THE HUMAN MICROBIOME

a new frontier in drug discovery

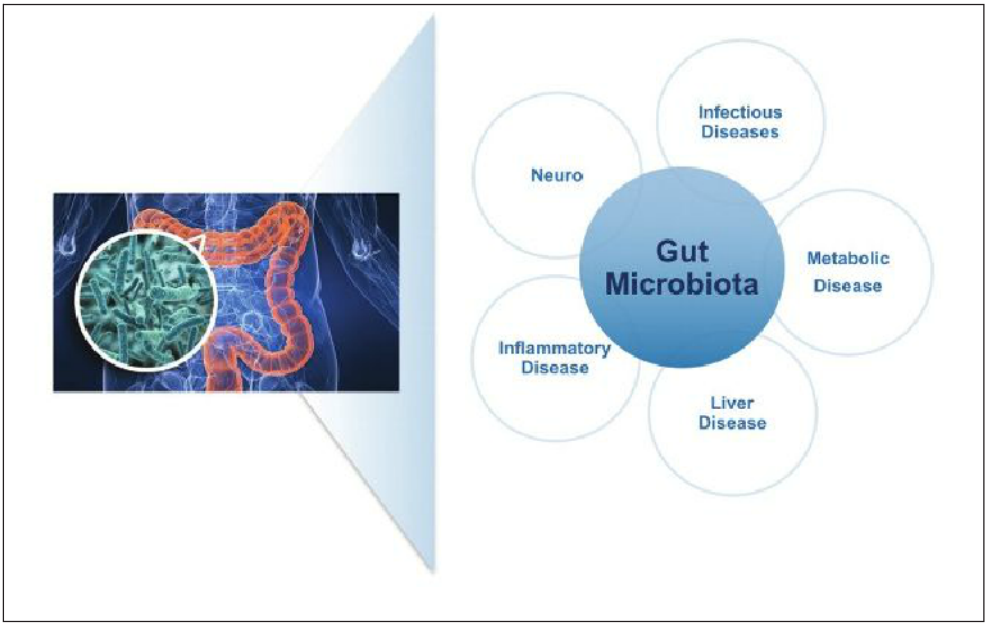
There is a new phenomenon sweeping the globe (or at least sweeping the imagination of people around the globe). It is the human gut microbiome and the role it may play in the future of human health.

By Lee Jones

Recent reports have indicated that the human gut microbiome may be implicated in a whole variety of health conditions such as metabolism, obesity, diabetes, immunity and autism. If you can name it there has probably been something written about the condition and the gut microbiome. One thing is for certain and that is that the gut microbiome has a role in protecting the human host from disease[1].

I too caught the bug (pun intended) as I first learned about the gut microbiome when I heard about faecal transplants. I became fascinated with how the microbes in faecal material could be used to solve an unmet medical need: people who had experienced multiple recurrences of a nasty gastrointestinal disease called *Clostridium difficile* infection (CDI) and who had no good alternatives. At the time, I did not know exactly what CDI was, but it sounded bad and seemed to be something that needed to be solved. I learned that the number of people getting the disease was growing and that it was harder to treat with existing antibiotics. It dawned on me, as I learned more, that my own family had fallen victim to this disease multiple times, and here, right before me, was a potential cure that had been demonstrated to work. I remember thinking that I might just be witnessing the dawn of a new era where the use of the human microbiome could revolutionise the way medicine would be practised in the future.

That was in 2011. At the time, one could find very little, if any, mainstream information about the human microbiome. Contrast that to today where in May 2016 the White House announced the National Microbiome Initiative with $121 million in Federal and $400 million in private funds to promote the study and use of the microbiome. This timeline from obscurity to the spot-



light for a technical initiative is like stomping on the gas in a sports car and going from 0-160mph in two seconds!

Prior to the Microbiome Initiative, over the past few years numerous companies have taken the concept of the microbiome as a therapeutic to the next stage by developing commercial products. Rebiotix is the first company to take a microbiome-based product as a drug through the FDA. Its product for the prevention of recurrent CDI is the most clinically advanced. Seres Therapeutics, which also has a CDI prevention product but one that is based on a limited microbial mix that forms spores, was the first microbiome company to go public through an IPO. Second Genome, Vedanta, Enterome and Synlogic, all microbiome companies, have recently closed financing rounds, signalling that the race is on to the market and that the market is real.

