

BC Innovations, Cover Story

CTLA-4 and PD-L1 blockade depends on specific commensal bacteria

Microbiome at the checkpoint

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The growing belief that the microbiome can be harnessed to treat cancer has arguably received its biggest boost to date with the publication of two papers in *Science* linking two of the hottest targets in cancer immunotherapy, [CTLA-4](#) and [PD-L1](#), with specific commensal bacteria. And in a sign of increasing commercial interest in the microbiome, the findings have been snapped up by [Enterome Bioscience S.A.](#) and newly-formed [Evelo Therapeutics Inc.](#), who announced partnerships with the respective academic groups behind the studies within a week of each other.

The microbiome has already gained acceptance in the mainstream as a player in gastrointestinal (GI) disease and an increasing body of evidence has linked it to other diseases, including autoimmunity, obesity and heart disease.

Hints of a role in cancer have come from research showing correlations between microbial bacteria such as *Helicobacter*, *Lactobacillus* and *E. coli* and cancers such as B cell lymphoma, stomach cancer or colorectal cancer.

Now, groups from [Institut Gustave Roussy](#) and the [University of Chicago](#) have shown the activity of checkpoint inhibitors is enhanced by - and, in one case, dependent on - the presence of specific bacterial strains in the microbiome.

The first study, led by Laurence Zitvogel at Gustave Roussy, demonstrated that *Bacteroides fragilis* is required for the tumor suppressive effect of CTLA-4 blockade in mice, and linked the strain with the action of [Yervoy](#) ipilimumab in melanoma patients. Enterome announced a framework partnership with the institute on Nov. 12, and CEO Pierre Bélichard told BioCentury the company is working on an agreement to use Zitvogel's findings towards a biomarker for checkpoint effectiveness.

[Bristol-Myers Squibb Co.](#) and [Ono Pharmaceutical Co. Ltd.](#) market the anti-CTLA-4 mAb Yervoy to treat melanoma.

Zitvogel is director of the Institut National de la Santé et de la Recherche Médicale (INSERM) UMR 1015 research unit on tumor immunology and cancer immunotherapy, housed at Gustave Roussy.

The second study, led by Thomas Gajewski, a professor of medicine and pathology at the University of Chicago, showed the commensal bacteria *Bifidobacterium* has antitumor activity and can improve the effectiveness of an anti-PD-L1 mAb in mouse models of melanoma. Cancer newco Evelo, which focuses on the microbiome, announced on Nov. 9 a partnership with Gajewski's lab, and said the company acquired an

option to license microbiome-based immunotherapies from the university.

Gajewski told BioCentury that while "the starting point was different" for the two studies, and the "exact bacterium that was isolated" was different, the "principle is the same, and that is that the composition of the gut can impact whether or not immunotherapies against tumors are efficacious."

Not-so-fragile bacteria

Zitvogel and colleagues focused on the relationship between CTLA-4 and the microbiome because they wanted to dig into the causes of Yervoy's side effects, which often involve immune responses at sites exposed to commensal organisms. Previously, the group had shown the microbiome is involved in the immune response stimulated by the generic chemotherapeutic cyclophosphamide.

"Toxicity related to CTLA-4 blockade is mostly in the gut," said Mathias Chamaillard, a co-author on the Gustave Roussy study. In addition, he said, patients can develop immunoglobulins against some bacteria.

Chamaillard is team leader at the Center of Infection and Immunity at the University of Lille.

In mice with depleted or absent microbiomes - produced by antibiotic treatment or germ-free housing - the anti-CTLA-4 mAb lost its ability to suppress sarcomas, melanomas or colon cancer.

Using a series of cell-based and RNA sequencing experiments, the team identified several *Bacteroides* species as key candidates for the microbiotal effect.

Next, the team re-introduced individual *Bacteroides* species into microbiome-deficient models and identified three that alone or in combination restored sensitivity to the anti-CTLA-4 mAb, with *B. fragilis* emerging as one of the most potent strains.

The researchers then re-introduced the bacteria to microbiome-deficient mice bearing metastatic melanoma or non-small cell lung cancer (NSCLC) tumors, by immunization with *B. fragilis* polysaccharides or adoptive transfer with *B. fragilis*-specific T cells, and showed the mAb regained its antitumor effect.

To test for clinical relevance, the team transplanted fecal microbes from metastatic melanoma patients who had received Yervoy into germ-free mice, and then treated the animals with the mAb. The transplants restored the tumor-suppressive effect of CTLA-4 blockade, and fecal samples rich in *B. fragilis* had the most potent effect.

Zitvogel's team also obtained sequencing data from patient microbiomes and found differences in composition between patients who did and did not respond to Yervoy, which Chamaillard said will be used to develop a biomarker.

