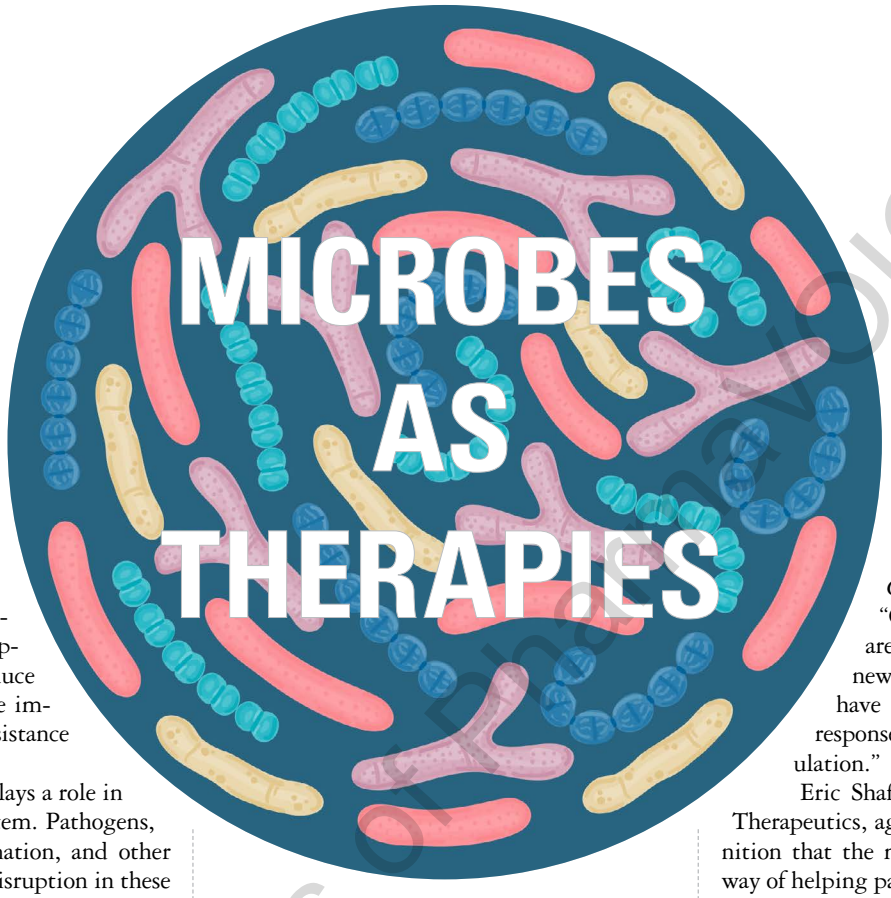
By Denise Myshko

MICROBES AS THERAPIES



Trillions of microbes — including bacteria, fungi, and viruses — call us home. These bacteria are on our skin, in our mouth, respiratory tract, urinary tract, and gut. These microbes are essential to human health, helping us to digest food, produce certain vitamins, regulate the immune system, and provide resistance against infectious pathogens.

In fact, the microbiome plays a role in just about every biologic system. Pathogens, antibiotic use, diet, inflammation, and other forces can cause dysbiosis, a disruption in these microbial ecosystems, which can lead to or perpetuate disease.

Interest in and understanding of the human microbiome and the implications for medicine have exploded, opening up a new paradigm of therapy — microbiota-based drug therapies, living therapies that are made up of microbes.

"Microbial biology in the intestine and in other parts of the body influences all of the major systems in the human body," says Bernat Olle, Ph.D., co-founder and CEO of Vedanta Biosciences, a clinical-stage company developing a new category of therapies based on human microbiota-derived bacteria. "We and other groups are showing that the interactions between microbes and different systems in the body impact many areas of biology."

There is a large amount of private and public research being conducted in the microbiome space. Large governmental research initiatives, including the Human Microbiome Project in the United States and the MetaHIT Project in Europe began a decade ago with the mission of generating resources that would enable the comprehensive characterization of the human microbiome and analysis of its role in human health and disease.

Now large biopharmaceutical companies and start-up companies alike are researching and developing therapies based on this understanding. The first of these research projects is looking to treat *Clostridium difficile* and other hard-to-treat infections. As the understanding of the microbiome has grown, the potential application has expanded to include GI inflammation, women's health, and even neurological indications and cancer.