What happened to drive a relatively unknown concept to global prominence in a few years? With regards to the gut microbiome, I believe that it had a lot to do with the ubiquitous nature of the source of the microbes (human faecal material) and the ability of anyone to acquire it and use it in research. And not just bench research, but human clinical research; including the citizen scientists trying it themselves in the privacy of their own homes (and of course, sharing the do-it-yourself techniques on the internet in all their glory!).

The fascination with the potential of gut microbiome-based therapies, and the broad and fast scope of research, has led to the birth of a new industry, including new therapeutics and diagnostics for human and animal health and disease, new test methods, new libraries of knowledge about previously unknown microbes and new methods to collect and manipulate vast quantities of data. From what I can imagine, we have only seen a tiny piece of what will surely grow over the years, opening up all sorts of opportunities.

So, what is the gut microbiome? What functions does it perform? What happens when the gut microbiome gets disrupted? How does that happen? These are just a few of the questions that scientists and others are trying to answer. Let us start with Human Microbiome 101.

Microbes and other organisms inhabit our surfaces and collectively outnumber our own human cells. The vast majority of these are a community composed of trillions of micro-organisms that reside in the human intestinal tract and make up the human gut microbiome. These micro-organisms live in concert with each other and their human host and share important functions including processing otherwise indigestible components of our diet and making essential nutrients that allow us to live[2]. In other words, without the gut microbiome, we as humans would not survive.

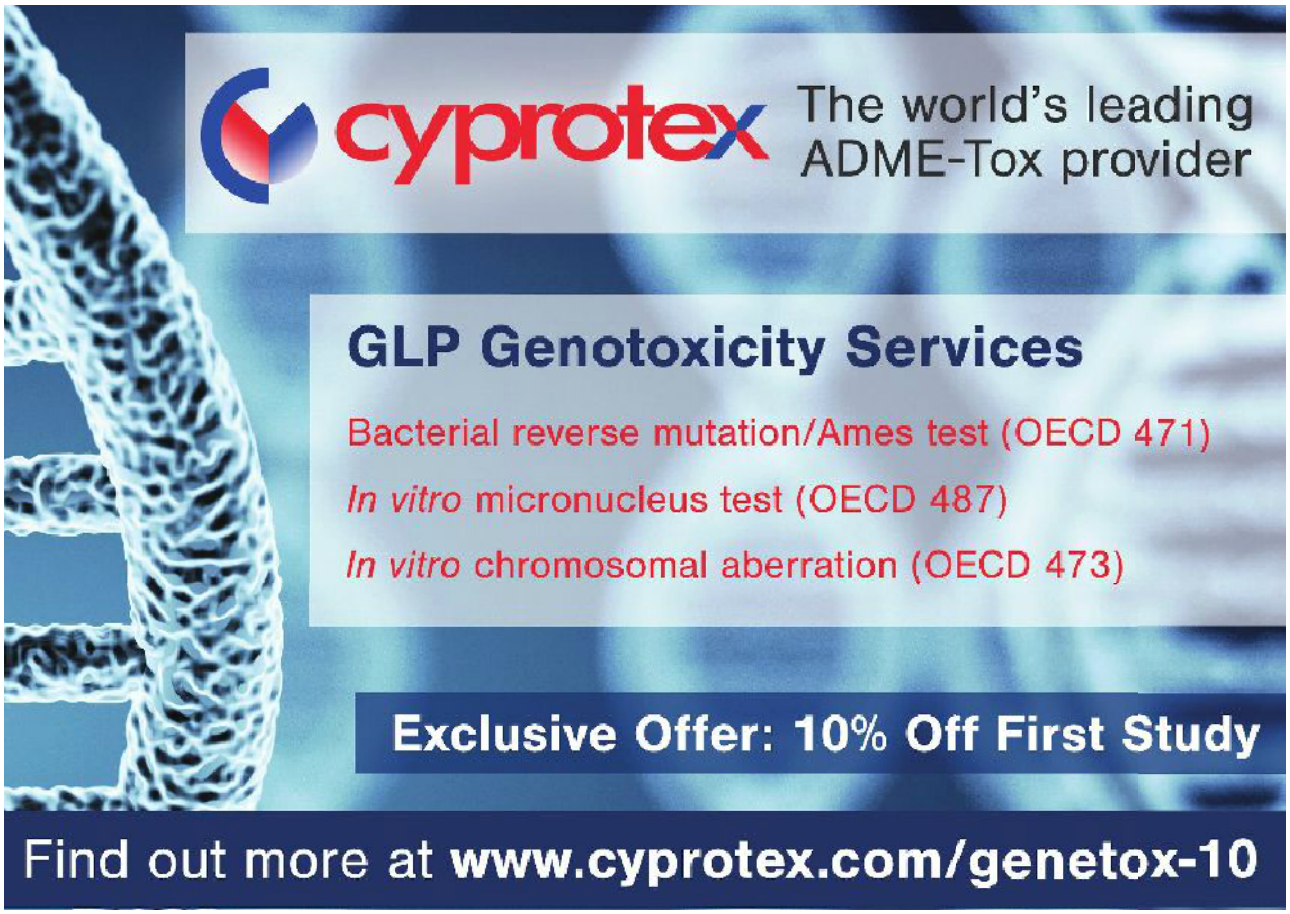
Interestingly enough, a healthy human's gut microbiome, as measured by the microbiota in the faeces, are dominated by two bacterial phyla: Bacteroidetes and Firmicutes. Does that mean that all human microbiomes are alike? Well, while there are differences at the species level to the point

