Commentary: Duncan Peyton

Live biotherapeutics change the pharma landscape

In recent years, the human microbiome has been a focus of academic and industrial research to understand how the community of microorganisms in the gut could have an impact on human health. The gut is a home to tens of trillions of microbes including at least 1,000 different species of bacteria. The microbiome has been implicated in the maintenance or modification of an ever-growing number of human systems, including the immune, metabolic, nervous and endocrine systems. Improvements in gene sequencing technologies and other 'multi-omics' analytics have made it possible for scientists to study both entire microbial communities and specific components of the microbiome, and unpick how they interact with their human host in health and disease. This understanding has also led to the development of an entirely new type of pharmaceutical product.

Live biotherapeutic products, or LBPs, are a new class of drug derived from the microbiome. The US Food and Drug Administration defines LBPs as biological products that contain live organisms such as bacteria and which can be used to prevent, treat or cure a disease. They can be drawn from nature, or be genetically modified. They are not vaccines or gene therapy vectors, but rather an entirely new therapeutic modality.

In this article, I describe how our company, 4D pharma Plc, is exploiting this new therapy type and in particular, how LBPs can be used to treat cancer. 4D pharma has a portfolio of clinical and preclinical drug candidates across a range of indications from oncology to asthma to central nervous system (CNS) conditions. Clinical programmes include Phase 1/2 studies in a number of solid tumours, a Phase 1/2 study in asthma and a recently completed Phase 2 trial in irritable bowel syndrome (IBS).

Clinical strategy

4D pharma's approach to LBP discovery is focused on understanding and leveraging the natural functionality of bacteria and host-microbe interactions for therapeutic effect. Our drug candidates are single strains of non-engineered commensal bacteria, originally isolated from the gut microbiome of healthy human donors. They are grown at our in-house GMP manufacturing facility, freeze-dried and produced as an oral capsule specifically formulated to deliver the drug to the intestine. When the freeze-dried bacteria encounter the conditions of the intestine (like pH, temperature, and water content) they are reanimated and become active.

We have built a library of live bacterial isolates, with near-complete coverage of the breadth of the genera present in the human microbiome. Using our proprietary discovery platform, MicroRx, we interrogate our library to identify specific strains displaying functional profiles of interest for use as therapeutics. Our emphasis is on causation, not correlation. This means that we look for functional signatures relevant to a target disease in a rational drug discovery approach, irrespective of a taxonomic classification or natural abundance in the human microbiome. We could be looking for activity against a specific target molecule, a known pathway or human cell type implicated in the disease. We do this to a high degree of specificity, looking at individual strains or isolates not just the species or genus level. This is important because even different strains of the same species can have significantly different levels of activity.

Once we have identified lead LBP candidates, we go beyond showing that they work, we want to understand *how* they work. This includes identifying the key molecules produced or modified by the strain and characterising their impacts on the human host in detail. We do have extensive genetic engineering capabilities, but we use these as tools to study the mechanisms of action of naturally occurring bacteria rather than engineering new functionality into strains. This central focus on, and in-depth understanding of, function is unique to 4D pharma among companies developing microbiome therapeutics.

4D pharma's lead immuno-oncology programme MRx0518, which is currently being evaluated in three separate clinical trials, is an example of this 'function first' approach. The drug candidate was initially identified through a broad immunostimulatory screening programme incorporating a number of *in vitro* human immune cell assays. This not only identified the immunostimulatory activity but also characterised it through the particular profile of cytokines, chemokines and other molecular signatures induced in cells treated with the compound, or its supernatant.

The anti-tumour activity was then tested in multiple mouse models of solid tumours. Mice orally administered MRx0518 showed reduced tumour growth, including models that are considered resistant to other cancer therapies such as immune checkpoint inhibitors. Furthermore, the compound showed activity in combination with immune checkpoint inhibitors in mouse models.

Beyond demonstrating the ability of an oral LBP to reduce tumour growth, 4D pharma also conducted extensive biomarker analyses in these animal models to characterise the effects of MRx0518 on the immune system and how these relate to anti-tumour activity. MRx0518 increased the infiltration of the tumour microenvironment by specific immune cell subsets including T cells, CD8+ T cells, cytotoxic cells and natural killer (NK) cells. These subsets are known to be associated with better responses to treatment, particularly with checkpoint inhibitors, and better clinical outcomes in patients.

But what was the mechanism by which MRx0518 induced these immuno-stimulatory effects? This required more work. Using a combination of immune assays, genome mining and genetic engineering, the company identified the flagellin protein, a subunit protein of the flagellum, a whip-

like structure that gives a bacterium the ability to move independently. This protein is a potent agonist of toll-like receptor 5 (TLR5), a receptor which triggers activation of the innate arm of the immune system. This leads to increased production of certain cytokines and chemokines – immune signalling molecules – and the activation of immune cells including dendritic cells and natural killer (NK) cells. Comparisons of the MRx0518 flagellin protein to that of closely related bacteria indicated that the flagellin produced by the 518 isolate was a particularly potent immune activator. Further analysis identified differences in the flagellin protein structure and genetic sequence of MRx0518 compared with related strains.