WHILE MICROBIOME RESEARCH IS STILL IN ITS INFANCY, INTEREST AND INVESTMENT IN THIS FIELD OF RESEARCH HAS GROWN QUICKLY. EFFORTS ARE ONGOING TO STUDY HOW CHANGES IN THE HUMAN MICROBIOME CAN IMPACT OUR HEALTH.

"My anticipation is that there will be a large and growing data set about how microbes influence health and disease in many different disease areas," says Karim Dabbagh, Ph.D., CEO of Second Genome, a preclinical stage biotechnology company with a mission to redefine disease in the context of microbiome medicine.

"Our expectation is that the first approval will be for *C. diff* infections," he says. "Once the regulatory pathways are elucidated, this will open up new ways to treat diseases that have only had partial therapeutic response for the general patient population."

Eric Shaff, president and CEO, Seres Therapeutics, agrees there's continued recognition that the microbiome could be a novel way of helping patients in a variety of different indications. Seres Therapeutics is developing biological drugs that are designed to treat disease by restoring the function of a dysbiotic microbiome.

"We will continue to see momentum and exploration of the microbiome," he says. "The microbiome could represent a new opportunity to help patients and we are seeing resources from industry and academia continue to flow into these opportunities."

Unmet medical needs are driving research in the microbiome, says Ken Blount, Ph.D., chief scientific officer, Rebiotix, a Ferring Company, and a late-stage clinical microbiome company focused on harnessing the power of the human microbiome.

"There are a number of medical concerns or diseases that aren't adequately addressed with current standards of care, and the scientific field and drug developers are recognizing that resident microbiota are likely to be key drivers of some of these unmet medical needs," he says.

Even though microbiome research is still in early stages, there is growing confidence in the biology, says David Donabedian, Ph.D., co-founder and CEO, Axial Biotherapeutics, a biotechnology company building a class

FAST FACT

IN 2018 ALONE, MORE THAN 2,400 CLINICAL TRIALS WERE TESTING THERAPIES BASED ON MICROBIOME SCIENCE COMPARED WITH 1,600 THE PREVIOUS YEAR.

Source: Seventure Partners, a French VC investor

of gut-targeting therapeutics for neurodegenerative diseases and neurodevelopmental disorders.

The potential seems unlimited due to the millions of microbes that live on our skin, inside our gut, and everywhere in the human body, says Erin Mistry, senior managing director, head of value, access and HEOR, Syneos Health Consulting.

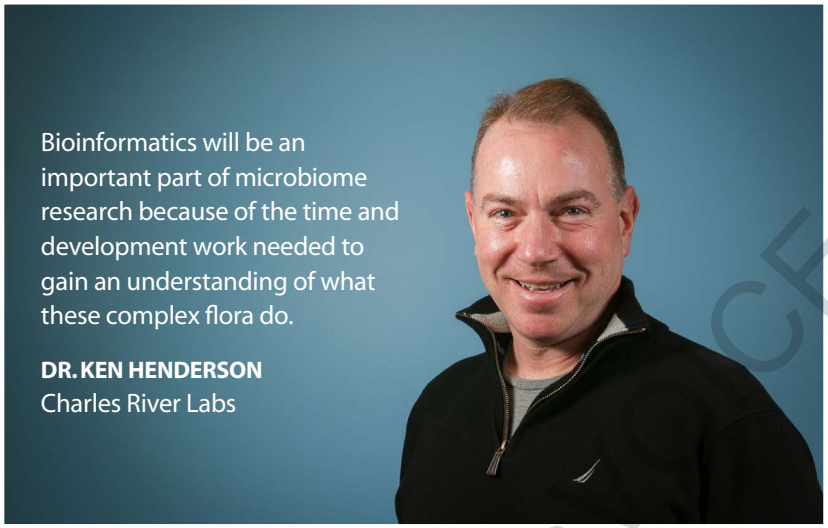
"The microbiome has been linked to a variety of conditions, ranging from inflammatory bowel disease to diabetes, multiple sclerosis, autism, cancer, and AIDS, which in turn has created an explosion in microbiome research, she says. "The microbiome therapeutic pipeline is growing, with the gut microbiome leading the way in research although oncology indications are gaining traction."