where no two individuals are exactly alike, it is now understood that functionally most people are similar, and most people have a common set of microbes at some level that look like everyone else's[3]. Our microbiomes are influenced by what we eat, where we live, who we live with, how old we are and what pets we have – so the composition is bound to be different between us[4]. Where the observed difference in microbiome composition is much larger is between those people who are generally healthy and those people who are generally ill.

The NIH Common Fund Human Microbiome Project (HMP) was established to generate research resources to enable the characterisation of the human microbiota and the analysis of their role in human health and disease. The foundation of the work done in the first phase (2007-2012) was to characterise the microbiome of approximately 242 healthy individuals from the US. The generated data set has been used as a basis for the representation of a healthy person's microbiome and is often used to characterise a microbiome as 'healthy'[5]. Actually, I am not sure that there is a singular 'healthy microbiome'. What I do believe is that sick people, particularly people who have experienced multiple episodes of CDI where I have experience, have a gut microbiome that is dominated by a different set of microbiota than those people who have not experienced the disease.

Using CDI as an example, the occurrence of the disease can be attributed to the disruption of the gut microbiota where the person who has a compromised gut microbiome gets exposed to the *Clostridium difficile* (*C. diff*) organism and is unable, due to a lack of the normal microbiota community, to resist the subsequent infection. How does the microbiome get disrupted?

For CDI, this occurs primarily as a result of antibiotic usage where the antibiotics are taken to treat an underlying condition, such as a urinary tract infection. When this happens and *C. diff* is introduced into the altered gut environment, the *C. diff* replicates and produces toxins. At some point, the toxin wrecks havoc on the lining of the large



intestine and causes profuse watery diarrhoea – the hallmark CDI symptom.

To reduce the number of *C. diff* organisms and therefore toxin production to stop the diarrhoea, the standard-of-care is to dose the patient with more antibiotics. For some people this works well and their normal microbiome restores itself, preventing further CDI. Unfortunately, for many people their gut microbiome is so compromised that just diminishing the *C. diff* number is not enough; the disease comes back after antibiotic therapy is discontinued. If you were to be one of those unlucky individuals, you might be condemned to a life of on-again off-again antibiotics, permanent antibiotic therapy, possibly having your colon removed, or death – none of which are desirable to say the least![6]

Fortunately, it is this very instance, for recurrent CDI, that microbiome-based therapies have demonstrated an ability to prevent future recurrences of the disease by restoring the damaged microbiome to a more normal state. As mentioned earlier, the normal microbiome provides a measure of protection to the person and prevents *C. diff* colonisation and subsequent toxin production. The earliest and crudest form of a microbiota-based therapeutic are faecal transplants.

