

Oncologists tap the microbiome in bid to improve immunotherapy outcomes

A pioneering Merck & Co.-funded study is set to explore the ability of the microbiome to boost immuno-oncology therapy outcomes, and other companies are quickly gearing up to enter this clinical space.

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When Hassane Zarour first saw the mouse data suggesting that the microbiome can control the efficacy of cancer immunotherapy drugs, he knew he wanted to test microbiome-moulding strategies in human cancer clinical trials. But Zarour, an immunologist at the University of Pittsburgh, hit a slew of scientific, regulatory and funding roadblocks as he tried to get such a trial up and running. “When I first proposed the trial, people laughed at me,” he says. Three years on, times have changed. Zarour and others are racing towards the clinic (TABLE 1). “It’s become hot stuff now.”

Zarour’s proof-of-concept trial — currently on track to be the first of its kind — is set to launch in March 2018. With financial support from Merck & Co., the clinical trial will explore whether oncologists can boost the effects of Merck’s PD1-blocking checkpoint inhibitor pembrolizumab in melanoma by transplanting faecal microbiota from PD1 responders into the guts of non-responders. Other academic groups and biotech firms — including the Parker Institute for Cancer Immunotherapy, the MD Anderson Cancer Center, Seres Therapeutics, Vedanta Biosciences and Evelo Biosciences — are setting up similar cancer trials of their own.

This anticancer opportunity has opened up with unexpected speed. “This was not something that I imagined us working on in 2012,” says David Cook, CSO at Seres. Instead, he thought early work would focus

on establishing stable microbiomes that can prevent *Clostridium difficile* infection or harnessing microbial immunosuppressive activity for indications such as inflammatory bowel disease.

This sharp pivot, however, creates considerable uncertainty over how to proceed. “We and everyone else are just trying to figure out how to tread that line of not waiting too long, and not jumping in too early,” says Luisa Salter-Cid, head of Immunology Discovery at Bristol-Myers Squibb (BMS). The community will therefore be watching the first proof-of-principle clinical studies for clues as to which bacteria are responsible for immunotherapeutic responses, how to deliver bacteria and which cancer patients will benefit most. Other ongoing studies could soon start shedding light on whether oncologists need to start stratifying patients on the basis of their baseline microbiome characteristics.

“This is such an evolving field,” says Theresa LaVallee, head of Translational Medicine at the Parker Institute. “What we’re looking for is really getting out there early and doing the bold experiments.”

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Bugs as drugs

Preclinical data pointing to the role of the microbiome in immuno-oncology started making waves in 2015, with reports from two independent research groups in *Science*. In one study, Tom Gajewski, an immunologist from the University of Chicago, reported that *Bifidobacterium* provides antitumour effects in mice, and that oral administration of these bacteria to mice, in combination with blockade of the PD1–PDL1 checkpoint, eliminates tumour growth. Laurence Zitvogel, an oncologist at the Institut Gustave Roussy, reported at the same time that *Bacteroides* bacteria drive responses to CTLA4 checkpoint blockers. When her team administered faecal microbiota from the stools of melanoma patients with a high abundance of *Bacteroides* to mice, the mice responded well to CTLA4 blockade.

A flurry of *Science* studies published earlier this year bolstered the initial results. Gajewski and colleagues analysed stool samples from 42 melanoma patients, and found that *Bifidobacterium longum* and a few other types of bacteria are particularly abundant in checkpoint inhibitor responders. When the team transplanted faecal matter from these responders into germ-free mice, the mouse responses to PD1–PDL1 blockade improved.

Separately, oncologist Jennifer Wargo, of the MD Anderson Cancer Center, and colleagues analysed the microbiomes of 112 melanoma patients on PD1 blockers and found

Table 1 | Select microbiome-immunotherapy cancer clinical trials

Sponsors and collaborators	Checkpoint inhibitor	Microbiome intervention	Cancer	Status
The University of Pittsburg and Merck & Co.	Pembrolizumab	FMT from PD1 responders	Melanoma	Opens in March 2018
Evelo Biosciences and undisclosed partners	Undisclosed	At least two single-strain treatment arms	Melanoma, colorectal, renal and others	Opens in Q2 2018
Parker Institute for Cancer Immunotherapy, Seres Therapeutics and MD Anderson Cancer Center	PD1 blocker	FMT from PD1 responders, and/or SER401 (an oral consortium of spore-forming bacteria)	Melanoma	Opens in 2018
Vedanta Biosciences	Undisclosed	Clonal bacteria consortium	Undisclosed	IND filing in 2018, opens in 2018 or 2019

FMT, faecal microbiota transplantation; IND, investigational new drug; PD1, programmed cell death protein 1.

microbial signatures that were associated with immunotherapy response. Deeper analysis of the microbiomes of 43 of these patients showed that responders had higher overall diversity, a higher abundance of Ruminococcaceae and enriched anabolic function compared with non-responders.