4D pharma designed a two-arm initial clinical development strategy for its lead oncology asset. Two very different trial designs, and their results, are answering different yet complimentary questions about the activity of MRx0518 in cancer patients.

A Phase 1b neoadjuvant study in which MRx0518 is given as a monotherapy for two to four weeks prior to surgical resection of a solid tumour has completed Part A. The trial was conducted with Imperial College London and was designed as a biomarker study with the goal of understanding the impact of MRx0518 on tumours and the immunology of the participants. Patients in the study had no prior treatments, or concurrent therapies. We have tumour and other biomarker samples at baseline diagnosis and after resection. In this respect, the trial design provides a clean look at the activity of MRx0518, with before and after samples for each patient.

The results show that treatment with oral MRx0518 for just two to four weeks induced a strong upregulation of certain immune cell subsets with known anti-tumour activity. These included the same subsets upregulated in the animal studies, which is a positive reflection on the MicroRx platform, our pre-clinical development, and is hopefully a good signal for the rest of our pipeline discovered using MicroRx. There was also an increase in the ratio of anti-tumour CD8+ T cells to immune-dampening regulatory T cells (Tregs) – a well-known prognostic factor in cancer treatment – and an increase in certain cytokines and chemokines such as IL-12 and CXCL10. Pro-inflammatory and anti-tumour gene expression pathways were also upregulated. These results not only demonstrate the single agent biological activity of MRx0518, but also indicate a mechanism of action that is complimentary to immune checkpoint inhibitors and potentially other types of cancer therapy.

The second trial of 4D pharma's initial two-pronged clinical strategy is a Phase 1/2 combination study of MRx0518 and leading immune checkpoint inhibitor Keytruda (pembrolizumab). The trial is in heavily pre-treated patients who are resistant to prior treatment with a checkpoint inhibitor and have no therapeutic options remaining. We want to show that the immunostimulatory activity of MRx0518 can re-engage the body's response to a checkpoint inhibitor. The trial consists of two parts. Part A, which included 12 patients with renal cell carcinoma and non-small cell lung cancer, has completed. It showed a clinical benefit in five (42%) patients, including objective responses in three (25%) patients and stable disease for more than six months in another two (17%) patients. Importantly, MRx0518 also showed a favourable safety profile, with no increase in adverse events over what would be expected for pembrolizumab alone. This is a major differentiator in oncology where therapies are invariably associated with toxicities.

In light of these results, the company has expanded Part B of the trial to include patients with triple negative breast cancer, squamous cell carcinoma of the head and neck, bladder cancer and microsatellite instability high tumours, in addition to lung and renal cancers. Part B is ongoing and we expect to complete enrolment by the end of 2021.

Expanding the clinical programme

A third oncology trial is currently being conducted in pancreatic cancer with the University of Texas MD Anderson Cancer Center in the US, with whom 4D pharma has a long-term strategic partnership. Pancreatic cancer is known to be non-immunogenic. The question is whether the immune stimulating activity of MRx0518 can turn 'cold tumours' into 'hot tumours,' something that is being explored by other companies with a variety of compounds. The clean safety profile of MRx0518 and LBPs as a drug class may mean they are particularly attractive in earlier-stage patients. This study will assess biomarkers of anti-tumour and immunomodulatory activity in addition to obtaining readouts on progression-free survival and overall survival. Initial data from the study is expected towards the end of 2021.

Recently, 4D pharma entered into a second oncology clinical combination collaboration – this time with Merck KGaA and Pfizer Inc. Under this arrangement, the company will evaluate MRx0518 as a first-line maintenance therapy in urothelial carcinoma in combination with Bavencio (avelumab), the first and only checkpoint inhibitor approved in this indication. This study expands the MRx0518 clinical programme in two important ways. First, it complements the last-line setting of the ongoing Keytruda combination study and moves MRx0518 into the first-line setting, and second, it expands the checkpoint inhibitor combinations for MRx0518 from anti-PD-1 (Keytruda) to also now anti-PD-L1 (Bavencio).

Forward thinking

As live biotherapeutics continue to advance through clinical trials and towards commercialisation, there will be a corresponding need for production capacity. In addition, the necessary expertise to work with strains with very specific requirements, in an efficient, highly reproducible and commercially viable way, is essential. We believe that 4D pharma is strides ahead of the field with its internal capabilities and GMP-certified facilities for LBP production for clinical development and early commercial-scale production.

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