Faecal transplants (FT) have been used for centuries to treat gastrointestinal diseases. In the US, the pivotal reference is a publication in 1958 by Dr Ben Eiseman and his colleagues, a team of surgeons from Colorado, who treated four critically ill patients with faecal enemas for pseudomembranous enterocolitis caused by what they thought was a *Staphylcoccus* infection (before *C. difficile* was a named microbe)[7].

Until recently, FT was offered as a treatment of last resort for patients who had experienced multiple episodes of CDI where antibiotics provided only a temporary relief of symptoms. The procedure was messy and time-consuming. A donor needed to be found (usually someone known by the patient), the faecal material gathered and the product 'manufactured' and delivered by the doctor. This normally had to happen within a few hours of the stool donation to ensure the viability of the microbes and there was little or no money to be made by the treating physician. Several case series were published over the years showing anecdotal effectiveness of the therapy with few reports of adverse events.

In 2013 that all changed with the publication in the *New England Journal of Medicine* of the first randomised controlled trial of FT. It demonstrated a significant benefit of the therapy over Vancomycin with limited side-effects[8]. Suddenly FT emerged as *the* treatment. Patients started clambering for the therapy because they were desperate for a cure. And it didn't stop there. Soon FTs were being used for nearly every condition – all despite a lack of evidence of safety and efficacy. Patients travelled across continents to get the treatment, and in some cases spent multiple thousands of dollars and lots of effort to track down treating physicians.

As with many new promising therapies, the hype has preceded the actual evidence, leading many to wonder whether, as a potential therapeutic, will the microbiome be a sustainable technology or will it become an unfulfilled promise buried under the sheer volume of noise being generated? I believe, from what I have witnessed in our own drug development programme, that it is promising and it is possible to develop it to its full potential, but only if there is some discipline implemented in the field as microbiota therapeutics are being developed.

In my frame of reference as a long-term medical technology executive, a therapeutic product only becomes commercially viable when it has undergone rigorous clinical trials for safety and efficacy as part of an FDA regulatory process, and has earned regulatory approval. It is proof of our promise to the patients that depend on us to deliver, to the best of our ability, products that will do them more good than harm.

Developing a microbiota-based therapeutic has been challenging. When we first started Rebiotix, we thought: "How hard can it be? The raw material is poop and people have been dealing with that forever." Just goes to show how wrong one can be. Human stool is a complex mix of live organisms

and dead, undigested food and human components that are shed on a daily basis.

We were interested in harvesting the live microbes, but no one knew how to do that or how to tell what and how many live microbes there were in the mix. In addition to that, we only wanted the 'good' microbes, not the 'bad' ones. It took us a year to develop the test methods that we could use to characterise the product. Without a test, we could not tell if the processes or materials we were using were helping or hurting the microbes we were trying to capture. Then there was the challenge of how to preserve the microbial mix we were able to extract, inventory the product and put it in a delivery vehicle so that the microbes could get into the patients we were interested in treating.

At the beginning of the process of developing our product, we started a dialog with the FDA to understand how the product would be regulated. This was important because each type of product has different requirements for quality systems, clinical studies, regulatory submission formats and other criteria. What we thought was a tissue transplant turned into a biologic drug product regulated by the FDA in its Center for Biologics Evaluation and Research (CBER) in the Vaccines division. This gave us the direction, but because we were the first company to take a microbiota-based therapeutic through the FDA, there were no standards or precedents to follow. We found the most challenging aspect of drug development with a highly-complex microbial mix was to define potency and develop validated test methods to assure that our drug product met the quality release criteria.

Over the past five years, the complexities around developing a microbiota-based drug have not diminished, even though progress has been made. The FDA, in its efforts to make sure that it did not stand in the way of a physician and their patient, unintentionally created a competitive nightmare for those of us that were developing our drug products and complying with the regulatory process. The FDA released a guidance in 2013 whereby doctors using faecal microbiota transplants to treat recurrent CDI were not subject to enforcement of the normal FDA rules. As a result, several stool banks began supplying untested and unregulated products to the market while newly-formed companies seeking to develop microbiome-directed therapeutics had begun the challenging (and costly) process of identifying potential technologies for clinical testing as true pharmaceutical products.

