Bitten by the Bug

A flurry of investments fuels microbiome drug discovery

It has become quite evident that the microbiome has the potential to impact drug discovery in vital ways. In addition to impacting study reproducibility and variability, the microbiome may influence drug efficacy or serve as a therapeutic agent.

To capitalise on the microbiome’s potential to improve human health, biotech and pharmaceutical companies are devoting greater resources to microbiome-related initiatives, spurring a spike in investments and collaborations. Incorporating this potentially disruptive technology into the highly-structured process of drug discovery requires new approaches, thinking and tools.

Betting big on the microbiome

There is no denying that interest in the microbiome as a focus of drug discovery is on a rapidly-rising trajectory. In 2018, more than 2,400 clinical trials involved testing therapies using the microbiome, versus 1,600 such trials in 2017. A recent *Nature* article estimated that more than $1.7 billion has been spent on human microbiome research in the last decade. Looking ahead, ResearchandMarkets.com reports that the global microbiome market is expected to reach $899 million by 2025, as investments pour into the study of microbiome-based therapeutics and the microbiome as a drug target.

To support the dramatic surge in microbiome-related drug discovery, there has been a corresponding rise in initial public offerings (IPOs), venture capital investments, mergers and acquisitions (M&As) and collaborations all boosting the funding of microbiome work. The IPO of Kaleido Biosciences in March 2019 is a prime example: it set the stage for expanding the company’s efforts to develop novel Microbiome Metabolic Therapies (MMTs™), which modulate the microbiome’s metabolic output and profile. Kaleido already has MMT candidates for metabolic, cardiovascular and infectious disease; in a clinical trial, one candidate demonstrated an ability to reduce ammonia produced by the gut microbiome, which is useful in developing therapies for diseases resulting from hyperammonemia.

On the M&A front, Switzerland-based Ferring Pharmaceuticals’ purchase of Rebiotix provided the company with access to the microbiota-based therapeutic that may be closest to market. Rebiotix is in Phase III trials with RBX2660, part of its MRT™ (Microbiota Restoration Therapy) drug platform that delivers live, human-derived microbes into the gastrointestinal tract. Designed to prevent recurrent *Clostridium difficile* (*C. diff*) infection, RBX2660 now has Fast Track and Breakthrough Therapy designations from the US Food & Drug Administration (FDA). Another noteworthy union was the merger of C3J Therapeutics and AmpliPhi Biosciences to form Armata Pharmaceuticals, a biotech focused on addressing antibiotic-resistant infections using therapeutic bacteriophages – viruses that are parasitic in nature and able to infiltrate, infect and destroy targeted bacteria.

By Dr Alexander C. Maue
Collaborations also are fuelling microbiome drug discovery, with three of the most prominent joint efforts focused on using bacteria consortiums – defined compositions of bacteria administered orally, which are emerging as a high-potential ‘bugs as drugs’ approach. Bristol-Myers Squibb (BMS) is partnering with Vedanta Biosciences to assess the efficacy of the immune checkpoint inhibitor Opdivo® in combination with Vedanta’s VE800, a bacterial consortium that activates cytotoxic CD8+ T cells. Similarly, Merck and Evelo Biosciences are evaluating the efficacy of Keytruda® in combination with Evelo’s EDP1503, an orally-delivered monoclonal microbial oncology drug candidate. The microbiome’s role in oncology immunotherapy is also the focus of a three-year, $20 million collaboration between Seres Therapeutics and AstraZeneca, combining Seres’ manufacturing capabilities and expertise in developing drug candidates targeting microbiome dysbiosis with AstraZeneca’s oncology portfolio. Taking a slightly different tack, a collaboration between Enterome and the Dana-Farber Cancer Institute aims to evaluate and develop gut microbiome-derived antigens for cancer immunotherapy. Dana-Farber’s preclinical models will be used for in vivo validation of bacterial antigens that mimic the antigens expressed by tumours and elicit a cytotoxic T cell immune response.

As significant funds are invested in microbiome drug discovery, the various players involved are employing a wide range of approaches and a diverse set of technologies. It seems the industry is taking different yet parallel paths that aim at a similar endpoint: understanding and leveraging the impact of the microbiome on disease development and treatment.

Mouse models are taking centre stage in these varied efforts. Despite known limitations that affect the translatability of microbiome research conducted in mice, mouse models are helping elucidate the mechanisms of the microbiome and enabling testing of therapies that could dramatically alter how major diseases are treated. Current initiatives demonstrate the utility of the mouse model in microbiome-related research, both in drug discovery and in the preclinical proving ground.

**Banking on bacteria consortia**

Several of the most promising initiatives in microbiome research are banking on the viability of bacteria consortia as effective disease therapeutics, whether as monotherapy or in combination with other drugs. Two of the leading players in this
space, Vedanta and Evelo, have obtained exclusive licences (from the University of Tokyo and the University of Chicago respectively) for specific applications of the bacteria used in their drug cocktails.

