**Understanding response to cancer therapy: time to do a gut check**

**-**Vancheswaran Gopalakrishnan, Jennifer A. Wargo

How human are we? Throughout our evolution, we have played willing hosts to trillions of commensal microorganisms, as numerous as our own cells, vastly outnumbering our genome, and inhabiting different niches within our bodies. Collectively called the microbiome, these commensals are anything but passive bystanders, and help us perform several vital functions such as digestion, metabolism, guarding against pathogen invasion, and maintenance of immune function. A growing armamentarium of next-generation sequencing tools such as 16S rRNA sequencing and Whole Genome Shotgun Sequencing (WGS), has afforded us the ability to perform comprehensive characterization of these microbiota, and comparisons between groups.

The scientific community continues to make massive strides in our battle against cancer through the advent of effective therapies harnessing the body’s own immune system to fight cancer, called immunotherapy. In melanoma (an aggressive form of skin cancer), they have led to durable cures in patients that would otherwise succumb to their disease rather quickly, but the majority of patients will ultimately become resistant to these therapies. There has been a tremendous investment by researchers across the world to understand this phenomenon, and we are beginning to appreciate the strong influence of gut commensals on the immune system, and on mediating differential responses to anti-cancer immunotherapy.

In order to study this phenomenon, we assembled a large cohort of melanoma patients who had advanced disease, and were beginning treatment with anti-PD1 immunotherapy. All patients were classified as responders (R) or non-responders (NR) based on tumor measurements that were done on serial radiologic scans. Oral (cheek swab) and gut microbiome (stool) samples were collected at the start of treatment, and, if feasible, an additional sample was obtained while on therapy. Characterization of the microbiota revealed significantly higher alpha-diversity in the gut microbiome at baseline in patients who responded to therapy compared to those who did not. As the name suggests, alpha-diversity is measure of how diverse a population is, with greater alpha-diversity indicative of a greater variety of the types of bacteria and more of each type. Of note, no differences were seen in the oral microbiome.

We were also interested in identifying compositional differences between groups. Specifically, we found an overrepresentation of the *Faecalibacterium* genus of the Ruminococcaceae family of the Clostridiales order in R, and the Bacteroidales order in NR. Additionally, WGS performed on a subset of samples, confirmed the aforementioned compositional differences, and identified differences in metabolic capabilities of the bacterial communities, with biosynthetic functions predominating in R and degradative functions in NR. All our findings were adjusted for established clinical and genomic predictors of response.

Tumor and blood samples were also collected at matched pre-treatment time points, to understand the nature of associations between gut bacteria and immune infiltrates. The presence of beneficial Ruminococcaceae in gut was strongly associated with increased infiltration by CD8+ killer T-cells both in the vicinity of the tumor and in the systemic circulation.

Lastly, to ascertain causality, fecal microbiota transplant experiments were performed using germ-free mice. These mice, which were initially devoid of any microbiota in their gut, were transplanted with stool from either a R or NR patient, using oral gavage. Subsequently, they were implanted with melanoma cells and treated with immunotherapy. Strikingly, mice that were colonized with R-microbiota grew much smaller tumors and responded significantly better to therapy compared to mice that were colonized with NR- microbiota. Characterization of stool samples, revealed transfer of the phenotype, with an enrichment of *Faecalibacterium* in R-mice. Immune-profiling performed on tumor and gut samples harvested from these mice also revealed an enhanced infiltrate of beneficial immune cells in mice that received R-stool.

Importantly, similar findings have been reported by other investigators, and differential bacterial abundances have been linked to enhanced responses to immunotherapy across several cancer types, mediated by critical changes in the immune system. While there is still a lot to learn, these findings have several important implications. The microbiome appears to shape a patient’s response to cancer immunotherapy, which opens up the potential to use it as either a diagnostic tool to identify who will benefit from these therapies, or as a therapeutic adjunct which can be modulated to improve existing therapeutic efficacy. Every person’s microbiome is unique and has been shown to be influenced by diet, exercise, antibiotic and probiotic use, and fecal microbiota transplantation, though this must be carefully tested in the context of clinical trials. We are involved in a concerted effort with Seres Therapeutics and the Parker Institute of Cancer Immunotherapy, to understand these phenomena better, and hope to launch a trial in 2018 which that combines immunotherapy with microbiome modulators in patients with advanced melanoma.