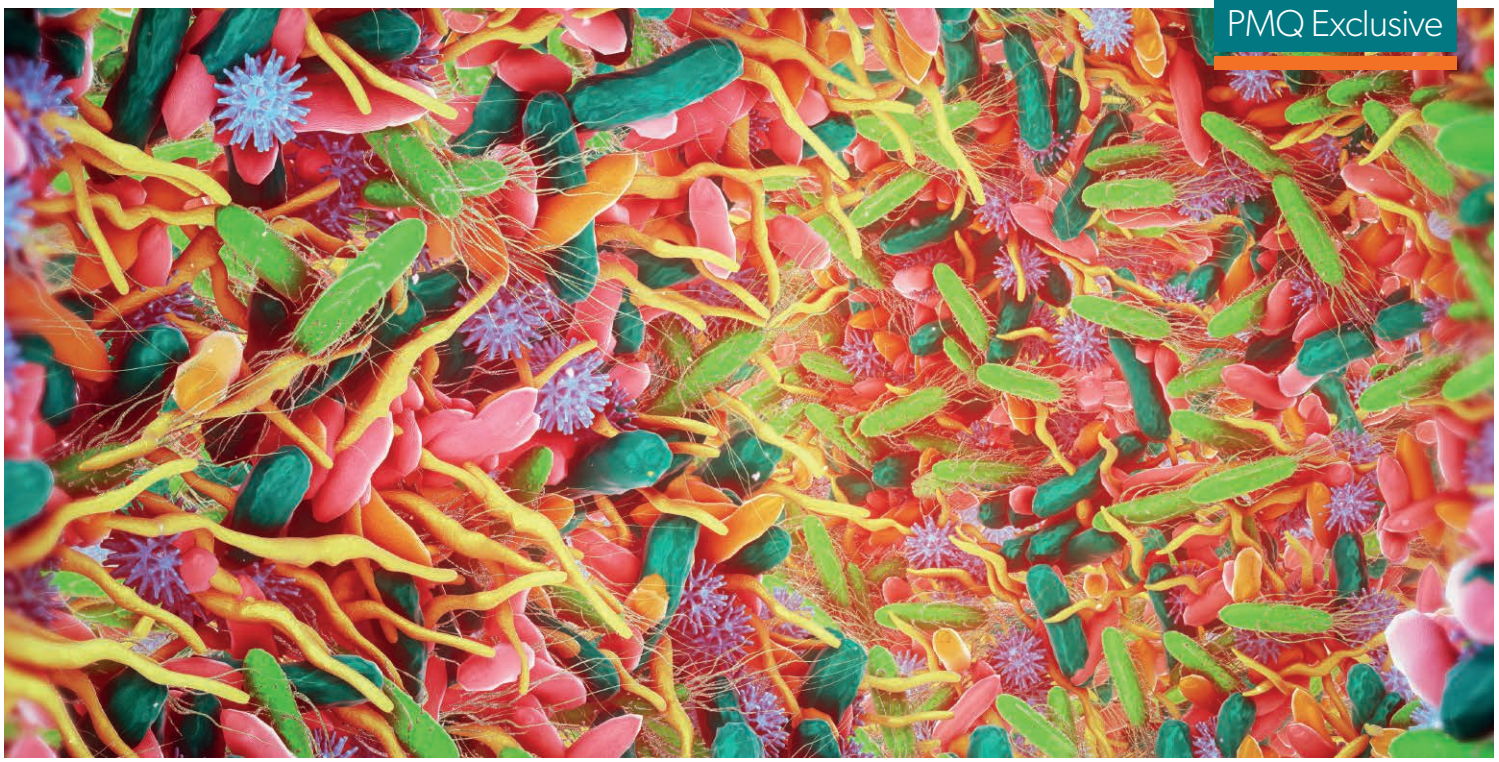


Microbiome Therapeutics Find Their Footing in Cancer

Based on their success treating *C. difficile*, drugmakers are using different strategies to develop microbiome cancer therapies, including using genomics tools to home in on patient-specific strategies.

By Caroline Hopkins

JENNIFER WARGO's rationale for focusing her oncology career on the microbiome boils down to the numbers. "We're only one percent human, when it comes to our total genomic content," she said. "We're actually 99 percent microbial."

