, as well as RM Global Partners (RMGP) BioPharma Investment Fund — so that the \$50 million minimum closing condition for the transaction has been satisfied prior to today's announcement.

Proceeds from the transaction will provide BiomX with substantial growth capital and the flexibility of a public listing to further accelerate BiomX's expansion as a leading microbiome product discovery company. BiomX is developing customized phage-based products designed to improve the appearance of acne-prone skin and eradicate harmful bacteria in chronic diseases. The company's pipeline includes preclinical candidates for acne-prone skin, inflammatory bowel disease (IBD), primary sclerosing cholangitis (PSC), and colorectal cancer (CRC). BiomX's product for acne-prone skin is anticipated to begin clinical testing by the end of 2019. The combined company will continue to be led by BiomX's experienced management team headed by Chief Executive Officer Jonathan Solomon.

The respective boards of directors of both CHAC and BiomX have approved the proposed transaction. Completion of the transaction, which is expected in October 2019, is subject to approval by CHAC stockholders and satisfaction of other closing conditions. Summary of Transaction
CHAC raised \$70 million in its

IPO which is now held in a trust account. Under the terms of the proposed transaction announced today, CHAC will issue 16.625 million shares and vested securities to current securityholders of BiomX. Certain BiomX shareholders may, subject to the terms of the investment agreements, receive up to an additional 6.0 million CHAC shares: 2.0 million shares if the share price exceeds \$16.50 by fiscal year 2021, an additional 2.0 million shares if the share price exceeds \$22.75 by fiscal year 2023, and an additional 2.0 million shares if the share price exceeds \$29.00 by fiscal year 2025.

After giving effect to the investor transactions being undertaken in order to meet the minimum cash condition, and assuming no redemption from CHAC shareholders, it is estimated that the current securityholders of BiomX will own approximately 73% of the issued and outstanding vested securities in the combined company at closing.

Post-closing, Mr. Solomon and three current BiomX directors will join Mr. Grossman and Dr. Amusa from CHAC on the seven-person board of directors. An additional board member will be designated by BiomX.

The description of the business combination contained herein is only a summary and is qualified in its entirety by reference to the definitive agreement relating to the business combination, a copy of which will be filed by CHAC with the Securities and Exchange Commission ('SEC') as an exhibit to a Current Report on Form 8-K. Additional information about

the proposed transaction will be described in CHAC's preliminary proxy statement relating to the business combination, which CHAC will file with the SEC.

28 MicroSintesis Announces \$16.4M Investment to Fund International Expansion and Material R&D Pipeline

MicroSintesis, a life science company focused on developing proprietary microbiome therapies announced the closure of a \$16.4 million minority investment from Toronto-based Northern Private Capital (NPC), an investment vehicle of CFFI Ventures, a company controlled by John Risley. The funding will be used to scale-up production of the company's products and build out the company's research capabilities and platform to deliver a new generation of microbiome products dedicated to improving livestock, companion animal, and human health.

Working in one of the newest areas of research, metabolites being produced by probiotics, MicroSintesis has identified a unique set of signal molecules that are part of the microbiome's own communication system and responsible for regulating its bacteria population. Using patented formulations of these molecules, the company has been able to shift bacterial populations in the gut from unhealthy to healthy to

Finance

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deliver a new generation of health products for the future.

The Company's research has been substantiated through 11 published papers, three clinical studies and more than 100 case studies. Over the past few years, it has built an impressive patent portfolio and a suite of proprietary assays for assessing its molecules' impact. To date, it has a library of over 50 probiotic production strains and has developed a proprietary process optimized for this next generation of postbiotic and probiotic products.

optimized for further clinical development.

Ginkgo has purchased 6,340,771 shares of Synlogic common stock as well as pre-funded warrants to purchase up to 2,548,117 shares of Synlogic common stock, both at a price of \$9.00 per share. Gross proceeds to Synlogic are approximately \$80 million. The transactions were executed and

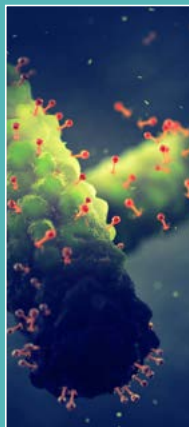
closed on June 11, 2019. At the closing, under the foundry services agreement, Synlogic paid \$30.0 million to Ginkgo for synthetic biology services to be provided over an initial period of five years which can be extended. Synlogic has exclusive rights to any Synthetic Biotic medicines that it develops as part of the collaboration and to intellectual property covering such products.

29 Synlogic and Ginkgo Bioworks Establish Transformational Platform Collaboration for GMO Microorganisms

Synlogic, Inc. (Nasdaq: SYBX) and Ginkgo Bioworks announced a platform collaboration to accelerate expansion and development of Synlogic's pipeline of Synthetic Biotic medicines using Ginkgo's cell programming platform.

The agreement provides an \$80.0 million equity investment at a premium in Synlogic by Ginkgo and entry into a long-term strategic platform collaboration. Synlogic will use Ginkgo's cell programming platform for building and testing thousands of microbial strains to accelerate progression of early preclinical leads to drug candidates

ALSO IN THE NEWS



Intralytix, Eliava Foundation and Ferring Sign Joint Collaboration Agreement

Intralytix, Inc., announced that it has entered into a joint collaboration agreement with the Eliava Foundation and Ferring Pharmaceuticals to develop and commercialize new and innovative bacteriophage-based drugs in the field of Reproductive Medicine and Women's Health. The collaboration expands on a growing global focus on the development of microbiome- and bacteriophage-based technologies to address unmet medical needs in different healthcare arenas.



Pherecydes Pharma and BIOASTER join forces to explore the use of phage therapy to treat complicated urinary tract infections



DayTwo Secures \$31 Million In Series B Financing To Scale Microbiome Research Platform To Address Chronic Health Conditions



Axial Biotherapeutics Announces \$10M Investment from Taiho Ventures



OxThera Initiates Extension Part of a Phase 3 Study of Oxabact in Primary Hyperoxal



DermBiont Raises \$8M Series A to Treat the Root Cause of Skin Diseases

MODELING HOLOBIONT EVOLUTION FOR A BETTER UNDERSTANDING OF SYMBIOSIS, DYSBIOSIS, AND HUMAN HEALTH

INTERVIEW BY KRISTINA CAMPBELL | PHOTO CREDITS UNAM

Dr. Alejandro Frank is a nuclear and molecular physicist. Professor and Director at the Center for Complexity Science, National University of Mexico (UNAM).

The list of presentations from any recent microbiome conference shows the diversity of approaches by which scientists are pursuing discovery of microbiome-focused therapeutics. And common to all of these approaches is the essential need to predict how microbial communities might work, in all their complexity, to influence host health.

Various mathematical models are used to capture aspects of complex microbial ecosystems and hosts. But which aspects? The give-and-take between hosts and microbial communities over time in response to a changing environment may be important for determining certain health-related phenotypes. So some scientists are arguing evolution of the **holobiont** - a host and its microbes considered as one unit - may be important to model for a better understanding of health - or more specifically, of how to manipulate the microbiome for a particular therapeutic outcome.

As humans have evolved, microbes have always been their 'guests'. And as microbial hosts, humans have developed microbe-controlling mechanisms (like the immune system) and have selected microbes that perform certain jobs for them. Humans and microbes thus depend on each other for survival - so can we gain a better understanding of how this symbiosis arose in the first place, and what might trigger a shift to a dysbiotic state?

Dr. Alejandro Frank (AF), a scientist at Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, in Mexico City (Mexico) recently addressed similar questions in a paper in *Frontiers in Physiology*, co-authored with Mexican colleagues, that proposed a mathematical model of the general mechanisms of holobiont evolution.

In this interview, Frank tells Microbiome Times about his group's approach and how models like theirs could be used to increase knowledge of both symbiosis and dysbiosis and of microbe-host interactions for health.

What unique perspectives can be gained by considering holobiont evolution?

AF: The process of evolution in the holobiont and the forces that drive it are (and should be) the same as in any other evolutionary processes. For instance, genetic mutation, genetic drift, and gene flow are clearly present, and influenced by natural selection.

However, these forces may apply at different timescales and rates for different parts of the holobiont, as the bacterial, viral and eukaryotic parts do not work in the same way. We believe that using this more complex, ecological approach, is what makes the holobiont a unique perspective.

With your recent paper, which aspect of holobiont evolution were you hoping to address?

AF: Our intention was to elucidate why and how symbiosis appears by using a relatively simple model of the process of mutation and coexistence between microbial communities and their hosts.

It was also our goal to understand

why the microbial communities help their hosts and why they tend to lose independence on the way, as many symbiotic microbes cannot live without their hosts. Host and microbes develop a co-dependence. We believe that understanding the process of the emergence of symbiosis and co-dependence is essential in understanding human evolution and health.

How did you model this mathematically?

AF: We first represented microbes and hosts as independent dynamical gene networks, where genes interact through gene regulatory interactions. We then allowed mutation to occur at different rates. Evolution and adaptation were simulated through the requirement of specific

collective 'functional' tasks. These mutations can either modify the way a gene regulates another, or change the target of the regulatory interaction.

Since the premise is that the holobiont is a collection of interacting networks, a mutation can make a microbial gene become regulated by a host gene and vice versa. We took into account

"...understanding the process of the emergence of symbiosis and co-dependence is essential in understanding human evolution and health."

DR. ALEJANDRO FRANK
UNAM



different approximate average timescales at which mutations occur in microbial and host networks. The performance of both hosts and microbes was measured independently by how well its gene expression can match a predetermined function assigned to each network.

What was your mathematical model able to predict?

AF: Apart from predicting dysbiosis when co-evolved networks are disconnected (simulating, for example, the action of antibiotics), one of the interesting predictions of our model is that when there are too many microbial communities contributing to the same host's task, the task is often poorly performed – think of too many people trying to change a car's tire.

This happens in spite of the fact that, in our model, a single microbial network contributing to a host's task can significantly improve its performance.

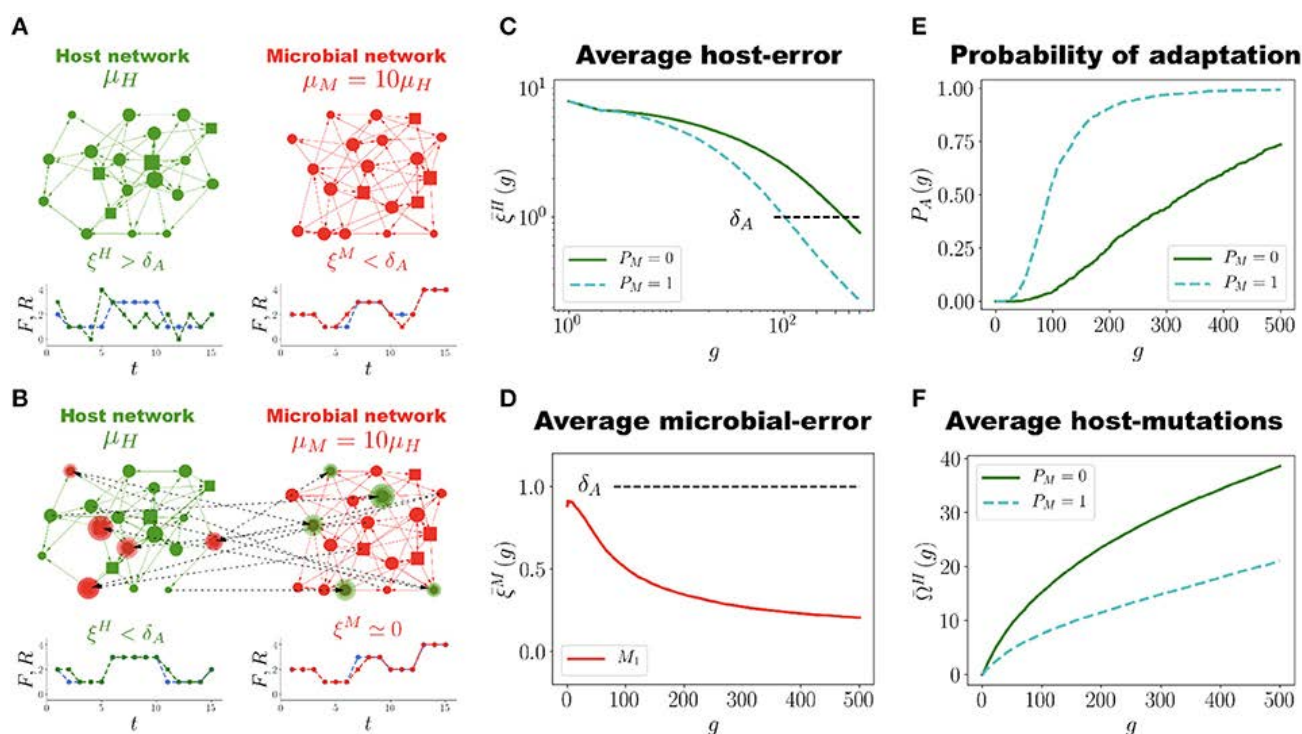
However, when specific niche constraints (either **territorial** or **metabolic**) are introduced, allowing only a few microbial networks to contribute to a given task, the overall performance of the host is rapidly improved in comparison with a host without the support of microbial networks.

