Microbiome-based therapies as anti-cancer agents

Over the last decade immunotherapies, most notably immune checkpoint inhibitors (ICI), have been transformational in cancer treatment and have resulted in long-term survival of patients in many cancers, including previously difficult to treat cancers and advanced solid tumours.[1] Consequently, the ICIs comprise one of the most important advances in the pharmaceutical industry, with Keytruda currently the world's third biggest selling medicine. Sales for this class of drugs are predicted to reach $55 billion in 2025. ICIs act by removing a protective cloak from the tumour cells, allowing the patient's own immune system to eradicate the tumour. However, remarkably for such a successful class of medicines, only 10-50% of the patients respond, even in cancers that are not intrinsically refractory to immunotherapy. There is therefore intense interest in identifying the mechanisms for non-response in the expectation that this will lead to approaches that extend the number of responding patients.[2]

From 2016 onwards, studies from several groups have shown the key role of the gut microbiome in ICI drug response.[3] Within the microbiome field this was not wholly unexpected since the gut microbiome has long been known to be critical in the correct functioning of the human immune system, from neonates onwards, and in regulating the adult human immune system.[4] Two types of early studies were important in demonstrating this association and the causal role of the microbiota.

One kind of study involves a standard microbiome experimental paradigm using 'gnotobiotic' mice to transfer phenotype. Faecal microbiota transfer (FMT) was undertaken from responding or non-responding patients into germ-free mice such that the mice then had humanised microbiomes akin to the different types of patients. When tumour models were established in these mice, it was shown that the ICI drug response phenotype was also transferred, corresponding with the patient source. In other words, germ-free mice engrafted with non-responder microbiome would not respond to anti-PD1 therapy. However the same treatment would be effective if FMT were carried out from responding patients. In the last 18 months, this type of study has been moved to a fully human setting with two human FMT studies in advanced melanoma patients. In both studies, patients that had previously failed to benefit from ICI therapy responded after receiving the microbiome from a patient that had previously responded.[5]

In the second type of study, five research groups analysed the gut microbiome in small clinical cohorts prior to ICI treatment, seeking to find a correlation with drug response. They identified certain bacteria that appeared to correlate with response. However the bacteria identified by each group were completely unrelated, casting doubt on the field.[6]

Within the microbiome field, the most advanced therapeutic approach uses the delivery of live gut bacteria to correct imbalances in the patient's gut microbiota or add therapeutic bacteria that transfer specific beneficial properties. These live biotherapeutic products (LBP) are now clinically precedented for *Clostridium difficile* treatment, using either donor-derived products or defined consortia of therapeutic bacteria, and in earlier stages of development for graft versus host disease, inflammatory bowel disease, and atopic dermatitis. While this emerging modality is still young, the principles of delivery and engraftment of bacteria in the gut have been established, the therapeutic potential is very broad, and the bacteria are deemed entirely harmless. Therefore companies have pursued LBPs with the aim of converting cancer patients from non-responders to responders. However the differences in the bacteria identified from clinical studies have led companies to primarily pursue LBPs identified by functional screening of bacteria rather than from clinical data. This inevitably places a burden on lab-based models to be predictive of clinical effects and restricts the mechanisms of the products to pre-existing hypotheses.

Clinic-based discovery

Microbiotica has developed a leading microbiome analysis platform to identify bacteria closely linked to phenotype from large clinical datasets, and use these bacterial signatures as combined biomarkers and therapeutic LBPs in a precision medicine approach. The platform is founded on developments at the Wellcome Sanger Institute which have been taken further to establish a unique platform combining mass culturing from patients, the leading gut bacterial reference genome database and leading machine learning technologies. Together these enable identification of previously missed bacterial signatures linked to phenotype for development as live bacterial therapeutics or biomarkers.

The Microbiotica platform was used to analyse the samples from a new microbiome study investigating the link to ICI response in advanced melanoma patients. The study, MELRESIST, was performed in collaboration with Cambridge University Hospitals and had longitudinal sampling and comprehensive clinical meta-data for a deep understanding of how the intestinal bacteria interact with ICI therapy. Profiling of the pre-treatment MELRESIST samples, followed by machine learning analysis, identified a discrete signature of gut bacteria associated with response. This analysis was extended using publicly available melanoma datasets to identify commensal species consistently altered in abundance in responding patients, as compared with non-responders.

To demonstrate the association of these species with outcome, an algorithm was developed based on the abundance of these species to predict whether or not a patient would respond to ICI treatment. The signature was 91% predictive of response across four cohorts of malignant melanoma patients based solely on their microbiome prior to therapy. It was also very predictive in a non-small cell lung cancer study (ROC curve AUC 0.82). The association of the bacteria with response in renal cell carcinoma was weaker (ROC AUC 0.74), which probably reflects the immunological differences between carcinogen-driven cancers and those with a lower mutation burden.