Companies are developing a wide range of microbiome therapies – encompassing the range of a single genetically-modified microbe as the active ingredient, to a full spectrum naturally-occurring microbial mix to using the metabolites produced by microbes and not the live microbes themselves as the therapeutic entity. Microbial products are being delivered via colonoscopy, enema, nasogastric routes and via pills. Formulations are frozen, freeze-dried, stable at room temperature or refrigerated. Indications have expanded from recurrent CDI to ulcerative colitis, multi-drug resistant infections, autism and obesity, among others. Great and exciting developments in a short amount of time!

Though it remains to be seen which approach to the microbiome and which technology will emerge, the industry has been buoyed by recent clinical data that suggests the concept has considerable potential. As the first microbiome company to enter the clinic under FDA guidance, Rebiotix has completed enrolment in three clinical trials, including the first multinational, prospective randomised double-blind placebo-controlled trial of a microbiota-based drug in the world. The results of those studies thus far have demonstrated the clinical efficacy of the therapeutic to prevent recurrent

References

1 Marchesi, JR, Adams, DH, Fava, F et al. Gut. 2016;65:330-339. Doi: 10.1136/gutjnl – 2015-309990.

2 Gill, SR, Pop, M, DeBoy, RT et al. Metagenomic Analysis of the Human Distal Gut Microbiome. Science. 2006; 312(5778):1355-1359. doi:10.1126/science.1124234.

3 Arumugam, M, Raes, J, Pelletier, E et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174-180. doi:10.1038/nature09944.

4 Yatsunenko, T, Rey, FE, Manary, MJ et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222-227. doi:10.1038/nature11053.

5 The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207-214. doi:10.1038/nature11234.

6 Seekatz, A, Young, V. Clostridium difficile and the microbiota. J Clin Invest. 2014;124(10):4182-4189. doi.org/10.1172/JCI72336.

7 Eiseman, B et al. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44(5): 854-859.

8 van Nood, E, Vrieze, A, Nieuwdorp, M, Fuentes, S, Zoetendal, EG, de Vos, WM, Visser, CE, Kuijper, EJ, Bartelsman, JF, Tijssen, JG, Speelman, P, Dijkgraaf, MG, Keller, JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013 Jan 31;368(5):407-15. doi: 10.1056/NEJMoa1205037.

CDI in nearly 90% of the patients treated within the study protocols.

What will the future hold for Microbiome Therapeutics? The National Microbiome Initiative has three goals: supporting interdisciplinary research; developing platform technologies; and expanding the microbiome workforce. Assuming that it is successful, I would expect to see more collaboration between big pharma, biotech and academia to support microbiome product development and clinical applications. Several of these collaborations are already in process: Nestle and Seres Therapeutics, Johnson & Johnson and Vedanta, and Synlogic and Abbvie to name a few. Precision or personalised medicine will expand to include the microbiome. With that will come new diagnostic tools. Companies such as Enterome, UBiome and Genetic Analysis are already working on them. Platform technologies will be developed, new indications will continue to expand and products will become commercially available. Rebiotix is involved in a number of different feasibility studies for indications outside of CDI.

Finally, I anticipate that more investments will be made. I am excited to see what happens over the next 20 years and happy to be part of this once-in-a-lifetime opportunity to shape a brand new industry and potentially treat a host of diseases with a technology that, at its essence, has been inside of us – and under our noses – for as long as time itself. DDW

Lee Jones *is the Founder, President and CEO of Rebiotix, a clinical-stage biotechnology company focused on harnessing the power of the human microbiome to revolutionise the treatment of challenging diseases. Lee has more than 30 years of experience as a healthcare executive and entrepreneur with large and small companies and in academia. Lee founded Rebiotix in 2011, building the company around its pioneering Microbiota Restoration Therapy, a drug technology platform that is designed to rehabilitate the human microbiome by delivering a broad spectrum of live microbes into a patient's intestinal tract. Today, Rebiotix is the most clinically advanced microbiome company in the industry.*

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