How do you think your model might be able to advance research on the human microbiome and health?

AF: One interesting aspect is to find and characterize the microbiome niches (which may not necessarily be anatomically separated), where only a subset of microbe species are normally involved. If too few or too many microbe species participate, this could lead to a dysbiotic state.

The specificity of microbial niches is crucial for future treatment of pathologies. In general terms, it now seems clear that considering the health of our microbial communities as an essential part of our well-being may lead to important discoveries in health and other human traits.

Host and Microbial Network Coevolution:



We believe that mathematical research, including time-series, network, and complexity science approaches will help shed light into the many different ways our microbiome interacts and has evolved with our bodies, as there is probably no linear relation between microbial diversity, abundances, and health.

How will you continue to improve on your model?

AF: At this stage, our paper may be considered as a basic framework for the description and simulation of the adaptation and heritability of our internal ecosystem. We are currently investigating, among other things, the effect of changing environments, modelled by evolving

tasks for our networks.

The question of heritability of our microbiome is also a major issue to be addressed. For example, we plan to investigate the role of the so-called 'core microbiome' (the microbes or microbial functions essential for our well being). We believe the model can be incrementally improved and made to better reflect specific situations, such as our microbiome's role in antibiotic resistance, obesity, or other pathologies. niches (which may not necessarily be anatomically separated), where only a subset of microbe species are normally involved. If too few or too many microbe species participate, this could lead to a dysbiotic state.

What do you think is ahead for mathematical modelling of the holobiont?

AF: I believe several different approaches will be necessary. On the one hand, new experimental methods and discoveries constantly occur, which will surely give rise to new theoretical ideas, which will have predictions, and so on, hopefully creating a virtuous circle – as in all good science.

With that said: our Boolean network approach has proved very useful in other areas, so we strongly believe it may provide a significant path for inquiry and modeling.



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THE LATEST ADVICE ON WHEN TO CHOOSE 16S rRNA GENE SEQUENCING OVER SHOTGUN METAGENOMICS IN MICROBIOME RESEARCH

FEATURE ARTICLE BY KRISTINA CAMPBELL

For every study that includes microbiome analysis, researchers must decide which sequencing method is best suited to the question at hand. Generally, scientists choose between two options: amplicon sequencing and shotgun metagenomics. (Newer offerings of shallow shotgun sequencing notwithstanding.) Those inside and outside the field often reflexively consider shotgun metagenomics to be the superior choice. But does the ‘Cadillac’ sequencing method always give researchers better information?

Sequencing technologies have advanced rapidly in the past few decades. The ability to amplify and sequence phylogenetic markers, including the 16S rRNA gene, was foundational in allowing the structure of microbial communities to be explored. 16S rRNA gene sequencing (called ‘16S’ for short) provides information on relative abundances of bacterial taxa in a microbiome—and it is generally considered accurate to the genus level. Most of the existing published literature on human microbiomes describe 16S analyses.

Recently, however, more published studies make use of information gained through shotgun metagenomic sequencing: a method that directly recovers total genomic information from a sample—and for bacteria, reliably provides up to strain-level resolution. Not only does it provide data from all members of a microbial community (bacteria, but also fungi, viruses, and other groups), but it also gives insights into function: what the microorganisms are genetically equipped to do.

Microbiome-related technologies and best practices continue to change rapidly. So Microbiome Times editors asked scientists at globally leading microbiome analysis service providers to give an update on when 16S rRNA gene sequencing may be the better option.

“16S is still the standard,” says Dr. Brett Finlay, scientific co-founder of **Microbiome Insights**. The majority of the company’s clients, spanning across academia and industry, request 16S analyses for their studies. Finlay says the strength of 16S is that it gives, “a good overview picture, and relative abundances of microbial composition”. He notes, “[Shotgun] metagenomics is not as useful for relative abundances but is good for

strain identification and metabolic potential.”

Furthermore, it stands to reason that since the great majority of previous studies in the microbiome field have made use of 16S, new studies using 16S allow more direct comparisons to—or replications of—prior work.

Undoubtedly the cost-effectiveness of 16S contributes to its wide use, but the service providers say even if researchers had unlimited resources, 16S may be the top choice under certain circumstances.

Clinical Microbiomics’ Chief Scientific Officer Dr. H. Bjørn Nielsen

says, in particular, certain low-biomass microbiomes are best to access through 16S amplicon sequencing. He explains, “By low-biomass I’m thinking of eye swaps, forceps GI biopsies, and similar.” This is because in situations with very low biomass communities, the data will reflect only a portion of the microbiome, leading to an incomplete understanding of the microbial community. Host DNA may be abundant, and would bury the microbial signal in a shotgun metagenomic dataset.

Nielsen notes that in the past,

skin, oral, and GI mucosal brushed biopsies may have posed a problem for shotgun metagenomic sequencing, but this is no longer the case if the samples are collected appropriately.

Diversigen founder and Chief Science Officer Dr. Joseph Petrosino agrees, adding that 16S is appropriate, "in cases where the DNA quality is so poor that metagenomic library prep is not effective, but 16S polymerase chain reaction is still possible. [But] library protocols are improving, so these types of samples may be becoming rare."

Petrosino details specific scenarios in which 16S rRNA gene sequencing is a reasonable option: (1) for high level (OTU/genus level) stratification of samples, subjects, or groups in a cohort—especially when the number of samples is large or if timing is of the essence; and (2) for projects in which deeper whole genome sequencing is possible, but only on a subset of samples. 16S can be used to help decide on which samples should get deeper attention through metagenomic sequencing or other - omics analysis.

In addition, Nielsen highlights soil communities as a special case. "Some soil types are still a challenge and one may still recommend 16S amplicon sequencing there," he notes. "It's unclear if all soil types can be resolved with sufficient short-read shotgun sequencing, or if one may need long reads."

Nielsen adds, "16S amplicon sequencing may also be best for studies of previously unexplored microbiomes... especially if only few samples are available from this microbiome."

One other consideration is relevant to sequencing choices, especially in clinical research: researchers who automatically go for the increased resolution of shotgun metagenomics may run the risk of 'missing the forest for the trees'. That is, having access to strain-level information

might bias researchers toward thinking specific strains are what contribute to a phenotype or outcome, when possibly relative abundances or other features of the overall microbial community are what matter more. An important question to address as microbiome testing moves toward clinical applications is whether critical microbiome-related information (e.g. for stratifying a patient as a 'responder' for a particular drug) can be captured by the more economical 16S, or whether it requires the higher resolution of shotgun metagenomic analysis.

None of the service providers predicted that the future of the field will be entirely about shotgun metagenomics. Rather, as the understanding of various human and environmental microbiomes progresses in fits and starts, they see both 16S and shotgun metagenomics analyses continuing to thrive, enabling researchers to understand microbial communities in unique and complementary ways.



UNTARGETED METAPROTEOMICS IS THE MOST RATIONAL APPROACH FOR FUNCTIONAL MICROBIOTA DISCOVERY & UNDERSTANDING

OPINION PIECE BY GILBERT SKORSKI
CHAIRMAN & CEO, PHYLOGENE

Introduction

The microbiota complexity has been discovered through untargeted sequencing approaches which showed us a complex new world. To understand how a holobiont works, untargeted approaches are available and essential as the traditional way of using targeted techniques to validate a hypothesis will ignore other parallel events which may impact a complex biological event.

Omics Landscape

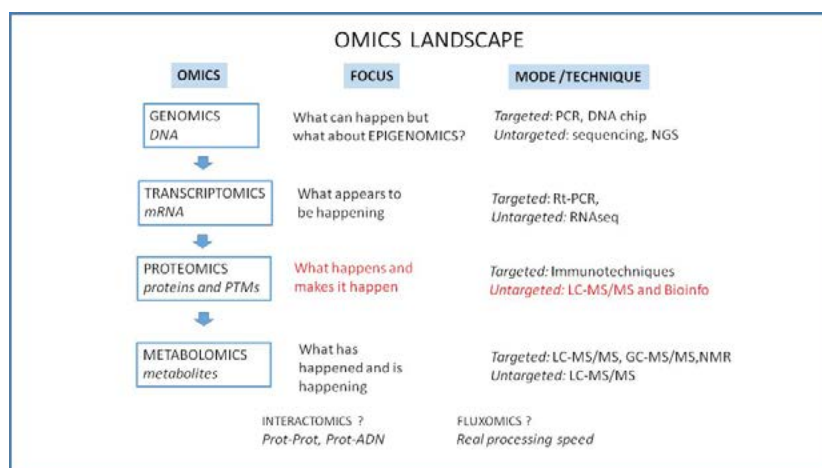
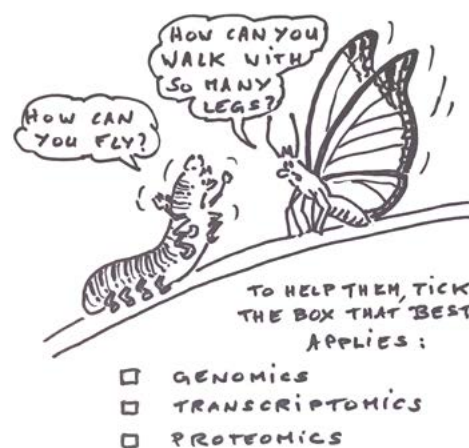


Fig1: Techniques are operational at different steps of the systems biology and provide different levels of information which are targeted specifically on analytes or only assign any signal to analytes through databases.