Wargo, the director of MD Anderson Cancer Center's Platform for Innovative Microbiome and Translational Research, or PRIME-TR, has been pushing the field to focus more on the ubiquitous microbes. Now, drug developers are starting to become interested.

Microbiome therapeutics are expected to grow to a $2 billion market by 2030. From big pharmaceutical firms to small biotech startups and the investors backing them, industry players are increasingly probing the microbiome's potential to diagnose, prevent, and treat cancer. At the moment, however, industry is testing a variety of strategies for advancing microbiome-based cancer treatments, raising questions around which patients should get these therapies and how best to administer them. And, at present, there's no consensus as to whether microbiome therapeutics are better suited for an individualized, precision medicine approach, or a broader, all-comers approach.

A new modality emerges

A large and growing body of mostly academic research demonstrates how the microbes in the gut, and in the tumor itself, can play a role in cancer development and patients' responses to therapy. But only recently has this research begun to stir the interest of drugmakers and regulators.

"This year was especially good for our field," said Nadim Ajami, the executive director of scientific research at MD Anderson's PRIME-TR.

In November 2022, the US Food and Drug Administration approved a live fecal microbiota therapeutic, a product of Ferring Pharmaceuticals dubbed Rebyota, for patients with recurrent *Clostridioides difficile* infections. "That was a big win, and our field needed a big win," Ajami said.

And in April, the FDA approved Seres Therapeutics' Vowst for preventing recurrent *C. difficile*. The oral capsule contains a consortium of bacteria intended for patients with recurrent infections. The success of the live fecal transplant

and the oral microbiome therapeutic have bolstered oncologists' and drugmakers' confidence that bacteria-filled capsules could similarly benefit cancer patients.

"There's precedent now [and] the FDA is aware of these products," Ajami said. "And they've been working with investigators around the world on investigational new drugs and trials."

By one estimate, out of 388 microbiome therapeutics in development globally as of March, 65 are oncology related and nine have entered clinical trials. In many of these development programs, drugmakers are combining microbiome therapeutics with existing cancer therapies such as immune checkpoint inhibitors, hoping to improve patients' responses to treatment or stave off recurrence.

Academic researchers have long been testing preclinically how the gut microbiome can be modulated to bolster immunotherapy response, but the approach only recently demonstrated efficacy in human trials, as detailed in two studies published in *Science* in early 2021. In one of those clinical studies, researchers at the Sheba Medical Center in Israel showed the feasibility of performing fecal microbiome transplants in melanoma patients whose cancers were refractory to immunotherapy and converting non-responders into responders.

In the other 2021 study, researchers at the University of Pittsburgh Medical Center and the US National Institutes of Health took fecal matter from seven advanced melanoma patients whose cancers had responded exceptionally well to Merck's Keytruda (pembrolizumab) and transplanted that fecal matter into 15 patients whose melanoma hadn't responded to the immunotherapy. In this trial, 40 percent of the patients who hadn't responded previously either began responding to Keytruda or experienced durable stable disease.

The science of microbiome therapeutics has "really bloomed in cancer and the oncology field because of these *Science* papers," said Lee Swem, CSO of a microbiome company called Federation Bio. Launched in 2018, Federation Bio is collaborating with MD Anderson to translate the success of the fecal transplant approach into an oral therapeutic.

Proponents of precision

While many of the industry players who've recently entered the microbiome space are trying to improve patients' immunotherapy responses with a broader, all-comers approach, others are using a precision strategy.