Vedanta is advancing its oral biologic candidate VE800 to clinical trials based on positive preclinical research findings in syngeneic tumour mouse models. A study by Dr Kenya Honda, scientific co-founder of Vedanta, demonstrated the VE800 cocktail of 11 human-derived bacteria boosted production of interferon-gamma-producing (IFNγ+) CD8+ T cells, which improved anti-tumour immunity and response to immune checkpoint inhibitor therapy in germ-free tumour models.

Previous landmark studies of the microbiome’s impact on checkpoint inhibitors focused on differences in the gut microbial compositions of responder patients versus non-responders – identifying clinical effects of the gut microbiome and validating them in mice transplanted with human microbiota. For instance, Gopalakrishnan et al observed a correlation between an effective response to anti-PD-1 therapy and the diversity and composition of the gut microbiota, using a melanoma mouse model. In contrast, the recent Vedanta study identified a defined collection of bacterial strains capable of improving anti-tumour response. Given that the 11 identified strains are not present abundantly in healthy humans, the findings have significant implications for the use of bacterial consortia as efficacious therapeutics. These preclinical findings paved the way for studying the impact of VE800 on immune checkpoint inhibitor efficacy in the clinic, an effort that Vedanta and BMS are now jointly undertaking.

Similarly, Evelo is in clinical trial phase with several monoclonal microbial drug candidates – orally-delivered single strains of microbes – after positive preclinical results. Its EDP1503 demonstrated activation of several systemic immune pathways that complement immune checkpoint inhibitors, with an increase in CXCL9 and CXCL10 production in the tumour microenvironment and enhanced NK and T cell infiltration to the tumour site. These findings set the stage for an Evelo-Merck clinical trial collaboration in which EDP1503 will be studied in combination with Ketyruda in a variety of tumour types, including metastatic melanoma, colorectal and triple-negative breast cancer. Monoclonal microbial drug candidates also are in testing for psoriasis, showing positive initial results. In August 2019, Evelo released data from a cohort of patients with mild to moderate psoriasis treated with EDP1815, a monoclonal microbial drug based on Bifidobacteria. After 28 days, patients showed a statistically significant (p<0.05) reduction in average lesion severity score (LSS), while those receiving a placebo had a mean increase in LSS of 0.25 points.

Harnessing the power of bacteriophages

Another emerging strategy in microbiome drug discovery is the use of bacteriophages (also called phages) as a tool for manipulating the microbiome for therapeutic purposes. The gut microbiome is known to harbour bacteriophages that interact both with each other and the human host. Researchers studied the effects of bacteriophages on the microbiome in gnotobiotic mice colonised with defined human gut bacteria, incorporating 10 species that represent the major phyla in the gut microbiome. The mice were subjected to predation by cognate lytic phages, capable of infecting and killing a bacterial cell, then multiplying and attacking nearby bacterial cells. Using metabolomic profiling, investigators discovered that the bacteriophages had altered the microbiome of the colonised gnotobiotic mice which in turn impacted the gut metabolome. The results demonstrate how the antagonistic effects of bacteriophages can modulate bacterial colonisation and potentially impact the host, adding to the growing body of evidence that manipulating the microbiome may be an effective therapeutic approach.

Armata Pharmaceuticals is among several companies leveraging bacteriophages as potential therapeutics, focusing on antibiotic-resistant infections such as *Staphylococcus aureus*. Preclinical work in mouse models has shown the effectiveness of phages against *S. aureus*. Two phages (K and 44AHJD) evaluated for efficacy against *S. aureus* in a mouse nasal colonisation model were found to lyse >85% of the clinical isolates tested. Data presented at the IDWeek 2018 conference demonstrated the efficacy of Armata’s lead candidate, AB-SA01, in the clinic as well. When AB-SA01 was used as an adjunct to antibiotics in 13 patients with severe *S. aureus* infections, 83% of patients achieved complete resolution or significant improvement of baseline signs and symptoms.

Another Armata investigational therapeutic based on bacteriophages (AB-PA01), targets the drug-resistant *Pseudomonas aeruginosa*. In two mouse models of pneumonia, treatment with the phages B-R656 and B-R1836 significantly decreased the bacterial load in the lungs (>6 log10 CFU and >4 log10 CFU, respectively) on day five.
Rethinking FMT

In the wake of the FDA’s safety alert in June 2019, the days of performing unapproved faecal microbiota transplantation (FMT) are likely over. However, FMT carried out under an IND is expected to remain a viable approach to manipulating the gut microbiome for disease prevention and treatment. It is anticipated that more rigorous testing will become a requirement to both understand and inform on the specific bacteria species present in the faecal matter prior to use.