Therefore the Microbiotica platform, combined with the MELRESIST study, has enabled the discovery of a

microbiome signature predictive of response across multiple cohorts. This resolved a significant issue in the field where different signals were identified by independent analyses of different studies. Microbiotica is progressing a therapeutic LBP and a biomarker for clinical use from these results.

Given the impact of the microbiome on immunotherapy, modulation of these bacterial species could increase the efficacy of ICI in melanoma and beyond. The Microbiotica signature was heavily weighted toward bacteria elevated (rather than reduced) in abundance, suggesting that the primary mechanism is activation of anti-tumour immunity by "good" bacteria rather than suppression by "bad" bacteria. This obviously lends itself to the development of an LBP comprising bacteria associated with response as a co-therapy for ICIs in order to deepen and broaden the benefit of these drugs. In-depth analysis highlighted the importance of nine commensal species at the core of the signature. These were independently predictive of response; highly significant in the machine learning algorithm; and amongst the most differentially represented species. These species are taxonomically diverse and represent the three main phyla present in the human intestines. Four of the nine are novel species that have not been previously described, underlining the power of the Microbiotica platform and how it was able to identify a cross-cohort signature where others had failed. Our product, MB097, is a therapeutic bacterial consortium composed of a representative strain from each of the nine species that has been rigorously profiled for safety and suitability for development.

Although the nine species comprising MB097 are strongly associated with clinical response to ICI, the ability of the consortium to drive an anti-tumour response was tested i*n vivo*. Syngeneic tumour models are the gold standard for *in vivo* testing of anti-cancer immunotherapies and have been extensively used in the development of ICI. These same models were also used to demonstrate the transfer of ICI responsiveness to humanised mice.

Bacterial therapeutic development

In a mouse syngeneic cancer model, MB097 caused a small but reproducible retardation in tumour growth. However, the combination of MB097 with anti-PD1 was significantly more potent than either treatment alone, suggesting a synergistic effect thereby validating the proposed effect of MB097 as an enhancer of ICI response. Transcriptomic analysis of the tumours indicates that MB097 upregulated a number of pathways and cell types essential to anti-tumour responses including antigen presentation pathways, natural killer (NK) cell function and cytotoxic T lymphocytes (CTLs). Importantly, these have also been associated with ICI efficacy.

To further explore the mechanisms found *in vivo* and to extend the observations to an *ex vivo* human system, the MB097 strains were tested in *in vitro* primary immune cell assays of dendritic cell, NK cell and CTL function. All of the MB097 strains in isolation potently stimulated human primary dendritic cells, as expected for bacteria. However MB097 strains induced either similar or higher levels of IL-12 relative to IL-10 when compared to known, very potent microbial stimuli. This suggested the dendritic cell phenotype was polarised to strongly prime cytotoxic T lymphocytes (CTLs). Indeed, the dendritic cells stimulated by MB097 strains were able to potently activate allogenic CTLs, as demonstrated by upregulation of perforin, Granzyme B and IFN-gamma. These CTLs were able to kill tumour cells *in vitro*. In addition, in a similar assay, the strains also drove NK cell activation and subsequent tumour cell killing. These data not only support the functional validation of MB097 in human models, but begin to dissect the mechanism by which the host microbiome influences ICI response.

The MB097 strains are currently being scaled up for manufacture with the aim of entering the clinic early next year. The clinically driven design of MB097 provides a strong pointer for clinical development. The species are associated with response to anti-PD1 containing therapy in multiple advanced melanoma cohorts. Indeed, further analysis of the original datasets indicates that 80% of patients failing anti-PD1 therapy have a microbiome that is not permissive of ICI response. The first in human study for MB097 will look at safety and tolerability but also efficacy in advanced melanoma patients who are failing to respond to ICI treatment. This will keep the trial as close to the discovery cohorts as possible and will enrich for patients most likely to benefit from MB097. Going forward, it is anticipated that the biomarker for ICI response that was derived from the same microbiome signature will identify patients that would benefit from the combination of MB097 and anti-PD1 before treatment initiation, i.e. first-line treatment.

In summary, the MELRESIST study combined with Microbiotica's platform has enabled the discovery of a microbial signature associated with ICI response across multiple cohorts. This has enabled a clinic-first precision medicine approach to designing a microbiome-based co-therapy for ICI use in advanced melanoma and potentially other indications such as lung cancer.

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