Knowledge Depends on Existing Analytical Techniques

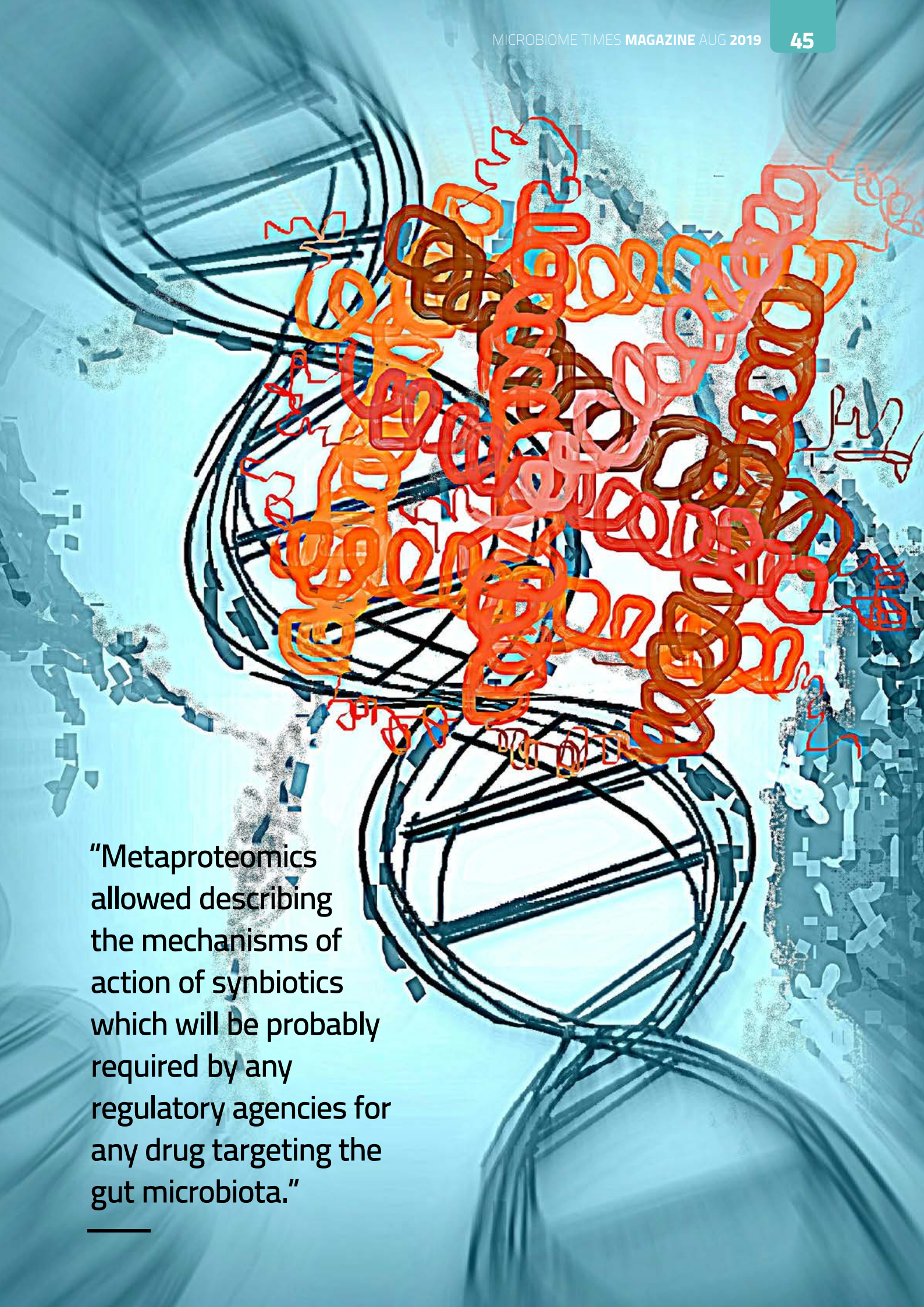
PCR was discovered around 1985 and allowed to detect specifically DNA strands. Since the years 1990, DNA sequencing techniques opened the door to untargeted approaches of genes. Now NGS allows sequencing a genome at low cost.



For over a century, mass spectrometry allowed detection of small chemical molecules. For 15 years now, mass spectrometry evolution allows to analyze and quantify proteins. The proteogenomics approach through the genes knowledge and with LC-MS/MS and databases evolutions allows identifying and quantifying proteins in an untargeted mode. Improvements in the mass accuracy, resolution, and sensitivity of MS instruments are enabling the rapid and reliable detection, identification, and quantification of proteins in complex mixtures.

These techniques are opening the door to improved methods for discovering disease-specific biomarkers with the potential to support early disease detection and even individualized therapies.

In an untargeted way (shotgun NGS) and quantitatively, a gene gives the information that the translated protein can structure or work in the cell, but we now know that epigenetics mechanisms may interfere or inactivate gene. Genomics stays reasonably at the informational level. (fig.1)



"Metaproteomics allowed describing the mechanisms of action of synbiotics which will be probably required by any regulatory agencies for any drug targeting the gut microbiota."

The transcript (mRNA), RNAseq at the quantitative shotgun level, tells us a protein may be translated but if and when remains an open question.

Only LC-MS/MS proteomics tells us which protein is there

Of course, no technique is able in an untargeted way to tell us if the protein is active and at what speed it produces metabolites, even if the post-translational modifications (PTMs) can be now reached by mass spectrometry (1). Also, no untargeted technique is able to know which protein interact with which other molecule.

Metabolites are produced by proteins and may be metabolized by other proteins. Metabolites may be tracked using LC for the liquid part, GC for the volatile part coupled with MS/MS or NMR but it works rather in a targeted way as databases are poor up to now (2, 3): of course, there is no structural correspondence between the gene and the metabolite as there is between the gene and the protein.

Shotgun sequencing allows finding all the known genes in a sample, genes which account for around 2% of DNA, the remaining being the 'junk DNA' which is now known to be of primary importance. Based on genes sequences databases, LC-MS/MS proteomics can easily identify the functional proteome and may also reach the signaling proteins using sample pre-fractionation. Proteomics has also the 'dark proteome' which accounts for around half of LC-MS/MS spectra and corresponds to disordered protein regions but which seems to be essential for the proteome activity disordered regions.

Up to now, metabolomics remains limited in an untargeted mode as only 10% of spectra obtained in mass spectrometry can be identified (2).

Although transcriptomics, proteomics, and metabolomics all generally measure the products of gene expression, one should not necessarily expect exact quantitative correlation among them. Measuring the level of a transcript reflects the production rate of its protein product, but does not accurately predict the concentration or stability of the protein product. In fact, the correlation of mRNA abundances with their corresponding protein abundances, while reasonable for some core metabolic processes in some microbial systems, in general is poor or non-existent in most biological systems examined to date, suggesting proteomics data is likely more indicative of biological phenotype than mRNA. (4, 5)

What is LC-MS/MS untargeted quantitative metaproteomics?

Proteomics means the study of expressed proteins in a cell, a tissue, an organ or an organism at definite time and conditions. The proteome is the entire set of these proteins. Metaproteomics refers to all the proteins of the ecosystem, mainly the host and its microbiotas, named holobiont. An LC-MS/MS instrument analyses continuously the peptide fractions obtained from HPLC after enzymatic digestion of a protein extract. The mass spectrometer does every second an MS spectrum followed by further MS/MS fractionation on the most intense components. This information generated on the different proteins segments is compared to mass maps and spectra available in databases and it allows the identification of

proteins present in samples. This identification is made by using proteogenomics (6) approach which makes the bridge between genes and proteins. The peptide pikes area ratios gives a relative quantification.

Relative quantitative proteomics or metaproteomics refers to the analysis of two or more groups of samples by globally and quantitatively comparing their proteomes. Mass spectrometry has the unique ability to measure changes in complex protein mixtures.

As the identification is a process without targeting particular analytes, this is a hypothesis-free approach.

The analytical workflows are now simple and linear, in a first intent without need of 1D nor 2D prefractionation to reach 5000 proteins in a sample. One challenging difference of metaproteomics compared to proteomics remains the size of the identification databases. The human side contains 23000 genes, on which at least 1000 genes per microbial species are filled out. (Fig. 2)

Analytically, LC-MS/MS relative quantification of proteins is now a reliable technique (7) with a dynamic range of more than 5 logs, inter-assay CVs less than 20% (8) and Limit Of Detection at the attomole level. The main variability is brought, as for most techniques, by the sample protein extraction (9) which is minimized in relative quantification of same kind of samples.

Associated Bioinformatics

The current proteomics LC-MS/MS output data reach easily more than

5000 identified proteins. More than 10000 proteins can be reached easily in metaproteomics. The relative quantification of two conditions reveals tens to hundreds of under- or overexpressed proteins.

This requires heavy data processing pipelines (10) which will analyze all impacted proteins in correspondence with the metabolic pathways in which they are involved. It is so possible to understand the biological events induced by the disease or the dysbiosis.

Usually, a taxonomic analysis, a functional analysis by taxon (Homo sapiens, Fungi, Bacteria and Archae) and inter-functions correlations can be produced which give access to links between signaling/metabolic pathways, potential association of functions to particular taxons, potential interspecific relationships between microorganism and between microorganism and human (11). As a synthesis, the mechanism of action can be formulated (12).

Multi-omics as Complementarity Factors

Although untargeted LC-MS/MS proteomics data provides information about protein production, it cannot accurately predict a protein's activity or functional state. To achieve a more complete understanding of metabolic activities, it is interesting to integrate -omics data. It is important to realize that while DNA and proteins are relatively stable, transcripts and metabolites often have very short half-lives; thus, there are dramatic temporal information differences in these omics measurements. By integrating these large-scale datasets, cellular metabolism can be examined at an upgraded information level. For complex samples such as gut microbiomes, this integrated omics information has potential to provide a detailed molecular view at a higher resolution (4). For example, having the information of major impacted taxons after a 16sDNA profiling will focus the metaproteomics database queries on these taxons.

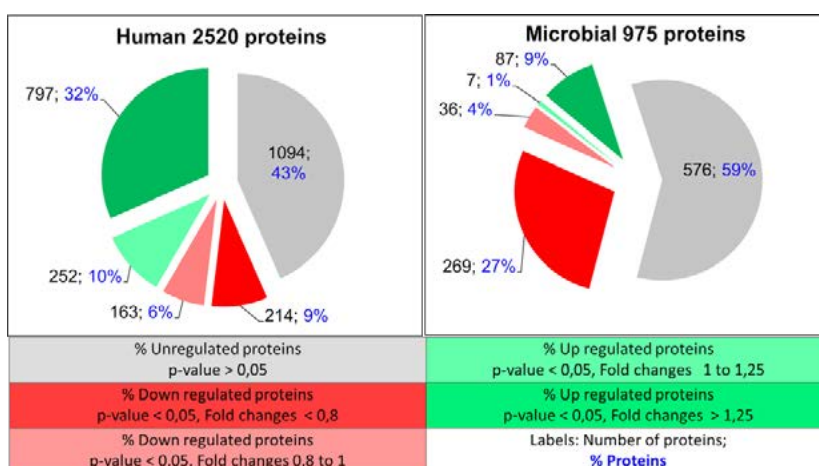


Fig 2. Proteins distribution for relative quantification Male/Female on skin: According to fold changes and p-values, statistical variation of abundances was shown for 1011 human proteins and 356 microbial proteins (including fungi and bacteria). Monneuse and all. ESDR 2017

Understanding the effects on host and microbiotas

Metagenomics studies initiated with the Human Microbiome or MetaHit projects brought huge knowledge about the individual variability of the microbiotas, the importance of the diversity and quantity of the gut microbiome at the taxonomic level, and relationship between a taxonomic state of a microbiota and diseases. Anyway a huge gap remains to understand the working of the holobiont such as the relations between microbiota and host, the impact of a diet, a probiotics, or a disease. The taxon, genus and species impacted by any dysbiosis and the 2 millions of identified genes in the gut microbiome does not tell much on what really occurs.

Already in 2012, the Human Microbiome Project (13) mentioned that the taxon variability was not in relation with the relative stability of functions at the genomic level.

Metagenomic studies have shown that gut microbiotas share a stable set of core functions, in spite of a large inter-individual structural/compositional variability. However, since sequenced genes are not necessarily expressed, metagenomics cannot provide reliable information on which microbial functional traits are actually changing in response to stimuli from host metabolism, immunity, neurobiology, diet, or other environmental factors which induce a substrate change. (12)

But this type of information can be gathered by functional metaproteomics, which displays higher sensitivity to perturbation and may therefore better reflect host-microbiome interactions (14) and mechanisms of action (15).

This way, several correlations were

identified between human and bacterial proteins (16) as both human and microbiota proteins are followed.

Using this approach on stool samples it is even possible to functionally relate human proteins to bacterial extracellular vesicles and to identify the peptides of interest reflecting taxonomic and/or pathway changes, which enables further biomarker discovery (17).

In another study on prebiotics and probiotics (synbiotics) diet in an obese mice model, it was shown first that high fat diet could induce a striking change in the functional activities of the gut ecosystem, which is characterized mainly by an increase in cell motility, and amino acid, carbohydrate and lipid metabolisms as well as a decrease in energy and nucleotide metabolisms. The symbiotic diet significantly reversed 11 KEGG pathways that are involved in carbohydrate, amino acid, and energy metabolisms, biosynthesis of other secondary metabolites, transcription, translation, replication and repair, as well as transport and catabolism (18). Metaproteomics allowed describing the mechanisms of action of synbiotics which will be probably required by any regulatory agencies for any drug targeting the gut microbiota.

