Probiotics as potentiators of cancer immunotherapy.

Shireesh P. Apte*

Editorial Board, Journal of Excipients and Food Chemicals

Editorial

KEYWORDS: Microbiome, immunotherapy, cancer, immune checkpoint inhibitors, probiotics, excipients

Whether the composition of the gut microbiome modulates cancer immunogenicity is no longer a moot point; numerous publications attest to the affirmative in this still evolving paradigm. Oral administration of effect isolated bacteria suppressed implanted melanoma growth to the same degree as immune checkpoint blockade (ICB) therapy in murine models. Animal fecal transplantation studies demonstrated that certain species of live commensal bacteria increased systemic tumor specific T-cell responses and intratumoral $CD8^+$ T-cell accumulation independently of bacterial translocation into the lymphatic circulation. These effects derived from upstream immune activation, partly achieved by upregulating gene transcription, at the level of major histocompatibility complex (MHC) class II host dendritic cells (DCs) that induced T-cell proliferation at lower antigen concentrations. The effects were abrogated in CD8 T-cell depleted animals, suggesting the involvement of a host immune component.

Due to interspecies competitive exclusion, i.e., the alteration of one or several bacterial species due to expansion (ingestion) of other(s); it is difficult to unravel an indirect effect acting *via* modulation of the abundance of other bacterial species; from a direct causative effect of positive regulation of anti-tumor immunity. However, regardless of which genus and species is actually responsible, the fact remains that the composition of the microbiome can affect the prognosis of treatment with ICB therapy.

As a corollary, the immunostimulatory effects induced by cytotoxic T-lymphocyte antigen, CTLA-4 or the programmed death ligand, PD-L1 blockade could be amplified by certain species of the intestinal microbiome; dysbiosis of the gut microbiota influences the outcome of cancer immunotherapy. The therapeutic efficacy of Anti-CTLA-4-mAb was abrogated in mice reared under germ-free (GF) conditions and attenuated in patients treated with broad spectrum antibiotics.

Among the most studied species of bacteria shown to potentiate checkpoint inhibitor blockers in animal models are *Bacteroides.fragilis*, *Bifidobacterium.breve, Bifidobacterium.longum, Bifidobacterium adolescentis, Akkermansiacea muciniphila* and those from the genus Ruminococcaceae. A search using the keywords "cancer" and "probiotics" returned 39 studies from the website http://www.clinicaltrials.gov. One study protocol in particular, NCT03358511, titled "engineering the gut microbiome to target breast cancer", sponsored by the

*Corresponding address: 5204 Coventry Court, Colleyville, TX 76034, Tel: 817 501 2984, E-mail: shireeshpapte@msn.com

Mayo Clinic, involves the administration of probiotics, three times a day, for 2-4 weeks prior to surgery in operable stage I-III breast adenocarcinoma tumors ≥1.0 cm. The particular probiotic, Primal Defense Ultra® Probiotic Formula, is an over-the-counter probiotic that provides 15 billion colony forming units of 13 species of beneficial bacteria, including *Saccharomyces boulardii, Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium lactis, Bifidobacterium bifidum, Lactobacillus rhamnosus, Bifidobacterium breve, lactobacillus casei, Lactobacillus salivarius, Lactobacillus acidophilus, Lactobacillus brevis, Bifidobacterium longum,* and *Lactobacillus paracasei*.

The advent of such clinical studies suggests that the clinical benefits of commensal gut microbiota engineering are significant to attempt replication in the clinic with an (as yet) sparse mechanistic understanding, similar to CAR-T therapy, whose mechanism(s) of action is still a work-in-progress. However, contrary to the latter, where selected chimeric antigens can be expressed on engineered T-cells that exert their effect independently of MHC antigen display, the microbiome is composed of taxonomically different ~ 35 x 10^{12} bacterial cells, which sets a much higher bar regarding an understanding of how a synergistic effect with ICB therapy is achieved.

A knowledge of the mechanisms by which commensal gut bacteria may influence the immune response to carcinogenesis is accumulating, but is still poorly understood, and could be critical in designing productive clinical studies, especially in light of the intersection of the microbiota-T_{reg}-T_{eff}-ICB axis. CTLA-4 blockade expands $FOXP3^+$ and $CD4^+$ T_{reg}-cells in a dose-dependent manner. The conundrum of how and why the phenotype of T_{reg} cells function as a cancer promoter in some studies and a suppressor in others, was found to be dependent on prior exposure to gut bacterial antigens and the presence of IL-10; allowing for a wider interpretation of the 'hygiene hypothesis' to include gut microbiota dependent differential susceptibility to neoplastic disease.