Ms. Mistry says companies investigating this new frontier will benefit from careful attention to maintaining collaborative interactions with the regulatory bodies regarding trial design and regulatory pathways, and consideration of commercialization challenges early on — including thorough market landscape assessment, education, and evidence needs of multiple stakeholders — to help prepare physicians, patients, and payers for this new category of therapy.

Ken Henderson, Ph.D., senior director of laboratory scientific diagnostic services at Charles River Labs, says his company has seen an up tick in proposals for studies related to the microbiome and live flora.

"Different researchers are targeting the microbiome to see how it influences inflammatory bowel disease," he says. "There's been great progress in understanding which bacteria increase or decrease in individuals who have inflammatory bowel disease, as well as what phage diversity might be present."

In the future, he says, bioinformatics will play an important part in microbiome research because of the time and development necessary to gain an understanding of what these complex flora can do.



The Microbiome and Infections

The microbiota in our gut, which includes largely bacteria, some yeast, and some viruses, are critical for healthy function. They have been recognized for modulating disease and wellness, and when they become imbalanced, depleted, or disrupted it can lead to various diseases.

Microbiome-based therapies for *C. difficile* infection are expected to pave the way, ushering into the clinic a new class of therapies and treatment approaches. *C. difficile* is a bacterium that can cause symptoms ranging from diarrhea to life-threatening inflammation of the colon. This is one of the top three most urgent antibiotic-resistant bacterial threats in the United States, according to the Centers for Disease Control. It is the leading cause of U.S. hospital-acquired infection and is responsible for about 29,000 deaths each year.

The current standard of care is to treat with antibiotics. While antibiotic treatment may resolve the acute infection, it can worsen dysbiosis in individuals, potentially making patients more susceptible to a recurrence of *C. difficile*.

Fecal transplants — transferring fecal matter from a healthy person — has been used for decades by doctors, and the practice has increased over the last five years after a study was published in the New England Journal of Medicine showed the treatment addressed the infection and restored the microbiome. But fecal transplants are not without risk. In June of this year, one person died and another became ill after a transplant.

Several biopharma companies are researching and developing other methods for delivering healthy bacteria to treat *C. difficile*, although these approaches also have their challenges.

Seres, for example, is continuing with its lead program, SER-109, for recurrent *C. difficile.* SER-109, an oral, donor-derived microbiome therapeutic candidate, failed to meet clinical endpoints in a Phase II trial in 2016. The company remains committed to the research. Seres began a Phase III trial in 2017 and continues to enroll patients. The company expects top-line study results in early 2020.

"With SER-109, we seek to repopulate the ecosystem with a healthy consortia of bacteria, which outcompetes the *C. diff* toxin and doesn't allow *C. diff* to take hold and cause a recurrence of infection," Mr. Shaff says.

The majority of the microbes in the gut are commensal, mutualistic, good microbes, ones that do important things with respect to overall health," says Mathew Henn, Ph.D., executive VP, microbiome R&D, at Seres Therapeutics.

"We firmly believe that the therapeutic modality that can have the greatest impact on human health is actually a consortia of bacteria," he says.

Seres is also conducting a Phase II trial of SER-287, an oral, donor-derived microbiome therapeutic candidate designed to normalize the gastrointestinal microbiome of those with ulcerative colitis. This trial, initiated in December 2018, is expected to enroll 201 patients with mild-to-moderate ulcerative colitis. This study could serve as one of two pivotal trials to enable a BLA submission. Seres expects to report study top-line results in the third quarter of 2020.

For this indication, the company is looking at a class of bacteria known as firmicutes, which are believed to be important in the health of the gastrointestinal tract, the integrity of its epithelial lining, and the modulation of the immune system. "Half of our immune system lines our GI tract," says Kevin Horgan, M.D., chief medical officer, Seres Therapeutics.

He says a Phase Ib study provided en-

The microbiome could represent a new opportunity to help patients, and we are seeing resources from industry and academia continue.

ERIC SHAFF
Seres Therapeutics

couraging results and achieved a statistically significant outcome with respect to complete remission. "In particular, this study showed that our microbiome therapeutic takes up residence in the GI tract, what we call engraftment, and that this may be a predictor of its clinical efficacy."