Zitvogel, in a [study of 249 patients with epithelial cancer](#), showed meanwhile that antibiotic use around the time of PD1–PDL1 blockade significantly lowers progression-free survival and overall survival. A sub-analysis of 100 patients highlighted the role of *Akkermansia muciniphila* in immunotherapy efficacy. The team also showed that when they transplanted faecal microbiota from immunotherapy responders with epithelial cancers into mice, they could improve the effects of checkpoint blockade in the animal model of disease.

Collectively, these results make immunological sense. The microbiome is a [key controller of the immune system](#), influencing everything from susceptibility to autoimmune diseases to [flu vaccine](#) response rates. Although the community still needs to work out a detailed and comprehensive explanation of the mechanisms of benefit in cancer, researchers are ready to accept that the gut microbiome can help prime immune cells to better seek out malignant cancer cells around the body.

From a clinical perspective, microbiome-modulating strategies also offer a clear theoretical advantage over other anticancer therapeutics. The bugs that appear to provide benefit are all commensals — bacteria that evolved over thousands of years to live in harmony with the human host. Whereas combinations of checkpoint inhibitors with small molecules and antibody drugs carry high toxicity risks, microbiome-based approaches should be relatively harmless.

That means microbiome-based companies can focus on the translational science. “There are shared patterns of microbial changes that

are associated with responses. And in animals, we can manipulate the immune response by using the microbiome. Those observations are solid,” says Bernat Olle, CEO at Vedanta. But as to the best way to translate this into humans, he adds, “it’s mostly just speculation”.

Most notably, published and proprietary research — generated by analysing narrow human patient populations, on various diets, with unique research methodologies and distinct mouse models of disease — point to different microorganisms of interest. On a higher-level order, *Akkermansia*, *Bacteroides*, *Bifidobacterium*, Ruminococcaceae and other bacteria are all on the table. And different strains of bacteria within individual species possess entirely different biological functions and effects from one another. So, which bugs will make the best cancer drugs?

Stools as tools

Zarour is taking an unbiased approach, collecting faecal microbiota from pembrolizumab responders and delivering this via colonoscopy to mould the microbiomes of human pembrolizumab non-responders. After all, he explains, the preliminary preclinical studies suggest that faecal microbiota transplants (FMTs) hold promise. And the human data that have been collected to date are from such small populations that they may be missing the real

relationships. “There are so many variables. This approach just allows us to transfer a group of bacteria without worrying about the mechanisms,” says Zarour. “Right now we just need to show proof of principle.”

But FMT has its downsides. Some of the bacterial species and strains that thrive in donor stools have immunosuppressive properties that can offset the benefits of the protective co-commensals. FMT can also lead to the inadvertent transfer of pathogens into patients with already compromised immune systems. And from the regulatory and commercial perspectives, FMT changes from donor to donor and represents a manufacturing and quality control nightmare.

Cook, in light of these limitations, is ready to focus on the spore-forming bacteria that are associated with the mucosa. The published data, as well as Seres’ proprietary results, suggest that these bugs are responsible for checkpoint inhibitor-boosting activity, he says. Seres has yet to disclose the characteristics of their lead candidate in this space, SER401. The firm’s lead candidate for *C. difficile* infection is made by purifying a select set of bacteria from healthy candidate donor faecal matter, and encapsulating these into a standardized and oral product. Their second-generation phase Ib candidate in *C. difficile* by contrast consists of a rationally designed microbial cocktail of 12 bacterial species that are produced by anaerobic fermentation.

Seres is [working with the Parker Institute and the MD Anderson Cancer Center](#) to launch a trial that will test SER401 later this year. The collaborative group are finalizing trial details, but are considering trialling SER401, FMT from long-term checkpoint inhibitor responders and a placebo control treatment. “That should really inform us as to the best microbiome drug to take forward,” says LaVallee.

Olle is on track to take the faecal out of FMT entirely. Vedanta makes their product in

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the lab by combining clonal bacterial strains together into strictly designed and defined consortia. His team is working with a network of academic groups to identify a consortium of bacterial strains that induce cytotoxic CD8⁺ T cells in the gut, from where they can traffic to tumours around the body. Vedanta plans to advance a product into the clinic in combination with a checkpoint inhibitor in 2018 or 2019.

By cutting the need for stool donors out of their product, Vedanta can ensure that its products are consistent from batch to batch. And by carefully curating a consortium of bacteria, it hopes to harness the pleiotropic effects that complex microbial ecosystems seem to offer. “If you get too reductionistic with the approach, the biology that you’re chasing may wash out,” cautions Olle.