The prognostic value of T_{reg} also differed with the dose administered and the tumor stage; tumor infiltrating T_{reg} allowed for immune escape early in tumor progression while increasing survival in late-stage or metastatic disease. Additionally, different intratumoral $CD8^+$:T_{reg} ratios and the presence of *effector* $FoxP3^+$ cell subsets within the $FOXP3^+$ T_{reg} population were proposed as explanations. Microbial proteins might be sufficiently similar to tumor antigens to elicit an immune response *via* antigenic mimicry, cross-reactivity or *via* toll like receptor (TLR) associated pathogen associated molecular patterns (PAMPs). Microbial polysaccharides, such as those containing zwitterionic domains also modulate T-cells. Intra-epithelial or lamina propria located T-cells, primed by microbial protein or polysaccharide antigens could translocate to extra-intestinal sites to recognize tumor associated antigens (TAAs).

Engineered abundance of select commensal species by ingesting probiotics may exert an indirect effect by changing the abundance(s) of other (as yet unknown, undetermined or unconsidered) gut bacterial species, whose functionality, in turn, may depend on (as yet unknown, undetermined or unconsidered) factors. The 16s RNA gene is a good working standard, but a far from ideal marker for molecular diversity. Consequently, results are reasonably accurate to the order or phyla level, but need algorithms to extrapolate down to the species level. Reported microbiome compositions assume that their targets are amenable to existing sequencing and annotation methodologies, the compositions reported hence correspond to the *known* microbiome. The differences in the *known* microbiome of responders and non-responders to ICB therapy may hence have as much to do with causation/correlation as with pure chance (observational bias or the streetlight effect); although the latter can be significantly reduced by administering species subsets from responder and non-responder subjects to GF mice.

Lastly, the usual caveat of animal studies not being extrapolable to humans is compounded exponentially because 85% of gut-colonized microbes in mice are not found in humans, and the microbiome in no two individuals is exactly the same. Taken together, the

probability of near-term clinical success of microbiome altering probiotics in conjunction with ICB therapy may be realized; but is likely to be uncertain and unpredictable.

Judiciously engineering the gut microbiota has the potential to decrease the responder variability, acquired resistance as well as the irreversible adverse effects (irAEs) of ICB therapy, thereby increasing its efficacy. However, 'judicious' in this case would necessarily have to incorporate confounding or data-sparse factors. Differentiation from placebo with this multi-factorial paradigm is expected to be challenging and will significantly depend on limiting (or segregating) (poorly understood) mechanisms and/or effects that work at cross-purposes of all the players involved as well as keeping the microbiota composition of patients on the treatment and placebo arms constant for the study duration. Since diet influences the gut microbiome composition, diet guidelines may need to be included in the study protocol.

The good news is that since probiotics contain commensal bacteria that are relatively benign, recourse could be taken to anecdotal data to identify a cocktail that appears to synergize with ICB therapy. Assuming correlation, microbiome composition of responders versus non-responders to ICB therapy may identify commensal bacteria that could increase the proportion of responders. Such an approach is currently underway with Seres Therapeutics probiotic, SER-401, that is based on research demonstrating that responders had more *Ruminococcaceae*-order and fewer *Bacteroidales*-order species in their microbiome. A Merck sponsored clinical trial involves transplanting faecal microbiota from PD-1 responders into non-responders, thereby bypassing the mechanism-gap and observational bias in microbiome manipulation. Vedanta biosciences plans to advance what they designate as a *rationally defined consortium* into the clinic. This comprises human derived, purified bacterial mixture of six or more live bacterial strains belonging to *Clostridium* clusters IV and/or XIVa and capable of inducing proliferation/accumulation of T_{reg} cells. Evelo biosciences conceptualizes "monoclonal microbials"; specific single microbial strains that lead to robust and reproducible cytokine release profiles and phenotype changes to the immune system, independently of background microbiome and independently of diet. Clinical trials of probiotics and ICBs in conjunction with cyclophosphamide; a gut permeability enhancer and lymphatic translocator, or in conjunction with select antibiotics, to decrease ICB inhibitory commensal populations; may significantly improve clinical outcomes.

The connection between cancer and bacteria dates to the late 19th century following William Coley's attempts to cure sarcomas by local injection of bacteria, the so-called "Coley's toxin". In the 1970s, Morales demonstrated that Bacillus calmette-Guerin (BCG) was effective in preventing recurrence of non-muscle invasive bladder cancer, despite trouble obtaining funding; with one reviewer commenting; "BCG is not only ineffective and dangerous but a throwback from the stone age of tumor immunology".

The composition of the microbiome undeniably plays a role in the host-immune response to carcinogenesis. Over-the-counter probiotic dietary supplements can alter low-level taxonomic species microbiota composition to generally mimic the most-investigated and beneficial immune-sensitizing bacteria in animal studies. Probiotics manufactured for FDA certification can alter high-level or low-level taxonomic gut microbiota composition to mimic that found in responders to ICB therapy, or to reproducibly alter the immune phenotype in predetermined ways respectively. It remains to be seen, whether changes at either, or both, level of taxa, in and of themselves, are sufficient to reproducibly demonstrate additive or synergistic effects with ICB therapy.

Probiotics, considered as innocuous and pharmacologically inactive substances are poised to come into their own as potentiators of anti-cancer immunotherapy.