Rebotix is another company focused on developing microbiome-based therapies for treating gastrointestinal infections, including *C. difficile*, inflammatory bowel disease, and liver disease. The company's lead product is RBX2660, which is an enema formulation currently in Phase III clinical development for the reduction of recurrent *C. difficile* infection. RBX2660 has been granted FDA fast track, orphan, and breakthrough status designations.

A Phase II study published last year compared two doses of RBX2660 with placebo. "During this study, we saw a benefit of RBX2660 compared with placebo; we also found there was no difference between one or two doses, so one dose was sufficient to provide clinical benefit," Dr. Blount says.

The company also recently completed an analysis of a larger open-label trial where 79% of those patients were recurrence free after eight weeks.

To have an effective immune response to different pathogens and cancers requires a balance of both immune stimulation and immune suppression.

DR. MATHEW HENN
Seres Therapeutics

Additionally, the company recently completed a Phase I trial of RBX7455, an oral capsule formulation for the reduction of recurrent *C. diff* infection.

Vedanta is another company researching microbiome-based therapies for *C. difficile* infection, in addition to programs targeting cancer, allergy, autoimmune disease, and multi-drug resistant organisms. The company is conducting a Phase II study of its lead therapy, an oral live biotherapeutic product for recurrent *C. difficile* infection. The multi-center, randomized, double-blind, placebo-controlled study is designed to evaluate the safety and efficacy of two doses of VE303 compared with placebo in patients with rCDI. The study is expected to enroll up to 146 patients, and the primary endpoint will be the prevention of infection recurrence at eight weeks.

Dr. Olle says Vedanta is able to bypass the limitations of fecal transplant by giving patients a product that is not directly harvested from feces, but manufactured from pure clonal cell banks that are synthetically grown through fermentation and then mixed together in a capsule. "The bacteria are live and viable, but they come from pure cell banks and they always have the same composition and activity," he says.

Vedanta's pipeline also includes the recently announced Phase I study of VE202 with Janssen Biotech for inflammatory bowel disease. The study is enrolling up to 160 healthy volunteers in two locations in Europe. VE202 consists of clonal human commensal bacteria strains selected for their ability to impact the number and activity of regulatory T cells in the gut mucosa.

The Microbiome and The Immune System

Over the last few years, research has shown that the majority of the immune system cells are located in the gut. We now know that bacteria educate our immune system, and a diverse microbiome is critical for helping the immune system balance reaction and tolerance.

Research in the microbiome field has been groundbreaking in immunology because traditionally immunologists studied the thymus, Dr. Olle says. "We have become more knowledgeable about what happens in the gut, and specifically the community of 1 to 10 trillion different bacteria, which are patrolled by cells of the immune system. It's become clear that the gut is the key interface in the human body to understand how the immune system works."

Dr. Olle says this understanding has opened up a new way of looking at allergy, autoimmune diseases, and even cancer. "We're taking these learnings and developing medicines based on live bacteria, focusing on some of the key players orchestrating those interactions."

One of the company's research programs has identified gut bacteria that can induce immunoregulatory responses and protect against allergic intestinal disease. This research and independent findings show that these immunoregulatory bacteria can potentially have protective effects against a range of food allergens, suggesting they work via antigen-agnostic mechanisms.

Vedanta has recently begun enrollment of a Phase Ib/II clinical study of VE416 in adults and adolescents with a history of peanut allergy. The trial will explore VE416 as a monotherapy, and in combination with an oral peanut immunotherapy, over the course of several months. The randomized, double-blind, placebo-controlled trial is slated to enroll up to 40 patients who are 12 years of age and older. The primary endpoints are safety of VE416 and the amount of peanut protein tolerated during a double-blind, placebo-controlled food challenge following the treatment.

Second Genome is another company leveraging its drug discovery platform to address immunology and microbiology. The company was founded in 2010 based on technology that was developed at Lawrence Berkeley labs to analyze microbial composition in environmental samples. Over the years, the company has evolved to apply its technology to understand microbial composition and function in the context of human health and disease and translate that into therapeutic applications.

"We can analyze at a very high granularity level the microbial composition at a strain level to know the exact identity of the strains that are involved in the context of a human contrast, which is healthy versus disease," Dr.