Gajewski and his partners at the biotech Evelo are however ready to double-down on single-strain ‘monoclonal microbial’. “We’re not trying to change the gut microbiome at all,” explains Evelo CEO Simba Gill. “What we’re trying to do is take advantage of the fact that microbes have evolved to interact with and modulate the systemic immune system through the gut.” As such, he adds, the company doesn’t need to worry about reshaping a patient’s entire microbiome or understanding the complex systems biology of how a cocktail of bacteria can achieve an effect.

Evelo has screened hundreds of bacteria to find specific strains that drive reproducible effects on the immune system. Preclinical data suggest that transient but high-dose exposure of these strains to gut-associated lymphoid tissue leads to robust and reproducible cytokine release profiles and phenotypic changes to the immune system. Oral administration of these single microbial strains drives potent pleiotropic and cancer-controlling effects in animal models of disease.

“The monoclonal microbial acts as a pharmacological agent, that has defined and reproducible effects for a set period of time,” says Gill. “Our product will work independent of background microbiome, and independent of what they eat, in the same way any drug does,” he says.

Evelo is set to start clinical trials this year of at least two separate oral products — one bifidobacterial strain, and one strain from an as yet undisclosed species of bacteria — in combination with a checkpoint inhibitor in patients with various cancers.

BMS’s Salter-Cid notes that the different strategies are not mutually exclusive. “I can envision a rationale where a few different strains or consortium of strains have similar effects,” she says.

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Instead, the approaches speak largely to each drug developer’s perspective on how to balance complicated and incompletely understood biology with manufacturing, regulatory, commercial and intellectual property considerations. Ultimately, adds Olle, the science will lead the way. “One way or another, this work is going to be done in the clinic, and I think we’ll learn useful things from it,” he says.

Researchers will be watching particularly keenly for hints as to whether microbiome-modulating strategies act equally across different cancer indications.

Zarour and the Parker–Seres–MD Anderson group are both launching their first trials in melanoma — a highly immunogenic cancer that was the ideal proving ground for both CTLA4 and PD1–PDL1 blockers. Much of the seminal preclinical data that set this field alight involved melanoma, further supporting this indication as a first step forward.

But as yet there is little evidence to predict whether success in melanoma will translate into improved outcomes for patients with other cancers.

“I don’t think it will be a one size fits all,” cautions Cook. One cocktail of bacteria may well work across immunogenic cancers like lung, kidney and bladder cancer, he speculates. But for cancers that don’t respond to checkpoint inhibitors alone, such as colorectal cancer, the community may need to dig deeper. Evelo’s trials may shed some light on this, as the company plans to test its products in both immunogenic and non-immunogenic cancers.

Cook is also keen to manage effect-size expectations. “It’s a mistake to think that the microbiome is going to be a panacea and that we’re going to take checkpoint inhibitor response rates from 30% to 85%,” says Cook. But a modest benefit in response rate or durability, without much of an additional toxicity risk, would be a welcome win for patients, he adds.

A gut punch

The emerging field may yet cause broader reverberations across the drug development landscape.

Researchers have long known that diet and other factors can impact preclinical mouse biology, and have come up with ways to

manage and standardize these variables. The emerging findings suggest that researchers will have to figure out how to account for microbiome composition as well — not just in immuno-oncology, but potentially across all therapeutic areas.

Researchers at BMS have started to change how they do things in their vivariums, easing up on their use of bleach, and seeding laboratories with certain strains of bacteria to foster the right kinds of microbiomes in their animals. But widespread changes to animal breeding and housing may be on the horizon for animal vendors, academic research groups and industry scientists.

There may also be consequences for completed immuno-oncology trials. Early studies did not make any attempts to assess the composition of the microbiome or account for its effects. Negative findings from small clinical studies may therefore need to be revisited, and some combination trials may need to be rerun. For a community that is already struggling to prioritize and make sense of the findings from more than 1,000 checkpoint inhibitor combination trials, this makes for daunting work. (There is also evidence that [some strains of bacteria can metabolize and inactivate cancer chemotherapeutics](#), highlighting another reason why oncologists may need to pay more attention to the patient’s microbiome.)

But industry is starting to adapt. “Everybody is either contemplating or already actively collecting faecal samples as part of their immuno-oncology programmes now,” says Cook.

BMS, one of the companies that is now collecting stool samples across all of its immuno-oncology trials, partnered with Enterome in 2016 to start looking for microbiome-derived biomarkers. Their early work focused on finding microbial signatures that would predict who was at risk of immunotherapeutic toxicity, building on the 2016 finding that microbiome composition might be a marker of [checkpoint blockade-induced colitis](#). But the company is now looking for biomarkers that can predict immunotherapy efficacy as well.

“This seems to me to be the lowest hanging fruit,” says Salter-Cid. The regulatory path for the incorporation of biomarkers into drug approvals is well established, she explains, and the trials that could provide the necessary data are already under way.

“This space is moving very fast,” says Salter-Cid. “It’s going to be a key area of research for many of us in the next few years.”