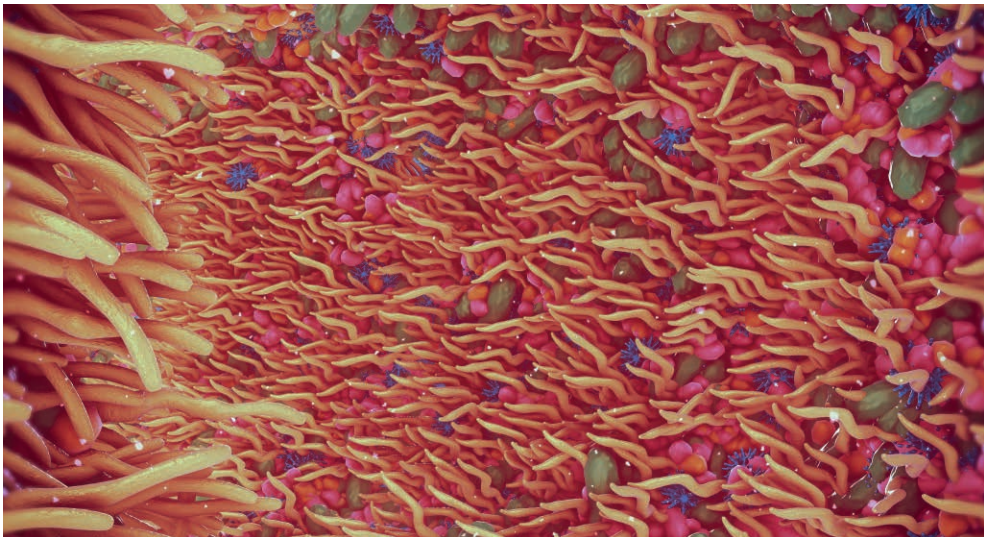
The French biotech Everimmune, for example, announced early this year it would launch a first-in-human trial of its microbiome therapeutic, Oncobax AK, to improve immunotherapy responses among patients with non-small cell lung cancer and renal cell carcinoma. Everimmune is homing in on a single species of bacteria, *Akkermansia*, and administering it only to patients with confirmed *Akkermansia* deficiencies.

In the trial, the firm is performing PCR-based sequencing of patients' baseline stool samples and giving only those with a bacterial deficiency the Oncobax AK *Akkermansia* capsule, after which they receive Keytruda or Bristol Myers Squibb's checkpoint inhibitor combination, Opdivo (nivolumab) and Yervoy (ipilimumab), depending on their cancer type. Everimmune, a Gustave Roussy Cancer Center spinout, is hoping to expand its first-in-human clinical trial from Europe to the US but at present has launched the trial at four sites in France and Belgium.

Everimmune insists its single-bacteria, patient-specific approach has advantages versus a bacterial consortium for all-comers. "Developing a consortium [of bacteria] is complex in terms of manufacturing," Everimmune CSO Romain Daillere said. "You have to be able to characterize all the unique bacteria in the product."

MD Anderson's Ajami acknowledged the single-bacteria precision approach could have advantages in terms of isolating efficacy and toxicity outcomes, but he also suspects *Akkermansia* might be an outlier when it comes to single bacteria strains that can elicit a powerful enough immune response to improve checkpoint inhibitor outcomes on their own.

"The reason behind using single bugs, which we call 'keystone species,' is they have very specific functions," Ajami said, acknowledging that studying *Akkermansia* or any other single bug in isolation and fully characterizing its genome, its proteins secreted, and cellular-bound properties is "definitely a lot cleaner." But he personally subscribes to the view that the microbiome is like a complex garden. "[It's] made up of many species that are competing against each other or are in balance with each other when they're healthy … Species A produces something Species B uses, which Species C then feeds off, and creates that sort of ecology," he said. "You need the players to create that homeostasis environment."



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Building on fecal transplants for broad benefit

Though firms like Everimmune are focusing on single-bacteria precision microbiome treatment strategies, other firms are building on the successes seen with fecal transplants to develop microbiome therapeutics benefitting broader patient populations.

"Fecal microbiome transplant studies have shown that something very powerful happens when you transplant an entire microbiome, and the effects are durable," Federation Bio CEO Emily Conley said. "If you go to a [fecal microbiota transplantation] recipient five years after their FMT transplant, a majority of the microbes are still present."

But while the transformative *Science* papers showed the fecal transplant approach could be

an effective way to improve patients' responses to immunotherapy, most recognize fecal transplants aren't feasible on a large commercial scale. As such, Federation Bio wants to take what the field has learned from fecal microbiome transplant studies about the power of a large, diverse, metabolically complete bacterial consortia and translate that into a therapeutic it can manufacture at scale under tightly controlled conditions.