When used under an IND and within strict conditions, FMT has proven useful in elucidating inter-patient differences in the response to a given therapeutic. Seres Therapeutics used this approach to validate tumour mouse models of two types – germ-free mice and those treated with antibiotics – that did not respond to anti-PD1 previously. Following FMT from healthy donors, the germ-free mice responded positively to anti-PD1 treatment, due to increased entry of cytotoxic CD8+ T cells into the tumour.

Preclinical studies such as these, using FMT in concert with mouse models, are helping to inform strategies for the development of microbiome-based therapies that are designed to improve the efficacy of other therapies. In one such example, a Seres live vaccine oral drug candidate (SER-287) is being used in a clinical trial in combination with anti-PD1 therapy in patients with ulcerative colitis.

Understanding transformative effects

The potential biotransformation effect of the microbiome is also emerging as a critical issue to consider in drug discovery. Numerous cases have demonstrated the potential for microbial biotransformation of a drug, altering its efficacy and/or toxicity. One of the most well-known examples involved the cardiac drug digoxin. Approximately 10% of patients who took digoxin did not respond to it because their gut microbiomes converted the drug to an inactive form, which was attributed to a single bacterial genus, *Eggerthella*. The use of antibiotics reversed this effect.

The gut microbiome’s ability to transform drug availability, efficacy and toxicity also was demonstrated with levodopa, which is the leading therapeutic for Parkinson’s disease and known to have wide variability and efficacy across patients. Scientists at Harvard University identified *Enterococcus faecalis* as the potential culprit, linking this bacterium to the metabolism of levodopa. *E. faecalis* absorbs levodopa and converts it to dopamine; another bacterium (*Eggerthella lenta*) converts the dopamine to meta-tyramine. The investigators hypothesised that the metabolism of levodopa may limit its bioavailability and reduce its efficacy, while causing some of the side effects associated with the drug.

Examples like these bring to the forefront the complexity of microbiome-based therapy, due to issues such as the potential for biotransformation and the variability of microbiota compositions from one person to the next. As more drugs are characterised by low solubility and/or permeability – spending more time in the gastrointestinal tract and likely having greater interaction with gut microbes – the potential impact of the microbiome on drug metabolism becomes of greater importance for study.

Putting the mouse to best use

Microbiome drug discovery holds much promise for the future of disease prevention and treatment, and the mouse model will remain a central tool in this endeavour, enabling investigators to test hypotheses in ways that are not practical in the clinic. The method used in the landmark studies that first linked the gut microbial composition of patients to their response to checkpoint inhibitor therapy – ie, observing a clinical effect then validating it in a mouse model – is a highly viable approach to translational microbiome drug discovery.

While homogenous genetics, a comparable physiology and accessibility to a wide range of model types make the mouse a sound choice for microbiome-related research, it is crucial to address the limitations of the mouse when designing studies for optimum reproducibility and translatability. Several considerations and best practices should be employed when using mouse models in microbiome-related drug discovery.

A key consideration is selection of the most suitable model. In addition to the germ-free models that have become almost synonymous with microbiome research, genetically engineered models that have been used successfully in other work have a role in studying the microbiome, particularly its impact on patient response to a given therapeutic. Where defined bacteria of interest have been identified – as in the case of bacteria consortia – generating a mouse model with a defined microbiome may be the right strategy. This approach affords the flexibility to study a host possessing the microbiota deemed relevant to a...
specific research application. Once a knockout or humanised germ-free model is colonised with the microbiota of choice, strict procedures for husbandry and housing must be followed to ensure the microbial composition remains intact. Another approach gaining traction is custom microbiota associations, which involves performing FMT from a patient donor to a germ-free mouse to study patient-specific gut bacteria and their effects on disease and to test drug candidates in the clinic. Finally, the use of a wild-type microbiome in a mouse model is an emerging approach that provides the advantages of studying disease development or therapeutic efficacy in a model with a more natural immune response than is found in lab animals.

Additionally, ensuring the microbiome of the selected model does not change over time is vital for research of any kind, but particularly when the microbiome is a key variable of interest. A wide range of factors can impact a mouse model’s microbiome unintentionally, ranging from husbandry practices, to mouse-specific factors such as gender and genetics, to the health standard and mode of birth. As a result, it is essential to employ globally harmonised health standards and standard operating procedures to maintain the desired microbial composition.

As investigators endeavour to learn more about the microbiome’s role in improving human health and strive to find new ways to harness its potential, investment in this field will likely continue its upward trend. New approaches and strategies will be required to fully realise the promise of the microbiome to elucidate the mechanisms of disease and help answer questions about why patient response to a therapeutic can vary so widely. The mouse model will remain front and centre as a tool for advancing researchers’ understanding of the microbiome and taking disease treatment to new levels.

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