FAST FACT

THE GLOBAL HUMAN MICROBIOME THERAPEUTICS MARKET IS ESTIMATED TO REACH $1 BILLION IN 2024, A CAGR OF 60.2% BETWEEN 2019 AND 2024.

Source: Visiongain

Dabbagh says. "We are investigating medical biological hypotheses where we can make a difference in the microbial composition and/or function."

Second Genome is developing protein and peptide drugs based on the hypothesis that microbial difference is functionally irrelevant.

"We are taking the microbial information and boiling it down to very specific molecules that are made by the microbes that can become drugs," Dr. Dabbagh says. "We are targeting those biological pathways' very specific receptors, enzymes, etc."

The company's lead product is SG-2-0776, which is being studied for IBD, including Crohn's and colitis. The company analyzed eight inflammatory bowel disease studies, two of which were done in collaboration with academic groups, to look for microbes that differentially present in healthy versus IBD patients.

"We hypothesized that microbes performed beneficial functions toward the immune system and toward the gastrointestinal barrier functionality itself, which tends to be disrupted in IBD patients," Dr. Dabbagh says. "We were able to then demonstrate that when we put these specific strains into animal models of GI inflammation they were able to restore the barrier function itself, basically attenuating the inflammation and repairing the lining of the gastrointestinal tract."

Company researchers then identified proteins and peptides that were secreted by these microbes that had effect on epithelial cells in the gastrointestinal tract.

"We were able to correct the biological defect that takes place, and that gave us a starting point for a drug discovery program."

This research of the gut's role in the immune system has important implications for cancer treatment as well. Over the last few years, the approval of checkpoint inhibitors such as Keytruda, Opdivo, and Yervoy have been a tremendous advance for patients. These drugs block the proteins that stop the immune system from attacking the cancer cells.

But recent research has shown that the estimated percentages of patients who are eligible for and who respond to checkpoint inhibitors are modest.

"The microbiome can have a meaningful impact both on immune stimulation as well as immune suppression," Dr. Henn of Seres says. "To have an effective immune response to different pathogens and to cancers requires a balance of both immune stimulation and immune suppression."

Vedanta also is looking at how the microbiome modulation can address cancer. Vedanta's research has shown that specific bacteria, either alone or in combination with approved checkpoint inhibitors, potentiate the immune response against tumors. "There seems to be a synergistic effect," Dr. Olle says.

Vedanta is working in collaboration to evaluate Bristol-Myers Squibb's immune checkpoint inhibitor Opdivo (nivolumab) in combination with VE800, a rationally defined human bacterial consortium, in patients with advanced or metastatic cancers. The company is beginning Phase I trials in the fourth quarter of this year in three cancer indications.

Another company in this space is Synlogic, a clinical-stage company applying synthetic biology to beneficial microbes to develop novel, living medicines.

"We are applying engineering principles and building circuits to perform certain human biology functions," says Scott Plevy, M.D., chief scientific officer, Synlogic.

Synlogic is designing microbiome-based medicines that work to detoxify dietary substances in patients with genetic defects of metabolism, boost a patient's immune response, and promote the body's ability to detect and destroy cancer cells.

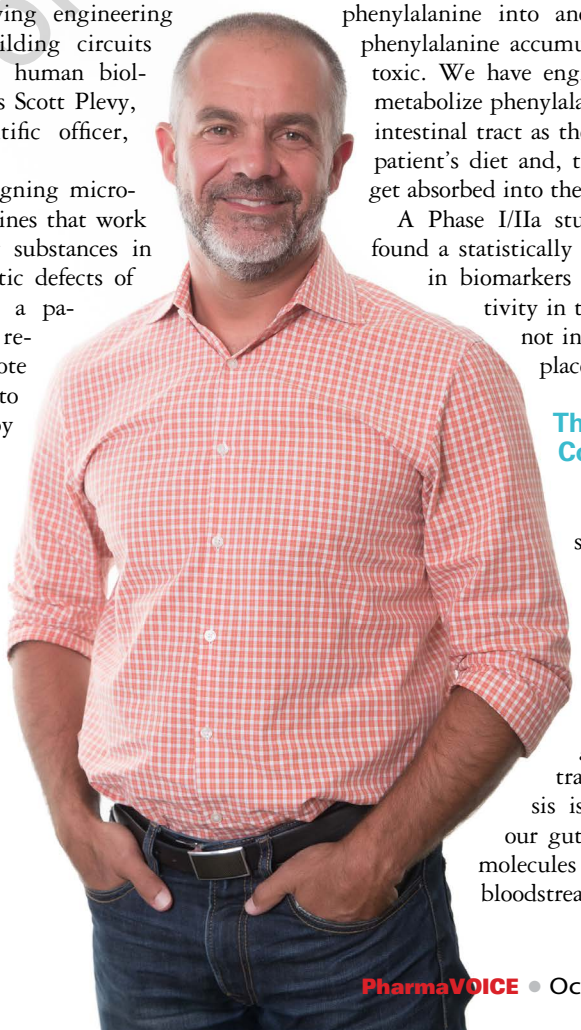
In August, Synlogic received FDA clearance of an IND application for SYNB1891. SYNB1891 is designed to activate the STING pathway, specifically in antigen presenting cells that naturally sense and engulf bacteria. Animal studies have shown this results in local ablation of cancer cells as well targeting metastases that spread throughout the body.