To accomplish this, Federation Bio has built a platform to grow multiple bacteria strains together, instead of one-by-one, and in doing so, create an oral bacteria consortium capturing the complex benefit of a fecal microbiome transplant. "Other approaches, historically, have taken one strain and grown it in a fermenter, then taken another strain and grown it in a fermenter, and another, and added them together," Conley said. "But that's extremely expensive and time consuming to the point where, if you want to go above 15 or 20 strains, it becomes totally infeasible."

Federation, which is already studying a 148-strain oral therapeutic in patients with a metabolic disorder called enteric hyperoxaluria, is targeting a similar number of bacterial strains in the therapeutic it's developing for immunotherapy-refractory cancers. The bacterial strains, derived from a fecal donor, are grown together in a co-culture, freeze-dried, and blended into a single capsule.

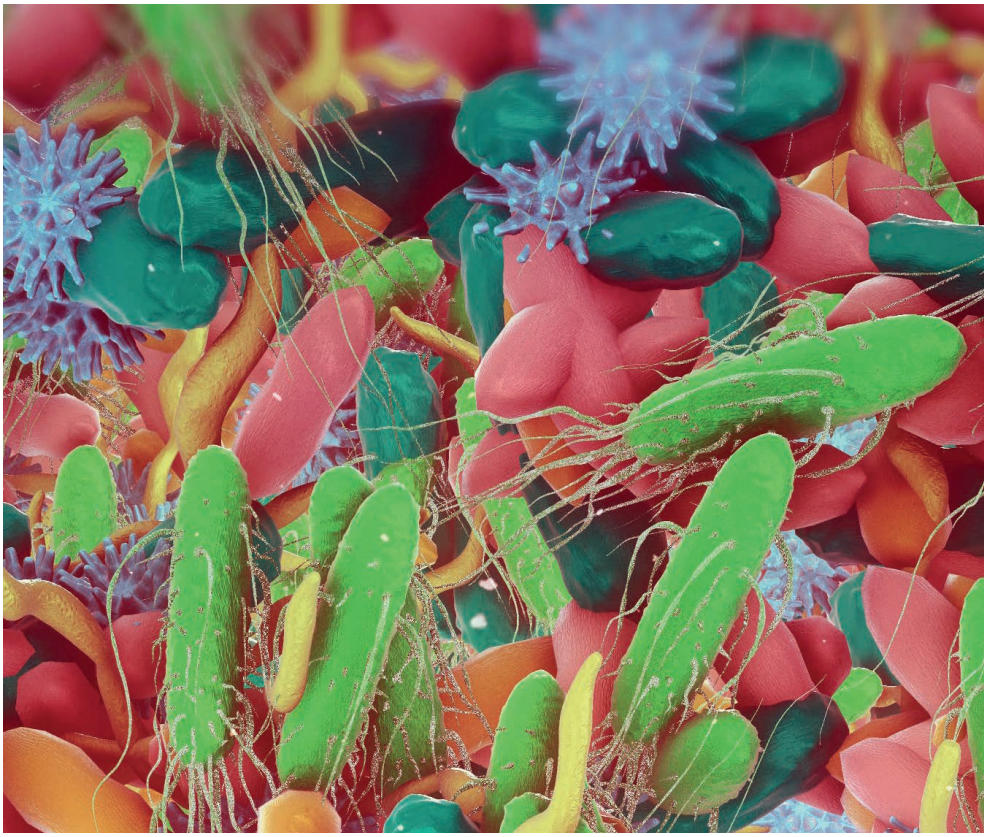
In the future Phase I study of its cancer microbiome therapeutic, Federation will likely enroll patients with multiple tumor types using a basket trial approach. The plan is to enroll immunotherapy non-responders, and "then, we'd give them this therapy to convert them from a non-responder into a responder," Conley said.

Federation wants to test the approach first in an all-comer population. It doesn't intend, at least for now, to sequence patients' current microbiomes to see if they have a deficiency of a certain bacteria and treat them with the therapeutic based on that information. However, the firm does plan to perform a metagenomic analysis of patients' gut microbiomes, both at baseline and after they receive the capsule, to inform future drug development.

"As we perform the metagenomic analysis of those recipients before, when they didn't respond, and compare that to what their microbiome looks like after they receive our therapy and do respond, we can start to understand the actual mechanisms at play driving this response," Swem said.