In Crohn's disease, metaproteomics and dedicated bioinformatics were used to follow a patient in 4.5 years (11) or a cohort of patients after resection surgery for 1 year (19). Almost all the functions observed across all individuals were observed in multiple phyla, these functions are not specific to any one phylum, genus, or species. There is a clear persistence of conserved metabolic functions across time and individuals. Finally, the gut microbiome's metabolism is not driven by a set of discrete linear pathways but a web of interconnected reactions facilitated by a network of enzymes that connect multiple molecules across multiple pathways (19).

Conclusion

Due to the proteogenomics shortcut, metaproteomics and dedicated bioinformatics is the most rational and pragmatic approach for discovery and understanding at the functional level. Based now on mature mass spectrometry technique, it provides an unparalleled ratio cost on result which is proposed as a service in skilled labs. Then it can be reinforced with other omics or PTMs investigations, or confirmed by multiplex targeted quantification such as Selected Reaction Monitoring in mass spectrometry to follow a set of biomarkers.

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TOWARD CLINICAL UTILITY OF METAGENOMIC DATA: INTERVIEW WITH DR. JOËL DORÉ

INTERVIEW BY KRISTINA CAMPBELL

More and more groups around the world are making use of shotgun metagenomic analysis for their microbiome studies. The resulting datasets are rich, but so far very little clinically relevant information can be gleaned from them. In this interview, Dr. Joël Doré, Research Director at INRA Micalis Institute and the Scientific Director of Metagenopolis, discusses how we're progressing toward clinical insights from gut microbiome data. He covers enterotypes of the gut microbiome, as well as the reasons for standardizing sample collection and analysis.

"Informing the clinician of the status of the microbiota in terms of richness is very important because it will allow the clinician to make strategic decisions in terms of clinical management of the patient."

How can we group people into different categories based on metagenomic analyses of their gut microbiota?

JD: We have now explored a large number of healthy individuals and patients, and we know that indeed there is high inter-individual variability; and there are clusters or groups of individuals that share commonalities or similarities in their microbiota, that have allowed us [to describe] what we call 'enterotypes' – a division of the normal population into what we can view as 'ecological settings' of the microbiome.

In 2012, we initially described three different enterotypes. This has been refined in a recent publication and we now have this [perception] of one of the enterotypes splitting in two separate entities, connected with the richness of the microbiota: one of the enterotypes will be low richness, and the other high richness. [Enterotypes] are also beginning to be reported as connected with health in several aspects: the connection with the richness in itself is an indication, but also one of the enterotypes, which is dominated by the genus *Bacteroides* and really low richness, is obviously connected with more fragile environments; it will be more prevalent in patients with more 'severe' conditions, so to speak.

This basically means that just profiling the microbiota of a general population or patients will tell you where they belong in terms of gut ecology and possibly connect that with a higher or lower risk of [disease] development, so that could mean a lot for nutritionists or for clinicians.

Are there any particular diseases that are of interest for using enterotypes to classify certain groups within it?

JD: So as I mentioned, in the case of the one enterotype with particularly low richness of the microbiome, i.e. low species diversity/gene count, it is connected (in terms of the scientific literature available today) with a higher risk of development of metabolic disorders or metabolic conditions such as diabetes and obesity, and comorbidities. When I talk of low richness, which is not necessarily focusing on enterotypes but splitting the population into high and low richness, this has been documented in many different conditions and we know that low richness is associated with a lesser response to treatment. It's true for dietary intervention in obesity; it's true for cancer immunotherapy (where we know that low richness is associated with a lesser response to the treatment); and it's true for the most severe conditions and aggravation in the liver pathway, towards the most severe outcome which is essentially acute on chronic liver failure in cirrhosis—a severe instance where you need to envision a liver transplant. These are just a few indications where we are convinced that informing the clinician of the status of the microbiota in terms of richness is very important because it will allow the clinician to make strategic decisions in terms of clinical management of the patient.

Can you tell us why is it important to standardize the sample collection and analysis if we're moving towards metagenomic data being useful in the clinic?

JD: This is actually something that I am very keen on. Standardization is critical. We know that there can be bias that we introduce in the process of generating data. If we want to be able to use the data for nutritional or clinical purposes, but if we have this accumulation of bias in the process, then the biological story we tell can, in the end, be very wrong.

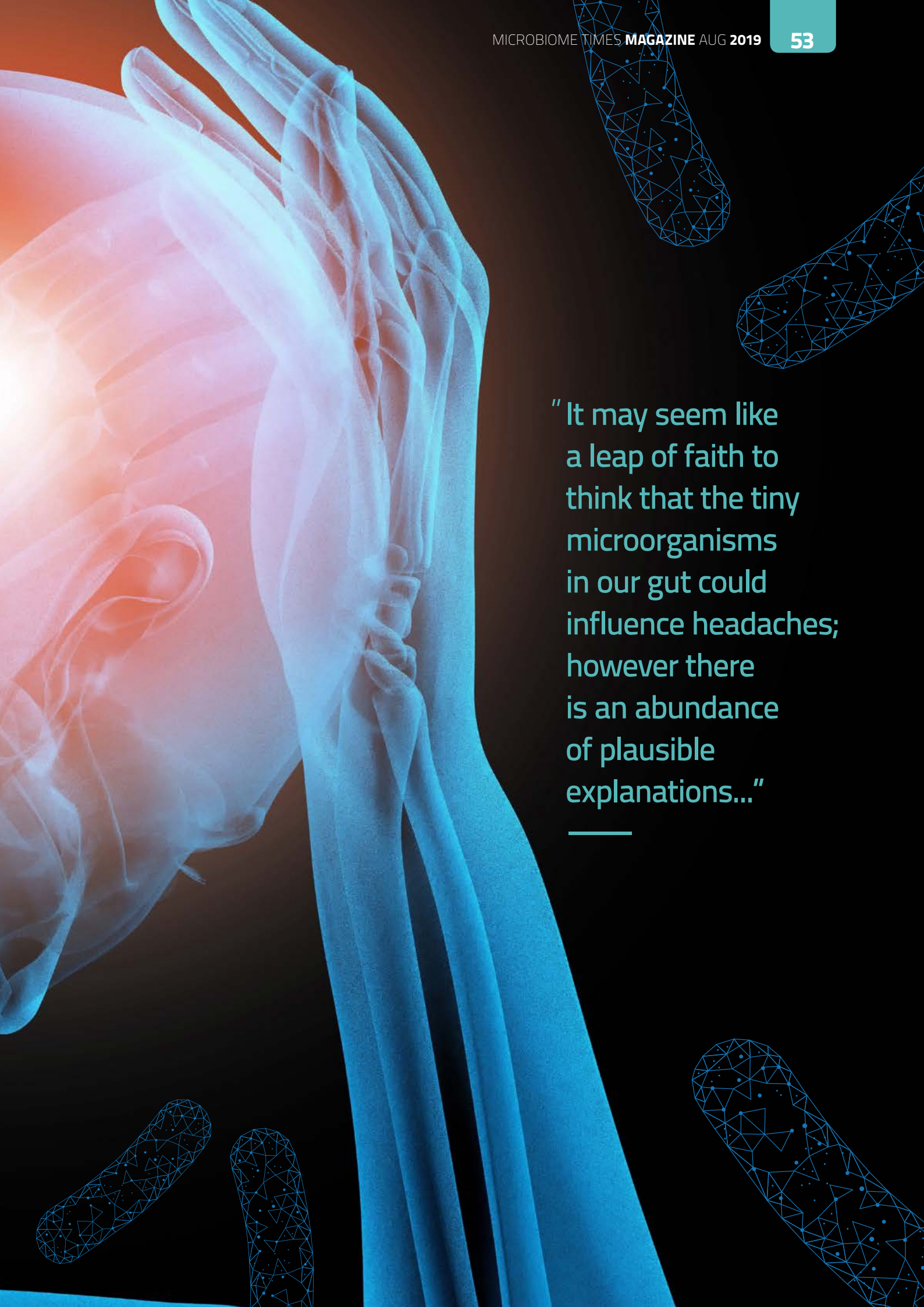
So we absolutely need to secure the robustness and the validity of the information we generate, and the best way to do that is to have highly standardized processes, starting from sample collection: recommending the proper way of collecting the material, shipping the material to the place it will be dealt with, [preparing] DNA from stool material or biopsies, and then sequencing the material to generate sequence data. All of those steps are critical. I would say that we have made good efforts when it comes to a full shotgun metagenomic sequencing (probably the best way to provide material or information to the clinicians to date), such that we are at the stage of writing and providing guidelines to be able to have a highly robust and reproducible determination at present – so it's a special moment in that respect! Standard Operating Procedures defined within the European program IHMS have been available online for downloading since 2015 (microbiome-standards.org).

This is not yet the case for 16S rDNA based approaches and efforts are still to be made to ascertain that two different labs or service providers will be telling the same biological story from the same sample set. The advantage today is that reference microbial cocktails are commercially available that can be used as reference.

MICROBES AND MIGRAINES: A NEW CHAPTER IN THE GUT-BRAIN AXIS

FEATURE ARTICLE BY ASHTON J HARPER
MEDICAL DIRECTOR, ADM PROTEXIN





"It may seem like a leap of faith to think that the tiny microorganisms in our gut could influence headaches; however there is an abundance of plausible explanations..."

Migraine is a common disabling headache disorder characterised by its severity and typical sensory disturbances to light and sound. The complex pathophysiology of migraine involves genetic, metabolic and hormonal elements which disrupt the ability of the brain to process incoming sensory information¹. It is estimated to affect 1 in 7 people (>1 billion worldwide) and was the 3rd most prevalent condition in the world in The Global Burden of Disease Study 2010². Severe migraines are ranked as one of the most disabling illnesses by the World Health Organization, comparable to dementia, active psychosis, and quadriplegia³. The direct (healthcare associated) costs in the US alone have been estimated at \$9.2 billion annually⁴, which is substantially less than the indirect costs incurred from both work absenteeism and presenteeism; potentially accounting for >90% the total economic burden⁵.

Migraines and the Gastrointestinal System:

Migraine headaches are commonly accompanied by gastrointestinal (GI) symptoms such as nausea, vomiting and diarrhoea and are often associated with GI conditions like irritable bowel syndrome, gastroesophageal reflux, and coeliac disease⁶. Abdominal migraine is a common functional disorder predominantly affecting children with a family history of migraine. Symptoms include severe intermittent central abdominal pain accompanied by typical migrainous sensory disturbances (photophobia and phonophobia) with or without headache⁷. Interestingly both the triggering and relieving factors are similar as for migraine headaches and both conditions are associated with infantile colic; a common cause of inconsolable babies which is linked to gut inflammation and dysbiosis (an abnormal gut microbial community)⁸. These correlations between migraine headache and the GI system may not come as a huge surprise for anyone keeping abreast of research in the gut-brain-axis; the bidirectional communication network connecting these organ systems. During our early development cells destined to elaborate into the nervous system of the gut (the enteric nervous system – colloquially referred to as ‘the 2nd brain’) originate from the same tissue (neural ectoderm) that develops into the brain⁹, and throughout life both structures remain intimately connected by the vagus nerve; the main component of the parasympathetic nervous system. The vagus nerve in party with the endocrine and immune systems represent the three major components of the gut-brain-axis¹⁰. Additionally there is mounting evidence to suggest that the microorganisms we harbour within our gastrointestinal tract may communicate with the brain via all of these pathways¹¹. This discovery offers tantalising new opportunities to both better understand our physiology and influence neurological diseases such as migraine.

Evidence of Microbiome Differences in Migraine:

Analysis of the gastrointestinal microbiome of migraine sufferers is currently extremely limited. In a 2018 pilot study researchers found a greater degree of gastrointestinal dysbiosis in adult women with migraine than without¹². In another study analysis of data from the American Gut Project identified significant differences in nitrate, nitrite, and nitric oxide reductase genes (bacterial origin) in the oral and faecal samples of migraineurs vs non-migraineurs¹³. Nitrate compounds are well known headache triggers and they may cause migraines via release of substances such as calcitonin

gene-related peptide (CGRP) (NB: a recent advance in pharmaceutical management of migraines has come from monoclonal antibodies that antagonise CGRP receptors)¹. Regarding dietary triggers for migraines some the most frequently identified culprits include coffee, alcohol, and chocolate. A clear explanation for this is yet to be found, however research has identified that, in the case of chocolate, those who ‘desire’ it versus those ‘indifferent’ to it have different gut microbial metabolic activities as identified by compounds measured in the blood and urine¹⁴, which suggests the possibility that our microbes may be influencing our dietary choices. Clearly further research is needed to elucidate microbial signatures related to migraine headaches; the possibility of identifying a predictive pre-attack microbial pattern would be of particular interest.