Synlogic has a clinical collaboration with Roche for the evaluation of SYNB1891 in combination with Genentech's PD-L1-blocking checkpoint inhibitor Tecentriq (atezolizumab) in patients with advanced solid tumors. Synlogic is initiating an open-label Phase I clinical trial to evaluate the candidate as a monotherapy and a combination treatment with atezolizumab.

Another Synlogic clinical program is a therapeutic to treat phenylketonuria (PKU), a rare genetic disorder estimated to affect 16,500 people in the United States. People with PKU are unable to process phenylalanine (Phe), an essential amino acid that enters the body as a component of dietary protein. PKU requires adherence to a strict diet to prevent the toxic accumulation of Phe in the blood and brain, which can impact neurological function.

"This is a great example of a dietary amino acid that's a part of the proteins that we eat," Dr. Plevy says. "Because these patients have a genetic defect in the enzyme that turns phenylalanine into another amino acid, phenylalanine accumulates and becomes toxic. We have engineered bacteria to metabolize phenylalanine in the gastrointestinal tract as they are exposed to a patient's diet and, therefore, it doesn't get absorbed into the blood."

A Phase I/IIa study of SYNB1618 found a statistically significant increase in biomarkers of SYNB1618 activity in treated subjects but not in those treated with placebo.



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DR. KARIM DABBAGH
Second Genome

The Gut-Brain Connection

Industry researchers say close to 30% of molecules that circulate in our bodies are in one way or another made by microbes in our gastrointestinal tract. The hypothesis is that microbes in our gut make a variety of molecules that end up in the bloodstream and can actually

cross into the brain and influence various activities in the central nervous system.

Dr. Dabbagh of Second Genome says studies have shown antimicrobial therapy with antibiotics and replacing the microflora of a patient with that of a healthy individual could actually improve symptoms in some autistic children, at least temporarily. This, he says, has led to the idea that microflora seems to potentially be implicated in many autism spectrum disorders.

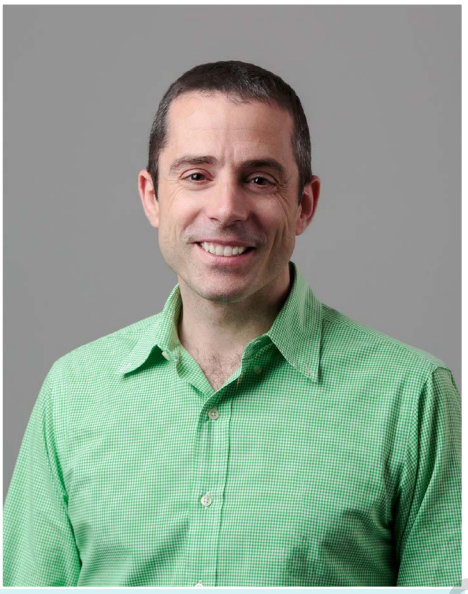
Second Genome is working with Stanford University School of Medicine and Oregon State University to develop a platform for discovery and validation of key metabolites produced by microbes in CNS disorders. In particular, the teams will study the relationship between the human microbiome and autism spectrum disorders.