Drugmakers eye a functional approach

Other drugmakers are less concerned about whether their microbiome therapeutics work for all-comers or in selected populations and more focused on ensuring the drugs perform their intended function each time they're administered. With environments as heterogenous as individual microbiomes, this isn't always a given.



A keystone species like the one Everimmune is working with, *Akkermansia*, is unique in that its function is well understood and can be isolated in a way that the bacteria involved in the complex interplay within microbiome consortia, or even fecal transplants, cannot be. But what if it were possible to engineer a specific microbe to have a desired function? That's what another recently launched microbiome-focused biotech, Persephone Biosciences, is attempting.

"What we've recognized over the last five years is, when giving a patient bacteria that are wildtype to the microbiome … there's no guarantee those bacteria are going to do what they're supposed to do," Persephone Biosciences CEO Stephanie Culler explained. "So, we're developing new tools to genetically engineer the microbes in our guts."

Right now, the plug-and-play technology doesn't really exist to genetically engineer microbes, Culler noted. Microbes are very oxygen sensitive, and they're novel. "They're difficult to work with, and there's a lack of tools," she said. "But we fundamentally think the ability to engineer these strains to precisely deliver the therapeutic function is absolutely critical to the success not only of our therapeutics, but to others."

Culler envisions Persephone's microbiome therapeutic will comprise prebiotics – natural fibers that feed specific beneficial gut microbes – as well as a consortium of engineered microbes to deliver the response across a wide population of cancer patients.

> "Will we use a personalized approach? That's probably how we're going to have to do it for now," MD Anderson's Jennifer Wargo said. "In the short term, it's going to be a targeted approach, but there could be a one-size-fits-all approach that could be used as a basic microbiome restoration."

The engineered microbes are in early development stages, but Persephone has already launched a large, longitudinal prospective trial, dubbed ARGONAUT, to generate the deep multiomic data it needs to support this program. In the ARGONAUT study, Persephone is expecting to enroll up to 5,000 patients with advanced solid tumors including non-small cell lung, triple-negative breast, colorectal, and pancreatic cancer, as well as patients at high

or low risk for colorectal cancer. The firm will longitudinally collect blood and stool samples and perform metagenomic whole-genome sequencing and multiomic analyses to home in on the specific microbiome compositions that impact patients' immune systems and responses to immunotherapy or chemotherapy.

Persephone has developed a platform, called a "microbiome avatar," to glean as many actionable insights as possible from the data collected within ARGONAUT. "It's essentially a microbiome on a chip," Culler explained. "As we collect stool samples from all these patients, we bank them so we could have them on a chip and then evaluate all of our interventions in the background of that cancer patient's microbiome."

The machine-learning platform will be a key tool in Persephone's efforts to genetically engineer microbes, according to Culler. "We can run sequencing analyses to know how we've shifted the microbiome; we can look at metabolites; and we can also do immune profiling," she said. "This is important [because] we want to have a certain immune context driven by our therapeutic so the patient can respond more effectively to treatment. We can now do that all *in vitro*."

While the engineered microbes are in their earliest development stages, Persephone is making strides enrolling ARGONAUT, which has attracted the attention of bigger pharma companies. In 2021, Persephone inked a deal with Janssen to apply insights from the trial to develop a microbiome-informed precision oncology approach for colorectal cancer patients. The partners are enrolling both colorectal cancer patients and participants at a heightened risk of colorectal cancer, analyzing their stool and blood samples, and using data from these analyses to develop tools to screen patients' microbiomes for cancer-specific biomarkers, and, in turn, inform early detection and guide treatment.

A different path

While many industry players are exploring ways to use oral microbiome therapies to improve patients' responses to treatment, Paris-based Enterome is trying leverage the microbiome to develop cancer vaccines. Enterome's investigational OncoMimics microbiome-derived therapeutic vaccines are powered by its database of 23 million bacterial genes derived from sequencing nearly 26,000 individuals' microbiomes. "We know these genes very well because we have done not only the full sequencing, but also the full functional annotation," said Enterome CEO Pierre Belichard.

The body's memory T cells are already programmed to recognize the bacteria colonizing the gut microbiome, and when these T cells see those bacteria outside the gut, they can recognize and destroy them. This, Belichard explained, is what keeps the bacteria in the gut from escaping the digestive tract and systemically infecting someone's body. Enterome is harnessing that ability to trick T cells into thinking they are attacking a known microbe outside the gut when they are actually attacking a tumor cell.