The Microbiome-gut-brain-axis and Migraine:

How is the gastrointestinal microbiome able to influence migraine headaches? It may seem like a leap of faith to think that the tiny microorganisms in our gut could influence headaches; however there is an abundance of plausible explanations, albeit complex and unproven. As mentioned previously the the gut-brain axis encompasses three main communication conduits – neural, hormonal and immune – and the involvement of bacteria in each of these will be evidenced below:

- Rodent models have clearly identified how bacteria in the gut are capable of altering brain function via the vagus nerve (neural). In mice with established anxiety the oral administration of a strain of Bifidobacterium

longum acts to normalise their behaviour, however this effect is only seen when the vagus nerve is intact¹⁵. In humans functional brain imaging (functional magnetic resonance imaging or fMRI) has demonstrated dysfunctional dynamics within pain pathways in the brains of migraineurs versus healthy controls¹⁶. Interestingly the activity in these pain pathways was altered by the oral administration of a multi-strain probiotic¹⁷; albeit in healthy women. The authors hypothesise that altered vagal signalling from the gut to the brain might explain this effect.

- In addition to vagal communication research suggests that probiotics may modulate central nervous system function through the production of a range of neurotransmitters (GABA, serotonin and acetylcholine) and their precursors¹¹. Serotonin and the serotonergic system are of central importance to migraine as identified by the well-known triptans class of drugs (serotonin 5-HT_{1B/1D} receptor agonists)¹. Migraine may actually be a chronic low serotonin syndrome, and evidence suggests that depletion of tryptophan (the precursor of

serotonin) exacerbates headache and associated migrainous symptoms¹⁸. Furthermore, tryptophan is predominantly metabolised along the kynurenine pathway (an important metabolic/ enzymatic pathway) producing a range of metabolites that have implications for migraine headache¹⁹. This is interesting from a microbiome-gut-brain axis perspective given the mounting evidence which implicates the gut microbiota in the regulation of kynurenine pathway metabolism²⁰.

- Stress (**hormonal**) has been identified as the most common trigger for migraine attacks²¹, and there is evidence to support an alteration in the stress hormone axis in these patients¹. Part of the stress response is the release of hormones including cortisol which is known to increase the permeability of the gut barrier allowing the passage of bacteria and pro-inflammatory compounds into the blood²². Lipopolysaccharide (LPS) is a cell wall component of gram negative bacteria with a well-documented pro-inflammatory effect (**immune**). The relevance of LPS to migraine headache is highlighted by evidence that

it sensitises pain receptors in trigeminal sensory neurones (those involved in migraine headaches) in the face²³. Probiotic supplementation has been successful in resolving stress-induced gut leakiness in rodents¹¹, and reducing cortisol levels in humans²⁴, evidence which may, at least in part, help to explain their benefit in preventing migraine headaches.

Probiotic Trial Evidence in Migraine Management:

The first publication exploring the effect of probiotic species alone in the management of migraines was published in 2015²⁵. In this open label pilot study a 12 week intervention with an 8 strain probiotic (Ecologic Barrier, Winclove) achieved a significant reduction in both the frequency and intensity of headaches in 29 adult migraineurs. In a subsequent randomised controlled trial (RCT) utilising the same probiotic these beneficial outcomes could not be confirmed; although it is worth noting that the active group did see a significant improvement in headache specific disability scores²⁶.

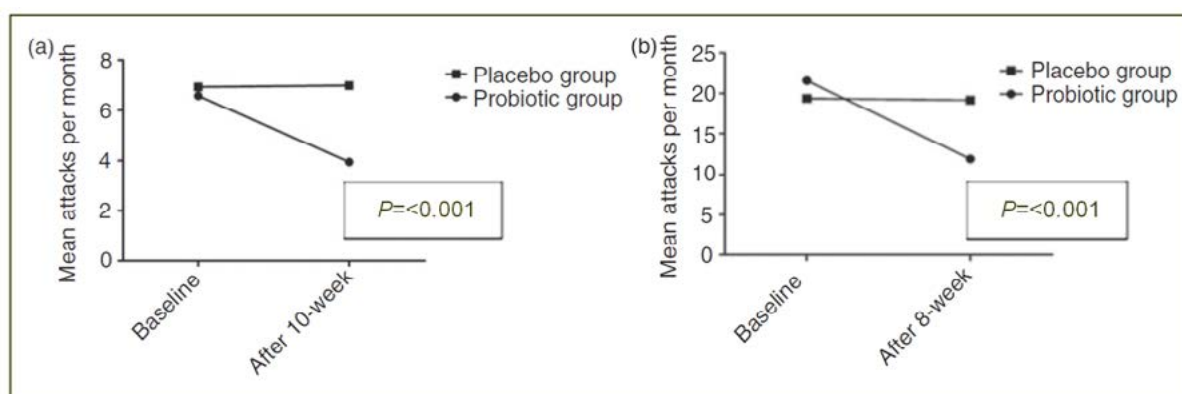


Fig 1: Comparison of headache frequency (attack/month) in (a) episodic migraineurs before and after 10-week supplementation with probiotic or placebo, and (b) chronic migraineurs before and after 8-week supplementation with probiotic or placebo after considering gender, age, BMI, dietary energy intake and means of headache characteristics at baseline in ANCOVA model (adapted from Martami et al 2019²⁷).

The only other RCT for this indication was published in 2019. In this study 50 patients with episodic migraine (≤ 15 headache days per month) and 50 with chronic migraine (≥ 15 headache days per month) were equally assigned to either a 14 strain probiotic (Bio-Kult, ADM Protexin) or placebo for 8 (chronic migraineurs) or 10 (episodic migraineurs) weeks²⁷. Overall patients in the probiotic arms experienced 40–45% reduction in the frequency of migraine attacks (Fig 1), a 30% reduction in migraine severity, and a 30% reduction in the use of pain-relief medication. This is the first RCT showing positive results in both chronic and episodic migraineurs with a probiotic adjunct. Although these results are promising they do require conclusive validation in a further large scale RCT.

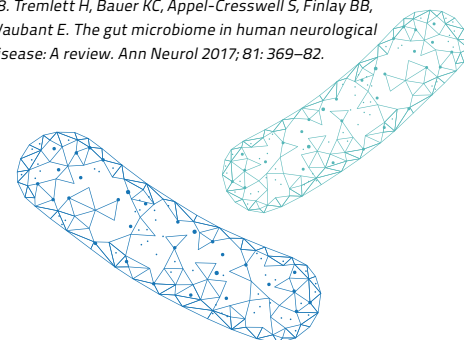
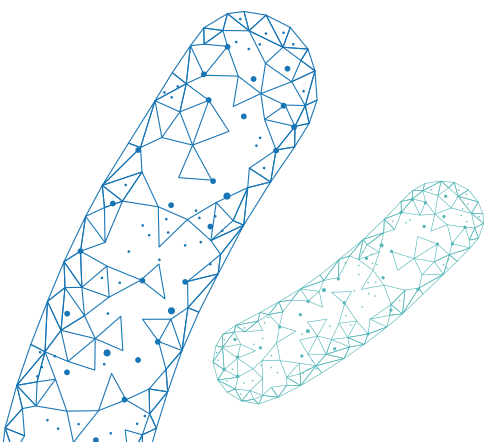
Future Perspectives and Conclusion:

Exciting research in the microbiome-gut-brain axis suggests that manipulation of our gut bacteria may enable us to alter brain function and potentially enhance the management of a range of neurological diseases^{11,28}. Migraines, given their high prevalence and devastating impact on quality of life, represent an important target in this field. The pathophysiology of these headaches is complex and remains to be fully understood; however there seems to be at

least some intriguing evidence implicating our gut bacteria in the process. Further research is required to firmly establish differences, if they exist, in the microbiome of migraineurs versus healthy controls, to enhance understanding of bacteria-relevant mechanisms, and to validate the promising trial results seen to date in further large and robustly designed studies.

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NEW RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL FINDS PROBIOTICS TO BENEFIT MOOD AND QUALITY OF SLEEP IN HEALTHY STUDENTS

ARTICLE BY NINA VINOT,
AREA SALES MANAGER, PROBIOTICAL

A collaboration between Probiotal (Biolab Research), the University of Verona and Microbion recently led to the publication in *Frontiers of Psychiatry* of new data on the potential of specific probiotics to support mental wellbeing¹.

The bidirectional relationship between the gut and the brain is now evident and previous studies on multispecies probiotics found positive effects on mood but they focused mainly on depression and anxiety²⁻⁴, whereas this study extends these findings with a more comprehensive and integrative assessment of Mood and Cognition.

This double-blind, placebo-controlled randomized trial on 38 healthy volunteers both male and female, aged 19 to 33 years, supplemented for 6 weeks either with the probiotic combination (*L. fermentum* LF16, *L. rhamnosus* LR06, *L. plantarum* LP01 and *B. longum* O4, 4 billion cfu/afu daily) or placebo collected data on 4 validated scales of mood, 4 validated scales of personality and 1 scale regarding sleep quality, but also on demographics, diet, physical activity, premenstrual syndrome and fecal samples, allowing to control for biases, and which will be further analyzed for future publication. Furthermore, compliance was assessed and improved thanks to a mobile application developed by Bussola Labs, reminding participants to take and scan their supplements each day.

The probiotics supplementation significantly improved mood, with a reduction in depressive mood state, anger and fatigue, as well as significant improvements in sleep quality and acceptance, which correlates with decreased depression sensitivity⁵. Interestingly, all these observed significant effects were maintained after 3 weeks of wash-out, showing prolonged benefits after cessation of probiotics intake.

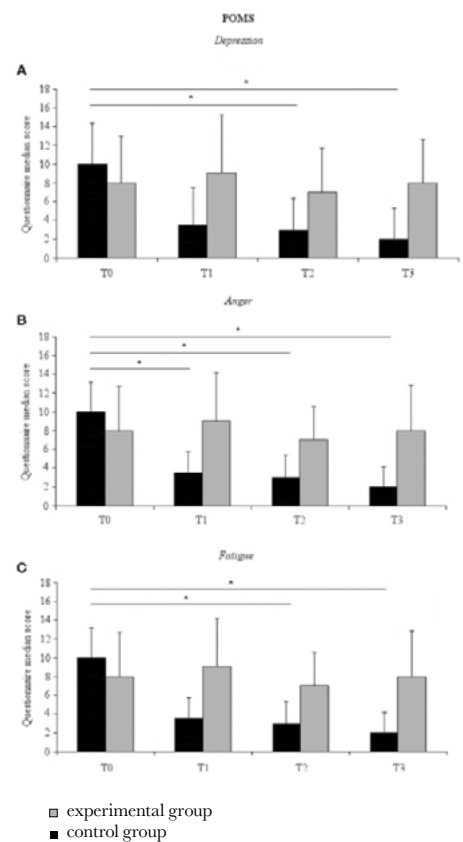


Fig 2: Median POMS sub scale score for the experimental (black columns) and the control (gray columns) groups at all-time points. (A) Depression sub scale; (B) Anger sub scale; (C) Fatigue subscale. Error bars represent 95% confidence interval. Asterisks indicate within-groups significant differences (Bonferroni corrected $p < 0.017$).

These results are coherent with previously described⁶ correlations between a good sleep quality and lower anxiety, depression, tension, anger, fatigue and confusion.

The personality-related aspects showed a few differences between the 2 groups in novelty-seeking, self-directedness, positive attitude and avoidance strategies, but not consistently across time points, making the differences difficult to interpret, and suggesting the stability of personality dimensions, which would appear to be complex and consolidated features.

Marco Pane, co-author of the study and R&D Director at Biolab Research, comments:

"These are strong and exciting findings because the probiotics intake managed to significantly and consistently improve several aspects of mood and sleep in a young and healthy population, which is among the most resilient population possible. They give positive expectations on how these results may translate to a clinical population. In fact this probiotics combination is now being tested in patients with major depression."