Chamaillard thinks the findings also clear up any questions about whether colitis produced by Yervoy is due to the drug's activity at CTLA-4, because suppression of CTLA-4 reduced tumors in antibiotic-treated mice given *B. fragilis* but did not cause colitis.

"This was quite surprising to us. We have a drug, we have the bacteria, and these bacteria promote the drug efficacy but do not contribute to the toxicity of the drug," he told BioCentury.

Environmental science

The University of Chicago study was designed to explore "why some patients respond well to immunotherapies and others less well or not at all.," Gajewski told BioCentury.

The team reasoned that the three most likely causes for differences in patient responses are genetics, tumor biology or environment. "And the environmental variable that's become easily tractable is microbiota,"

said Gajewski.

To test the effect of different microbiota, the team performed studies on genetically similar mice obtained from two different facilities.

In a melanoma model, mice obtained from [The Jackson Laboratory](#) displayed a much stronger immune-mediated tumor response with significantly higher numbers of CD8+ T cells and tumors that grew less aggressively than mice from [Taconic Biosciences Inc.](#) Co-housing the groups before implanting tumors eliminated the differences, which the authors said supported a role of the microbiome.

Gajewski's team then transferred fecal material from the Jackson mice into the Taconic mice, which resulted in slower tumor growth and higher numbers of CD8+ T cells than transplants of Taconic fecal material or vehicle. In addition, combining an anti-PD-L1 mAb with fecal material from Jackson mice produced better tumor suppression in Taconic mice with established tumors than the antibody alone.

Using RNA sequencing, Gajewski's group identified several commensal species of *Bifidobacterium* as the key players. In Taconic mice, *Bifidobacterium* increased tumor suppression and the accumulation of intratumoral antigen-specific CD8+ T cells compared with no treatment or with a *Lactobacillus* strain that is also present in the microbiome.

Finally, the team showed that *Bifidobacterium* in combination with the anti-PD-L1 mAb produced greater tumor suppression than either agent alone, which it considered one of the most important findings.

"So you could make borderline-responding mice to checkpoint blockade into major-responding mice by just giving one bacterium," said Gajewski.

Different paths

For Enterome, the partnership with Gustave Roussy provides it with a path to expand its microbiome focus into cancer.

The company was launched in 2012 on research showing the relationship between the microbiome and obesity, Crohn's disease and other inflammatory bowel diseases. Its lead molecule is EB 8018, a [fimH](#) antagonist for Crohn's disease that Bélichard said should enter the clinic in 2016. The company also has a Crohn's diagnostic slated to reach the market at the end of 2016.

Bélichard said the first step with the Gustave Roussy deal will be to use the sequencing data to develop a biomarker to guide treatment options.

In addition, Enterome wants to develop therapeutics based on specific bacterial proteins or metabolites.

"Some gut microbiome components do mimic tumor antigens," he said. He added that while the company is not yet ready to disclose details of the program, it is looking to develop a compound based on tumor-mimicking agents to support the immune response.

Bélichard thinks that approach will prove safer than "developing live bacteria" and introducing them into a patient's microbiota - a strategy pursued by some other microbiome companies that he thinks is risky.

"Bacteria that are commensal for you could be pathogens for other people, so it could be difficult to develop a magic bullet," he said.

Evelo CEO Simba Gill takes the opposite view and said Evelo will pursue bacterial mixtures for testing in humans first, while exploring in parallel the biology that could lead to a metabolic product or small molecule produced by bacteria. He said that because microbiome-based therapeutics is such a new field, "there's a lot of expertise and capability that's needed to translate the research-level findings into something that could be a real product."

Gill told BioCentury the goal is to find "microbial immune activators" that stimulate the immune system to attack tumors. The company wants to use its deal with Gajewski's group - and several other undisclosed academic collaborations - to research "which bacteria have what level of response and why."

According to Gajewski, it's highly likely there are more components of the microbiome with a role in cancer immunotherapy.

"I have a feeling that there's going to be a family of commensal bacteria that have overlapping properties. If we line them all up, hopefully we'll be able to figure out the biology," said Gajewski. "If we understand the biology, maybe we could even engineer the bacteria to make them better."

Companies and Institutions Mentioned

Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.

Enterome Bioscience S.A., Paris, France

Evelo Therapeutics Inc., Cambridge, Mass.

Institut Gustave Roussy, Villejuif, France

Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France

The Jackson Laboratory, Bar Harbor, Maine

Ono Pharmaceutical Co. Ltd. (Tokyo:4528), Osaka, Japan

Taconic Biosciences Inc., Hudson, N.Y.

University of Chicago, Chicago, Ill.

University of Lille, Lille, France

Targets and Compounds

[CTLA-4 \(CD152\)](#)

[fimH - Bacterial fimbrial adhesin](#)

[PD-L1 \(B7-H1; CD274\) - Programmed cell death 1 ligand 1](#)

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