Enterome accomplishes this by first identifying a target unique to a cancer tumor. Researchers sift through the company's database to find a bacterial antigen with a sequence closely mimicking that of the tumor-specific target antigens. Enterome then creates a bacterial peptide drug to boost the immune system and enrich the memory T cells against that specific bacterial antigen.

When the memory T cells encounter the bacterial peptide vaccine, which is administered outside the gut, they proliferate and mount an attack just as they would if the bacteria had escaped from the gut naturally. As the memory T cells programmed to eliminate these bacteria increase, so does the likelihood they'll engage in what Enterome calls a "cross-reactive response," where they attack and kill the tumor cells whose antigens look similar to the bacterial peptide antigens.

"We've seen, in 95 percent of the patients we've treated so far, that levels of these memory T cells circulating in the blood of the patient are enriching from 0.5 percent to 10, 20, sometimes 30 percent," Belichard said. "Those T cells are first directed against the bacterial peptide, but in turn, are acting against the human tumor-specific antigen to improve survival in these patients."

Several clinical trials of Enterome's OncoMimics peptides are underway, including the Phase I/II ROSALIE trial of the investigational therapeutic EO2401 alone or combined with BMS's Opdivo or Genentech's Avastin (bevacizumab) in recurrent glioblastoma patients. According to Belichard, adding the immune checkpoint inhibitor as a combination treatment could further boost anti-tumor effects. The firm is also exploring the approach in clinical trials for adrenal tumors and B-cell malignancies. Soon, it plans to launch studies in colorectal cancer patients with minimal residual disease based on circulating tumor DNA testing.

Belichard said Enterome is still figuring out the best strategy to select patients for these OncoMimics peptides based on tumor antigen expression. For now, the firm has chosen to focus on tumor types known to express established antigens. The therapeutic Enterome is developing for B-cell malignancies, for instance, mimics the antigens CD20, CD22, CD37, and CD268, which B-cell cancers typically express. The glioblastoma and adrenal cancer-focused peptides are designed to mimic the sequence of I13Rα2, BIRC5 (survivin), and FOXM1, and the "helper" peptide UCP2, all of which are established tumor antigens in these cancer types.

Importantly, patients need to undergo human leukocyte antigen testing to make sure they're HLA-A2 positive before enrolling.

The FDA has cleared Enterome's investigational new drug applications for its ongoing OncoMimics trials, and the firm has already reported early data from the fully enrolled glioblastoma study suggesting the peptide approach is well-tolerated and can generate strong systemic immune responses. The firm is expecting a readout from its B-cell cancer trial this year.

The next hill to climb

As drugmakers refine their microbiome cancer therapy strategies, the jury is still out as to whether these drugs will enter the market for an all-comer population or employ a precision approach to identify best responders.

"Will we use a personalized approach? That's probably how we're going to have to do it for now," MD Anderson's Wargo said. "In the short term, it's going to be a targeted approach, but there could be a one-size-fits-all approach that could be used as a basic microbiome restoration."

As is the story in drug development, it will take trial and error and clinical trials will fail and succeed before researchers and drugmakers can zero in on the best patient populations and therapeutic strategies for microbiome therapeutics, but Wargo and Ajami think that for the first time the technology is there to generate these answers.

"The technology's in place, but the critical tipping point will be showing the clinical value," Ajami said. "That's the next hill we have to climb." PMQ

Caroline Hopkins

Caroline is a senior reporter for *Precision Oncology News* where she covers the rapidly advancing world of personalized cancer medicine, including its business, regulatory landscape, and scientific research. Prior to joining *Precision Oncology News* in 2020, Caroline wrote for the American Society of Clinical Oncology's ASCO Daily News, among other oncology-focused publications. She has covered health, medicine, and science as a freelance journalist for *NBC News, Vox, National Geographic, Women's Health* magazine, and more. Caroline is based in Brooklyn, NY and has a bachelor's degree in English from Boston College and a master's degree in journalism from the Columbia University Graduate School of Journalism. You can find her on Twitter at @Ch_Hops.