The four strains were carefully selected based on preclinical in silico and in vitro characterization of different, complementary modes of action:

- Presence on the genome of genes coding for the production of gamma-aminobutyric acid, the main inhibitory neurotransmitter in the human central nervous system;
- Antimycotic activity against *candida* species and antibacterial activity against *E. coli*⁷. Fungal and *E. coli* infections are common in people with depression, and decreasing the pathogen load in the gut can also decrease the pro-inflammatory status they contribute to;
- Anti-inflammatory activity⁸: three strains were chosen due to their induction of anti-inflammatory cytokines including IL-10 and IL-4, and reduction of pro-inflammatory cytokines such as IL-6. The strains are able to modify the proteins expressed on the surface of monocytes, leading to their differentiation towards anti-inflammatory cells;
- Anti-oxidant power⁸: two strains significantly reduce superoxide anion production in PMA-exposed Peripheral Blood Mononuclear Cells (PBMCs)
- Protection and restoration of membrane integrity⁸: two strains are able to restore membrane integrity after a challenge, opposing leaky gut and the associated low grade chronic inflammation.

Inflammation plays a major role in depression⁹⁻¹⁰ and selected probiotics can down-regulate it in several ways, such as the above-mentioned anti-pathogen activity, direct regulation of immune cells and protection of membrane integrity.

Another important aspect of the study is the release and stability analyses on the probiotics, using both plate counting methods (with results in colony forming units) and flow cytometry (with results expressed as active fluorescent units), allowing the commercialization of the product also declared in afu.

The dietary supplement is available from Probiotical S.p.A., as BifiZen.

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"WHERE THERE'S MUCK, THERE'S BRASS" — MONEY IN THE MICROBIOME

OPINION PIECE BY ARNAUD AUTRET
INVESTMENT PRINCIPAL | LIFE SCIENCE AT M VENTURES

The human microbiota is plausibly related to numerous chronic diseases such as type 2 diabetes, irritable bowel syndrome and Crohn's disease, obesity and malnutrition, as well as autoimmune diseases (multiple sclerosis, psoriasis, rheumatoid arthritis, etc.) and allergies. Current studies have revealed that gut microbes also influence brain function and behaviour and are related to neurological disorders like dementia or Alzheimer's disease via the gut-brain axis. Additionally, there is increasing evidence from preclinical models and human cohorts showing that the diversity and composition of gut microbiota is associated with therapeutic improvement in various forms of cancer therapy.

Our scientific knowledge around the Microbiome is progressing at a rapid pace. However, understanding the complexity of the Microbiome is still a big challenge. Researchers in the field have been battling with the difficulties posed by the multifaceted puzzle represented by the Microbiome and practical application of this knowledge is still in its infancy. For example, most of the clinical evidence lacks the basic science to explain the mechanism of action behind it.

Financially, the field is also rapidly growing. BCC Research indicates that the Microbiome-based drugs market should reach nearly \$9.3 billion in sales by 2024. But while Microbiome companies have been successful in raising capital, translating Microbiome research into targeted and stable modalities with dedicated benefits for the human host has not yet been achieved. Microbiome-based therapies remain in their early stages.

- **How to fill the gap between expectations and concrete applications?**
- **How to translate observations and correlations into specific treatments?**
- **How to navigate into this highly complex ecosystem?**

At the M Ventures Life Science fund, we are deeply committed to investing in new technologies that will fill this gap, allowing researchers and companies to provide strong and stable data behind each potential product.

In our view, some specific areas are of interest:

1

Standardization (research, protocols)

The excitement and enthusiasm for Microbiome research seems to have outpaced any agreement upon experimental best practices. Normalized approaches must be put in place through all essential steps of a specific study: sampling, preservation and storage, DNA extraction and amplification, bioinformatics analysis, etc.

A non-formalized way to go through each of these steps can lead to experimental biases resulting in unreliable results. Proper experimental design and analysis parameters can provide greater resolution of species in complex metagenomic samples and improve the interpretation of data. Well-defined and controlled experiments are essential before claiming that some microbes are beneficial or

not or even deleterious.

This is also valuable for specific protocols like Fecal Microbiota Transplant (FMT). The goal of FMT is to restore the physiological balance by transferring gut microbiota from a healthy donor. There is no standard method to produce material for FMT, and there is a multitude of factors that can vary between institutions that offer this therapy. The underlying

reasons why an untargeted intervention like FMT is effective in some cases but not others are mostly unclear. Standardizing this protocol will enable comparisons between preclinical or clinical trial results of specific targets, for instance. This will then help researchers to design the right process according to patient conditions for improvement in clinical impact.

Importantly, in mid-June 2019, the Food and Drug Administration (FDA) informed health care providers and patients of the potential

risk of serious or life-threatening infections caused by multi-drug resistant organisms (MDRO) with the use of FMT. FDA has determined that donor stool screening for MDRO is needed for any investigational use of FMT. Again, this indicates that standardization of protocols is required.

2

Biodynamics

As discussed, Metagenomic approaches and data have had profound impacts on our understanding of the Microbiome. However, the bacterial and viral populations of a specific Microbiome are highly dynamic. The field does not have the technical capacity to deeply analyze these biodynamics. As a result, for example, real-time monitoring of microbiota function in situ remains challenging.

Tracking microbiota state, function, and intercellular interaction via imaging will help, for example, to dissect mechanisms of action.

This will dramatically improve the temporal resolution researchers can focus on, allowing a deep look into inter-species or host-microbiota interactions that are underlying the functionality of the ecosystem itself.

3

Sequencing

We are also currently investigating new sequencing tools, mainly targeting the gap between the two main methods of Microbiome analysis: the high-throughput 16S rRNA amplicon gene sequencing, a method that sequences one single gene present in bacteria; and the whole-genome shotgun (WGS) metagenomic sequencing, a more recent method that sequences all the genes (DNA) in all micro-organisms present. Both of these technologies have underpinned Microbiome research for more than a decade and they continue to dominate the field.

However, these techniques have specific limitations like the lack of quantitative functional annotations for the 16S sequencing or the high price of the shotgun tool. Developing a fast and accurate sequencing tool for cost-effective analysis will add a lot to the field.

In addition to the bacterial community analysis, other key components of the Microbiome ecosystem have not really been addressed up to now. Its ecological dynamics and interactions

are possible factors that have so far remained understudied. For example, if the Virome is an important component of the Microbiome, the study of the viral diversity is going to be difficult because there are no specific marker genes for viruses such as those found in fungi or in bacteria. High-throughput deep analysis of the Virome or Mycobiome will give researchers an interesting view on the modulation of the full ecosystem. This will also help in the design of personalized and stable treatments for patients as this will take into consideration all potential interferences that could occur.

4

Metabolomics

Beyond the microbial DNA contents, insights into the functional relationships in this symbiotic ecosystem remain challenging and require understanding. In our view, obtaining a more comprehensive analysis by identifying not only the microbial composition but also the metabolites (metabolome) in various conditions will be more insightful.

Microbes produce many of the body's chemicals, hormones and vitamins. The major metabolites produced include for instance short-chain fatty acids (propionate, butyrate, etc.), lipids (Sphingomyelin, Cholesterol, etc.), polyamines, or gases (e.g. CO₂,

CH₄). High-throughput analysis of the biological functions of host and microbiota co-metabolites will increase our understanding of the ecosystem. Faecal metabolic profiling has recently been developed and will be an extremely useful tool to understand the complex metabolic interactions between microbes and their host. When Meta-transcriptomics,

which is the analysis of the whole transcriptome of a microbial community, can be further optimized and standardized, it will also become an interesting way to correlate the presence of specific microbes to specific biochemical or small molecules and metabolic functions.

Getting access to the actual activity of the microbial population as

a functional readout will support our understanding of the impacts of Microbiome modulation on the ecosystem but also of its mid- and long-term effects on human physiology.

5

Bioinformatics & Predictive tools

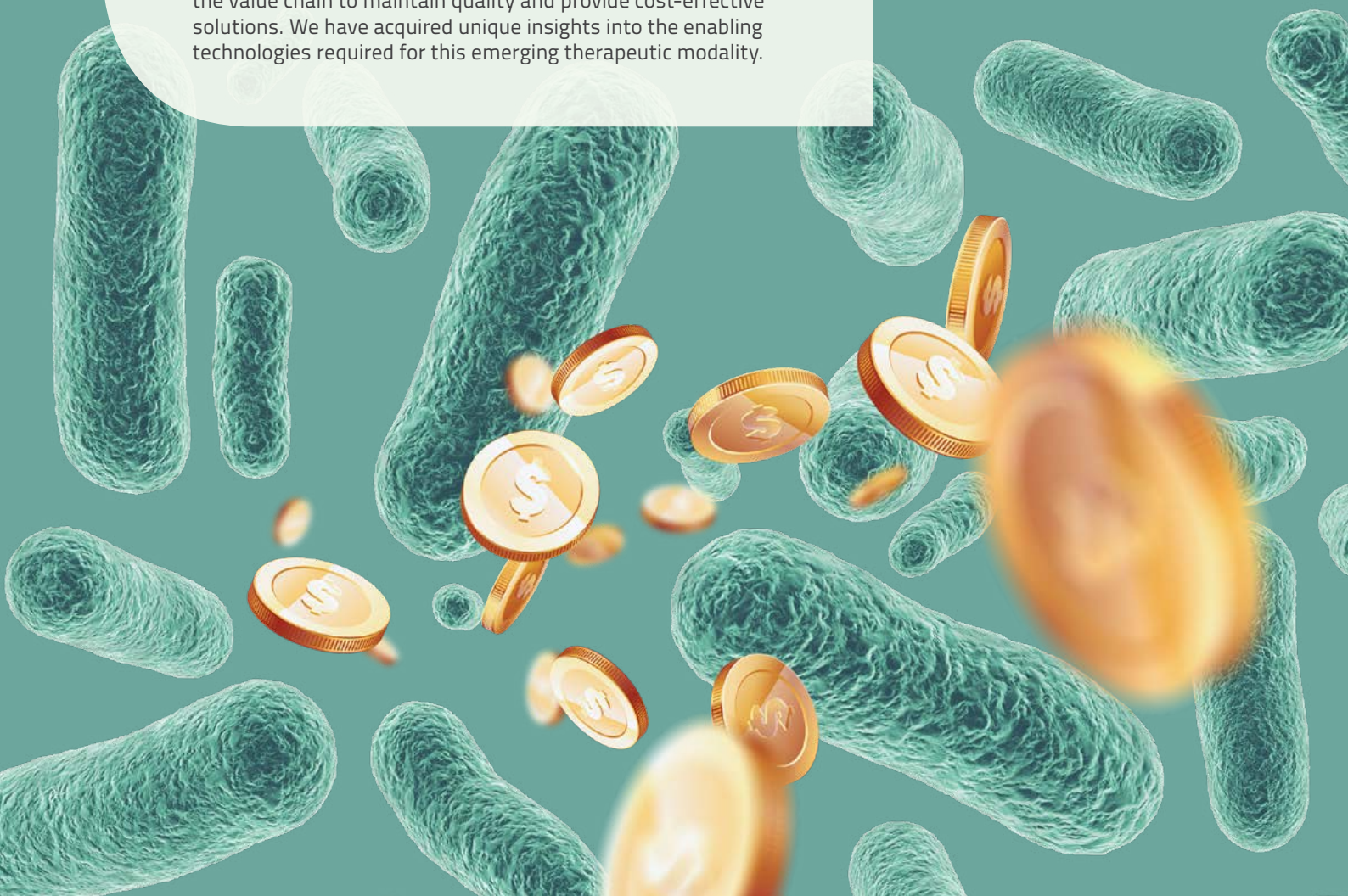
As discussed, Metagenomic studies highlighted that quantitative and/or qualitative microbial alterations occur in an increasing number of diseases. But these observations are still difficult to translate into operational insights. Bioinformatics tools (Data analysis, combination and correlation) could be of help to that end. The development and optimization of novel and powerful tools will support our understanding.