Second Genome is conducting clinical, genetic, and metabolomic analyses of the collected samples of pairs of siblings with one neuro-typic (non-ASD) child and one ASD child. The company will then use its PhyloChip technology platform to differentiate bacterial strains depleted in the ASD gut to ultimately discover novel bacterial strains and metabolites found in neuro-typic children that are greatly reduced in their sibling with ASD. Such metabolites could pass the blood-brain barrier and may affect the central nervous system and modulate behavior. Stanford University School of Medicine will then conduct in vivo experiments in animals to test the effect of these identified metabolites in CNS disease.

Axial Biotherapeutics is also studying the gut-brain connection, and company researchers believe that altered microbial metabolites may drive GI and behavioral symptoms in ASD subjects.

Axial's scientific co-founder, Sarkis Mazmanian, Ph.D., and researchers from the California Institute of Technology discovered that human gut bacteria potentiate numerous Parkinson's disease (PD)-like features in mouse models of disease and suggest that alterations in the human microbiome may be a novel and causal risk factor for PD. In a PD mouse model, gut bacteria were shown to promote hallmark disease processes, including motor and gastrointestinal dysfunction and inflammation of the nervous system, which resulted in alpha synuclein aggregation (both in the gut and brain). Colonization with microbiota from PD patients in PD mice enhanced physical (motor and GI) impairments compared with microbiota transplants from healthy human donors.

Dr. Donabedian says the company has developed gut-retentive, small molecules that can be taken orally to prevent the aggregation of alpha synuclein. "In addition to these molecules, we have developed a proprietary delivery system to deliver these molecules to the lower part of the GI tract because that's where the bugs reside," he says.



> Microbial biology in the intestine, as well as other surfaces of the body, influences all of the major systems in the human body. This has a very important implication for medicine.
>
> **DR. BERNAT OLLE**
> Vedanta Biosciences

The company is conducting a safety study in a subset of Parkinson's patients who were prescreened based on their microbiome.

In autism, Axial has developed a gut-selective, nonbacterial-based therapy that has shown in preclinical models the ability to improve barrier integrity and ameliorate core and noncore behavioral symptoms associated with ASD.

Dr. Donabedian says about 40% to 50% of children with autism have some form of gastrointestinal distress, such as constipation, diarrhea, abdominal pain, nausea, and vomiting. "In addition, epidemiological studies have noted differences in the microbiomes of autistic children compared with neurotypically developing children," he says. "Some autistic children tended to lack diversity and abundance in their microbiome. Many of these children also had what's called a leaky gut, which allows oxygen into the gut that may potentially further perturb the microbiome."

Axial conducted preclinical studies in mouse models of autism and found that these mice had elevated levels of certain metabolites that were produced by particular microbes. One metabolite in particular is called 4-ethylphenylsulfate (4-EPS). Axial initiated a cross-sectional study to determine the prevalence of 4-EPS in ASD subjects, and found a subset of ASD children had markedly increased levels versus matched healthy controls.

Axial's therapeutic AB-2004 is focused on research demonstrating that reducing systemic and brain exposure to problematic microbial metabolites may improve core and non-core ASD symptoms related to behavior and gut health. AB-2004 is a drug candidate that has demonstrated, in animal models, the ability to repair leaky gut and improve repetitive behavior and anxiety.

In July, Axial began dosing in a Phase Ib/IIa clinical trial of AB-2004. The trial is designed to examine the safety and tolerability of AB-2004 in up to 25 male adolescent subjects with ASD. PV

Challenges of Researching the Microbiome

- **Clinical Development and Trial Design Considerations.** Challenges in microbiome therapeutics R&D stem from the fact that a person's microbiome is changeable when perturbed and the precise mix of bacteria being administered therapeutically cannot be known.
- **Regulatory Wayfinding.** Regulatory frameworks have not caught up to microbiome-based therapies, and it is not yet clear through which route these therapies will be evaluated, although the FDA has issued draft guidance and signaled a willingness to discuss with the industry. Whether this results in the creation of a new regulatory pathway for microbiome-based therapies within the regulatory agencies remains to be seen.
- **Commercial Considerations.** Because microbiome-based therapies constitute an entirely new therapeutic category, there is much uncertainty about the best paths to commercialization. Media attention and hype may create unrealistic expectations among physicians, patients, and payers. Unrealistic expectations coupled with a fear of inadequate efficacy or