A crucial next step to develop specific Microbiome-based treatments

will be to develop bioinformatics tools for predicting the effects of external perturbations on complex microbial/viral populations. In silico analysis of the potential effects of a probiotic or a cocktail of phages for instance on the full Microbiome ecosystem will be of course of very high interest.

Conclusion

The Microbiome is a complex environment that is difficult to navigate. The Life Science business can provide high value to this emerging sector and M Ventures aims to invest strategically to that end. These novel therapies will require innovation throughout the value chain to maintain quality and provide cost-effective solutions. We have acquired unique insights into the enabling technologies required for this emerging therapeutic modality.



THE VEDANTA OPPOSITION: A HEALTHY SIGN OF A MATURING INDUSTRY

ANALYSIS BY CRAIG THOMSON | PATENT PARTNER, HGF LIMITED &

RACHEL FETCHES | INTELLECTUAL PROPERTY LITIGATION PARTNER, HGF LIMITED

NEITHER CRAIG NOR RACHEL HAVE EVER PROVIDED LEGAL ADVICE TO VEDANTA BIOSCIENCES

As we move towards marketing approval for microbiome therapeutics, the “value” of the microbiome industry is increasing exponentially. This will inevitably result in disputes between the leading competitors seeking to protect their key intellectual property (IP) rights, including patent monopoly rights. Whilst some may regret the ramping up of competitive behaviours, this should be seen as part of the healthy development of an increasingly commercially relevant industry.

Despite the increasing commercial value of patents in this area, Patent offices are relatively inexperienced at examining patent applications relating to therapeutic microbiome compositions. A granted patent provides a 20-year monopoly and to be valid, the invention must be new, inventive and sufficiently disclosed. Some early patents granted in this field may prove to have broader claims than is justified by their technical contribution or disclosure. Legal challenges are likely to narrow or knock-out such patents but at present, it is difficult for third-parties to be certain about their freedom to operate position.

A subtler benefit of such challenges is the effect they have on the way patents are examined. For example, when one strain of bacteria is demonstrated to treat IBD, examiners may have to consider if it is acceptable to claim the use of any member of the genus of that strain for treating any form of autoimmune disease? Decisions issued by the courts and patent offices in response to challenges from third parties will guide such considerations of examiners, leading to more consistent examination. Indeed, it is vital for the industry’s progress that we see consistently good decisions being made so that the microbiome patent landscape develops in a fair and commercially relevant manner.

The most significant challenge to patent rights in the therapeutic microbiome field to-date is the recently issued decision in the “Vedanta Opposition”. The patent (European Patent No. 2575835) was granted to the University of Tokyo (UoT) in October 2016. It relates to work derived from the Honda lab and was exclusively licensed to Vedanta Biosciences, Inc. The CEO of Vedanta was quoted in October 2016 as saying

“This European patent is an important addition to our global intellectual property portfolio”, “The claims issued put Vedanta in a very favorable position to commercialize drugs based on bacterial consortia in the second largest market in the world.”

The principle claim was granted for:

“A composition for use in a method of treating or preventing a disease selected from infectious disease, autoimmune disease or allergic disease in an individual, the composition comprising, as an active ingredient, bacteria comprising spore-forming bacteria belonging to *Clostridium* clusters IV and XIVa in combination, which combination induces proliferation or accumulation of transcription factor Foxp3-positive regulatory T cells in said individual.”

Within the 9-months after grant, the European Patent Office (EPO) received six Oppositions from Seres Therapeutics, Nestec and four anonymous parties. The Opposition hearing lasted three days and almost 130 documents were considered. Ultimately, the patent was maintained by the EPO’s Opposition Division (OD) but in an amended form, with a narrower claim scope; this decision has been appealed and it will likely be at least 2 years until the Appeal is heard.

The principal amended claim accepted by the OD is as follows (amendments shown as tracked changes).

“A composition for use in a method of treating or preventing a disease

selected from infectious disease autoimmune disease or allergic disease in an individual by inducing proliferation or accumulation of transcription factor Foxp3-positive regulatory T cells, the composition comprising, as an active ingredient, bacteria belonging to the genus *Clostridium* comprising spore-forming bacteria belonging to *Clostridium* clusters IV and XIVa in combination, which combination induces proliferation or accumulation of transcription factor Foxp3-positive regulatory T cells in said individual."



One of the most commercially interesting aspect of the proceedings was UoT's strategic decision to voluntarily remove "autoimmune disease" from the claims, leaving infectious disease and allergic disease. This means that UoT forfeited its granted protection for the treatment of autoimmune disease in Europe but also avoided public analysis of the patentability of that aspect of the claimed invention. One possible motivation could be the publication of a Poster Presentation that disclosed the colitis model data present in the patent. This may affect the novelty and inventiveness of claims for the treatment of autoimmune disease using the claimed bacteria. UoT have since filed two divisional patent applications, which are not published as yet. We expect, however, that one of these new filings claims the treatment of autoimmune disease, kicking the analysis of patentability of that aspect of the claimed invention into the long grass.

The definition of the bacteria in the claims was fiercely argued during the OP. UoT argued that the application from which the patent was derived merely refers to the genus *Clostridium* as an umbrella term that could mean any bacterium in the entire phylogenetically and taxonomically diverse group of bacterial strains within in the recited "clusters". The OD

was not convinced. Whilst they accepted that the term "genus" was ambiguous, they concluded that the application as filed must have been restricted to only bacteria belonging to the genus *Clostridium*. This forced UoT to amend the claims such that any non-*Clostridium* bacteria that are members of the recited clusters are unlikely to fall within the claims.

The OD then considered if there was sufficient evidence that the bacteria of the claims were capable of inducing the claimed T-cell response and thereby treating infectious and allergic diseases. The OD held that there was sufficient evidence. The data in the patent demonstrated that 46 strains of bacteria of the genus *Clostridium* were able to induce a strong Foxp3+-T-cell response in the colonic lamina of mice. Forty-one of the 46 strains were found to belong to one of the recited clusters and based on this, the OD concluded there was no reason to doubt that the bacteria of the claimed clusters were responsible for the observed T-cell response.

The patent contained data of both systemic and local effects in an allergic model following treatment with the claimed compositions. Consequently, the OD acknowledged that there was sufficient evidence to support the claim for the treatment of allergic disease. No evidence demonstrating effects on an infectious disease model were provided. It may be counter-intuitive that induction of a T-cell response would be useful for treating infectious disease. However, reference was made to a review suggesting that Treg induction can play a role in minimising deleterious effects of an immune response to infection. Consequently, induction of Tregs

can perform a role in broadly treating the effects of infectious disease. UoT were able to establish the T-cell response created by the claimed bacteria and on that basis the OD held that there were sufficient data to underpin a claim to the treatment of infectious disease.

The last element of the claim raises one of the wider difficulties for prospective patentees in the microbiome field. This is the fact that existing Fecal microbiota transplant (FMT) therapies inherently disclose the claimed bacteria of the recited clusters and so could have provided the claimed therapeutic effect, even if this had not been appreciated at the time. Valid inventions in the microbiome arena will be based on the recognition that the use of bacteria of the claimed clusters has a new therapeutic effect. The claims are therefore purpose-limited – that newly appreciated purpose must be part of the claims. In this case, the microbial composition is used to treat infectious and allergic diseases **“by inducing proliferation or accumulation of transcription factor Foxp3-positive regulatory T cells in said individual”**.

Why this limitation matters is because of the potential difficulties of enforcing second use claims of this type in the national courts. While granted centrally, a European patent is effectively a bundle of national rights. Third parties who want to “clear the way” can both centrally oppose as well as bring validity challenges in the national courts. Patentees, however, must enforce their patent against potential infringers (direct or indirect) in the relevant national court(s). For second use claims, there is a tension between the principle that people must be free to use known or “old” methods or products against the desire to incentivise patentees to invent new uses for known compositions in return for a monopoly right. Certainty as to liability for infringement for third parties is also important. However, for direct infringement, the legal test of what the relevant intention is to infringe a second use claim is not well defined. Where such claims have been considered, the national courts have differed in their approach. What is the position of a provider of FMT if their patient has an allergic disorder, would they infringe the claims as amended by the OD? Even if they were not found to directly infringe, might they be infringing indirectly by providing “the means essential”, i.e., the transplanted matter. As the value of the microbiome field increases, all of this will need to be litigated in the national Courts.

Where does the OD’s decision leave Vedanta commercially? UoT have appealed the decision, which has a suspensive effect. Three of the opponents have also appealed the decision. The patent family’s commercial value pivots on the relative importance of autoimmune treatments (not now covered in the patent) to infectious or allergic treatment (currently covered in the patent). It will be interesting to see how UoT proceed with seeking protection for compositions for treating autoimmune diseases in the newly filed divisional applications. The claims of the granted patent may be upheld as granted, as amended or restricted further during the Appeal proceedings. The Boards of Appeal are more rigorous than the OD, so UoT can expect a rough ride. Although the parties’ grounds for appeal have not been filed yet, it is clear that the patent may be vulnerable. The evidence of therapeutic benefit in relation to infectious disease seems to be the most difficult for UoT to substantiate. Does the patent disclose sufficient data to select those infectious diseases that

would have a net benefit from Treg induction via administration of the recited bacteria? A restriction of this disease to treating excessive inflammation caused by the immune responses to infection may be more acceptable. The amended claims are focused on the invention having clinical effect specifically via the induction of Foxp3-positive regulatory T cells, and through the use of bacteria of the genus *Clostridium* in the recited clusters. If the Opponents can show evidence that the therapeutic benefit demonstrated in the patent is not from selecting those specific bacteria, and/or that it is not the recited T-cell induction that provides the benefit, the patent would be vulnerable.

Monitoring further developments as this case moves through the EPO’s Appeal procedures and any national court litigation, is likely to reveal more insight into what the future holds for patent protection in the therapeutic Microbiome field.

FOUR LESSONS MICROBIOME-FOCUSED COMPANIES CAN LEARN AFTER UBIOME'S RECENT TROUBLES

ARTICLE BY KRISTINA CAMPBELL

It all started with the sudden news in late April of this year that the FBI had raided the offices of direct-to-consumer microbiome testing company uBiome. This occurred in response to consumer and private insurer reports of improper billing practices. While the investigation is still ongoing, the company—valued at \$600 million—subsequently saw the resignation of its co-founders Jessica Richman and Zac Apte and its interim CEO John Rakow, and the layoffs of many of its staff.

Heads always turn when a frontrunner in a small industry faces ire. And while it may have been a classic case in healthcare technology of “a problematic quest for growth that [resulted in] a lack of quality control/compliance”, the possibility still exists for outsiders to paint other microbiome-focused companies with the same brush.

Microbiome Times editors asked a series of experts from medicine, venture capital, and product development/marketing what lessons the rest of the microbiome industry can take away from the uBiome troubles. What follows are four points to take away from the situation, as companies take the opportunity to re-orient toward best practices in this relatively young industry.

1

Be open with investors and view them as partners.

Arnaud Autret, Principal in the Life Science fund at M Ventures, warns against companies coming up with schemes to impress its investors. He says, “Because main investors are typically highly specialized in their segment, the management of the company should see them as partners. They are used to [facing] technical challenges and will support the company to find the right way to deal with its product pipeline.” He continues, “Management must be transparent, and need[s] to deal [in] the earliest possible way with potential problems or opportunities. Clear communication is vital.”

Autret’s colleague Joey Mason, Head of the Life Science Fund at M Ventures, agrees. He reminds companies that “interests of the management and the investors are aligned – reaching key milestones and creating value. The company needs to build an open and transparent relationship with investors, not only prior to legal duties but throughout the course of the year.”

2

When it comes to gathering data on your product, don’t underestimate microbiome complexity.

Scientific evidence to back up a diagnostic or therapeutic is essential—and companies need enough evidence to convince stakeholders of efficacy and safety. But new levels of microbiome complexity are constantly being revealed, making it difficult to know if the data you have is enough.

Dr. Stephen O’Keefe, physician and microbiome researcher from the University of Pittsburgh Department of Medicine, has taken note of some of the twists and turns as the field has progressed in recent

years. He gives an example: “[One recent study from Michigan found] specific [gut microbiota] signatures for normality versus polyps versus [colon] cancer... they could take a stool sample and predict whether that person had polyps or cancer. It got very exciting. They actually

proposed that it could be used as a clinical test.”

But that turned out to be an incomplete story: O’Keefe noted that his own work in a different population, individuals native to Alaska—who happen to have an extremely high incidence of colon cancer—showed completely different microbial signatures of the disease. He says, “They’re all at very high risk, but the very high risk signature is not the same as the one identified in Michigan.” This showed that more data were needed before development of a clinical test.

O’Keefe cites another challenge that will undoubtedly crop up for some microbiome-focused companies: “We’re measuring bacteria mainly. What’s happening to viruses and fungi and how they all interact together as well? So, we’re only looking at part of the

question when it’s much more complex than that.”

He adds, “Everybody’s now wanting to measure microbiota and so therefore costs of analysis are remarkably cheap, but that’s not the same with viruses or fungi, so you’ve got to spend a lot of money if you want to include those measurements as well. Most people don’t do that at the moment. It has to come at some stage because you can’t just look at part of the picture.”

3

Think carefully about regulatory aspects and reimbursement, and be prepared to change the plan.

Mason and Autret advise companies to get into the nitty-gritty of regulation and reimbursement as soon as possible when developing a product. Says Mason, “The regulatory pathway is critical—especially in the healthcare segment. The company must have a clear vision of what actions will be required, especially regarding the geographic region in which a new product will be launched as regulations are often quite specific.”

Furthermore, says Mason, “Decision-making around regulatory status of products is evolving, as seen recently with the Food and Drug Administration (FDA) regarding the use of fecal microbiota transplant. Companies in this specific field have to be agile, maybe more than in any other space.”

According to Autret, “One important issue of the market entry strategy for medical technologies is to achieve reimbursement. The application process is time consuming and companies sometimes struggle to achieve this step in an efficient manner. Again, this is something that should be taken into consideration at the earliest [possible stage].”

Kevin Cain, an independent consultant in pharmaceutical commercial development, market planning, and marketing research, says the US medical billing system can be particularly challenging to navigate, with its system of ‘CPT codes’ acting as simplified means for doctors

to document patient procedures for the purposes of medical record-keeping. Cain says, “You have to get prepared for the market. So you either have to know going in you’re using an existing CPT code or you have to get a new one.”

“You decide, is the existing code enough to reimburse for what the product is, or the test is? Or are we substantially different that we want to get our own code? And then what you have to do is prepare to get to market,” says Cain. “That whole process, you have to figure out pre-market: what it’s going to take to be successful, what your costs are going to be, what you think you want to charge based on what efficacy you think you’ll have with your product.”

He adds, “Medical billing is an art more than a science a lot of times.”

4

Consider unmet medical needs when deciding how to position your product.

One of the risks in a relatively new field like microbiome science is to advance a microbiome-focused solution to something that may not actually be a problem to patients or medical professionals. Rather, Cain stresses the need to meet a medical need that already exists.

“You have to look at what is the standard practice today? Do they see a medical need for that product and the new technology? What medical need does it fit?” he advises. “There’s all those trade-offs and you have to figure out, how do you position that

product to be successful?”

Cain continues, “Something could look really great, but it depends on the condition in the market and what gets treated... a product could [experience] failure if it’s not a significant improvement compared to what’s on the market.”

THE PRODUCT DEVELOPMENT JOURNEY OF A SMALL BIOTECH AND IT'S COLLABORATION WITH A CDMO

SPONSORED ARTICLE BY RICHARD ELLIS, HEAD OF BD, BIOSE INDUSTRIE & CHRISTOPHER REYES, CEO, BLOOM SCIENCE

The relationship between Biose Industrie and Bloom Science makes for a laudable collaboration from two very different parts of world. From the peaceful town of Aurillac in the middle of the massif central mountains to San Diego, the Pacific Ocean and the sunny climate of Southern California.

Biose established itself as the world leader in the development of Live Biotherapeutics, when CEO Adrien Nivoliez, PhD, and his group committed the company to focus on becoming the go-to CDMO in this rapidly expanding field of drug development. In part, this decision was based on growing evidence and opportunity to benefit human health via microbiome modulation. This decision has led to rapid growth for the company, from 55 to 130 people in 2 years with very little space left in the car park! This expansion has also been driven by the establishment of Biose's leadership in a global network of companies in the emerging Microbiome therapeutics space.

With the advent of microbiome therapeutics and LBPs there has been interest from around the globe with the local Aurillac hotels and restaurants receiving guests from across Europe, the US and Asia. These clients are visiting the Biose plant to understand the opportunity to manufacture both Drug Substance and Drug Product at clinical and commercial levels at Biose's GMP facility.

On the other side of the globe, San Diego has long been recognized as one of the epicentres of Biotechnology worldwide with UC San Diego, the Scripps Research Institute, the Salk Institute and the Sanford Burnham Prebys Medical Discovery Institute all in close proximity to drive innovation and support the local biotech ecology. In particular, there's a phenomenally strong academic foundation for microbiome innovation such as the Gallo Lab in skin microbiome, Rob Knight for Microbial Bioinformatics and the Gilbert lab for Microbial Ecology.

Bloom Science is a Microbiome start up, very much in the Californian

mould, with its founders steeped in therapeutic start-up tradition. Bloom's CEO Chris Reyes, PhD, previously founded a Biogen spin-out focused on monoclonal antibody therapeutics targeting cancer stem cells which was acquired within two years of inception.

Bloom Science was founded with the remarkable science coming out of the lab of UCLA's Elaine Hsiao, PhD. Their discovery platform elucidates novel connections between clinical outcomes from the ketogenic diet, the microbiome, and critical disease pathways to accelerate drug discovery. Bloom's lead program, BL-001, is a Live Biotherapeutic that alters dietary metabolic processing resulting in alterations in key neurochemicals, and consequentially, decreased seizure burden in multiple preclinical models of epilepsy. BL-001 is being developed for orphan and refractory epilepsy. As the ketogenic diet was developed nearly 100 years ago to treat seizures and has demonstrated efficacy in treating a host of neurological conditions, Bloom is also expanding their pipeline to other neurological conditions and oncology.

An original approach to product development

Bloom decided to approach the development and optimisation of the production process for this new drug from a different angle. Using a top down approach, they wanted to de-risk their program as early as possible by establishing a manufacturing process for their "starter" strains. In biologics drug development, CMC has the highest risk for failure amongst preclinical activities. With an early focus on manufacturing, Bloom could de-risk their program, evaluate

the manufacturing feasibility of their specific starter species and finally expand their process to additional proprietary isolates.

Manufacturing feasibility study

Biose Industrie possesses in-depth process development knowledge around many different aerobic and anaerobic strains. Biose has applied their vast knowledge gained in fermenting a large range of strict anaerobes to the development of Bloom's species of interest. Bloom's lead drug is comprised of two anaerobic species that are particularly sensitive to oxygen and that both have unique process characteristics. Fortunately, Biose Industrie can apply their proprietary methodology to develop the process and media needed to encourage strong growth while also maintaining good viability. Having achieved successful results for fermentation and lyophilisation in the laboratory, a process was developed to enable scale up of the two species successfully. In parallel, Biose developed internally all the analytical methods to ensure the future release of the batch.

Isolating and Identifying novel strains

Once we had developed industrialisation pathways for both species, Bloom began the arduous task of acquiring the specific isolated strains. This has been achieved through a combination of internal isolation from stool donors and acquiring strains directly from other sources.

Manufacturability screening

Fermentation is a complex affair and the handling conditions for strict anaerobes makes the whole process more difficult. In addition, the

manufacturing process for inter species strains is often variable.

As such the next step is to perform small scale manufacturing tests on the candidate strains to help obtain the optimal solution for the scale-up. So, under anaerobic conditions we apply the following process:

1. Resuscitate the candidates
2. Inoculation
3. Select the best candidate / species to begin small scale fermentation
4. Concentration
5. Freeze-drying with successful cryoprotectant solutions
6. Measure viability throughout the process

Globally we've achieved excellent results using this approach in this and other projects and we will be looking to successful progress in tandem with Bloom Science with the production of clinical material shortly.

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PEOPLE OF THE MICROBIOME



Azitra, Inc. Appoints Mark Sampson, Ph.D. as Chief Scientific Officer

Azitra, Inc. announced the appointment of Mark Sampson, Ph.D. as Chief Scientific Officer. Travis Whitfill, Co-founder of Azitra, will serve as Executive Director of Advanced Technology, and will Chair Azitra's Scientific Advisory Board. Dr. Sampson most recently worked with Botanix Pharmaceuticals

where he was responsible for development of the preclinical strategy and clinical development plans for new antimicrobial indications. Previously, he served as VP of R&D at Realm Therapeutics, a company developing therapeutics for dermatology and immune-mediated diseases. Dr. Sampson held prior research roles at Sterilox Technologies and Warwick International Ltd. He earned an undergraduate degree and Ph.D. degree in Microbiology/Biochemistry from University of Aberdeen, UK.



Microbiome Therapeutics Innovation Group Announces New Chairman

The Microbiome Therapeutics Innovation Group (MTIG) announced the appointment of a new chairman, Ken Blount, Ph.D., Chief Scientific Officer of Rebiotix, Inc. Dr. Blount replaces Tom DesRosier, Esq., Chief Legal Officer of Seres Therapeutics, who served as MTIG Chairman since the coalition's inception in 2018. Tom DesRosier will continue to serve on the MTIG Board of Directors.



Synlogic Appoints Scott Plevy M.D. as Chief Scientific Officer

Dr. Plevy is a gastroenterologist who most recently served as VP, Gastroenterology Disease Area Leader and IL-23 Pathway Leader at Janssen Research & Development, LLC, after a successful career in academia. He has served as the lead investigator on multiple early-phase clinical trials, published over 100 referenced papers and articles on a breadth of topics from disease-specific targets to basic immunology and molecular biology, and performed

translational research to advance the understanding of novel immunologic interventions in inflammatory bowel disease, other inflammatory conditions, and microbiome-related diseases. He earned an M.D. degree and an A.B. in mathematics from Columbia University and carried out his residency training in internal medicine at Brigham and Women's Hospital in Boston.



Enterome Appoints Jan Fagerberg M.D., Ph.D. Chief Medical Officer and Catherine Mathis Pharm.D. Chief Development Officer

Jan Fagerberg is a board-certified clinical oncologist with more than 25 years of experience in cancer drug development. He gained an M.D. and a Ph.D. (in immuno-oncology) from the Karolinska Institute and subsequently spent ten years in oncology practice and academic translational immuno-oncology research at the Karolinska Hospital. He joined Roche in 1999, ultimately serving as Therapeutic Area Expert Oncology. After Roche, Dr. Fagerberg served as the Medical Director at TopoTarget A/S and as the SVP and CMO at Micromet A/G. After this, he served as VP, Global Development and Managing Director at Amgen Research GmbH, and subsequently as CMO at SOTIO a.s. Dr. Fagerberg has published more than 60 articles related to immuno-oncology/oncology in peer-reviewed journals.



Catherine Mathis has over 25 years of experience in clinical research and regulatory affairs in the pharmaceutical and biotech industries. Prior to joining Enterome, Ms. Mathis served as COO and Head of Regulatory Affairs at ElsaLys Biotech. She also served for nearly 20 years as Head of Regulatory Affairs at Transgene, a French biotech company developing virus-based immunotherapies against cancers, and before this was at Sanofi-Pasteur and Ipsen. Ms. Mathis gained a Pharm.D. and then earned a Master's degree in applied and basic toxicology from the Paris Diderot University.

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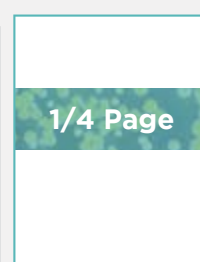
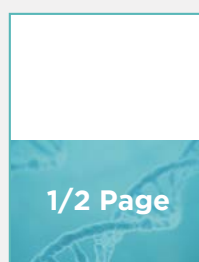
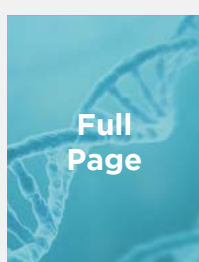
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