Live Biotherapeutic Products as Cancer Treatments

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ABSTRACT

Almost every aspect of cancer can be influenced by microbiota including tumor onset, progression, and response to therapy. The increasing evidence of the role of microbiota in human health and disease has reinvigorated the interest in designing microbial products that can affect cancer outcomes. Researchers have made numerous attempts to develop safe, engineered biotherapeutic products for cancer treatment using synthetic biology tools. Despite the progress, only Bacillus Calmette-Guérin is approved for human use. Here, we highlight the recent advances and current challenges in using live bacteria as cancer therapeutics.

Evidence for microbial treatment of cancer is ancient, dating back to 2600 BC. The Egyptian physician Imhotep developed a poultice that, when applied to a tumor, caused infection and subsequent tumor regression. While this unknowingly developed the first precursor to immunotherapy, the first explicitly bacterially based cancer therapy was invented in the late 1800s by Busch and Coley, who independently tested *Streptococcus* and *Serratia* infection in patients affected by terminal cancer. While controversial and sometimes lethal, this strategy cured 30% of treated patients, giving them 10+ years of tumor-free survival. Improved methods evolved over time; for instance, replacing live bacteria with inactivated bacteria or antigens. A current example of this approach is Bacillus Calmette-Guérin (attenuated *Mycobacterium bovis*) in treating bladder cancer. Notwithstanding the initial excitement, the use of bacterial products declined due to their potentially hazardous nature (e.g., induction of systemic infections) and the advent of chemotherapy and radiotherapy. In the past few decades, the discovery of the influence of microbiota on tumor onset, progression, and response to therapy as well as technological advancements in synthetic biology and bioengineering have resurrected live biotherapeutic products as a potential treatment for cancer. Live biotherapeutic products range from transplantation of whole communities of organisms to introduction of single engineered or non-engineered bacterial strains. We highlight their benefits, discuss their potential side effects, and briefly describe how each of these approaches can be applied to cancer therapy.

Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) consists of the collection and preparation of a healthy donor's stool for introduction into the gastrointestinal system of an individual with disease. FMT is currently approved to treat nonantibiotic-responsive *Clostridium difficile* infections. Because the gut microbiome can modulate cancer therapies, FMT is now being tested in cancer patients to improve treatment outcomes or reduce treatment induced adverse events. For example, the composition of the gut microbiota differs between responders and nonresponders to anti-programmed cell death-1 (PD-1) immunotherapy (1). Gnotobiotic mice who received stool from responders experienced melanoma shrinkage after anti–PD-1 treatment compared with those who received stool from nonresponders, thus demonstrating that FMT from responders sensitize the tumor to immune checkpoint blockade (ICB) therapy (1). These results led to two clinical trials of FMT evaluating its safety and efficacy in patients affected by metastatic melanoma refractory to ICB or BRAF-targeted therapy (2). In these small single-arm clinical trials, 26 initially nonresponsive patients received FMT treatment with stool donated by two anti–PD-1 responsive patients prior to receiving therapy again. Remarkably, the donor microbiota caused sensitivity to anti–PD-1 in 25% of patients. However, whether FMT is effective in larger populations or has long-term benefits (e.g., tumor free and overall survival) in the context of cancer therapeutics is not yet known. It is noteworthy that the composition of the microbiome influences not only the efficacy of therapy, but also the occurrence of adverse events related to ICB, such as ICB-induced colitis. This insight has driven the exploration of FMT as a therapeutic option to not only enhance treatment outcomes but also to address adverse events in patients who are unresponsive to conventional immunosuppressive treatments.

Clinical trials currently underway in patients with solid tumors (NCT05502913, NCT04264975, NCT04577729) and hematologic tumors (NCT04935684, NCT03678493) may soon provide new insights into the use of FMT in cancer treatment. In most cases, a recipient's original microbiome composition returns after treatment with FMT within days. The manufacturing and screening of the final product can be expensive and may require recurrent administration to achieve a clinical response. Though this procedure is recognized as being safe, a case report indicated that significant morbidity and mortality from infections caused by extended spectrum beta lactamase (ESBL) bacteria and other organisms have occurred in immunocompromised individuals. Of note in this case report, the donor microbiota was not screened for ESBL organisms prior to administration. But perhaps the most frustrating aspect of FMT is that the patients' responses to the treatment are highly variable. This is driven by a poor understanding of how to identify the most effective donors–recipient pairs. Because of the cost of finding appropriate healthy donors and screening the donated stool for ESBL and other infectious agents, defined communities that can be administered orally (i.e., fecal pills) have become more appealing. Ongoing studies using this type of live biotherapeutic products will soon determine whether defined communities are as effective as FMT in treating cancer, or reducing therapy-related adverse effects, while minimizing the patient-to-patient variability of response.

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Probiotics

Probiotics are live microorganisms that can confer health benefits. They can be found in dairy and fermented foods or in concentrated pill formulations. A major advantage of probiotics is that they are easily produced in large amounts and dosed and administered like a drug, though most are currently used as complementary health adjuvants. Recently, many research groups have explored the connection between probiotics and immunotherapy. For example, studies in mice have shown that *Bifidobacterium longum* and *Bifidobacterium breve* can stimulate anti-melanoma CD8 T cells and control the tumor to the same extent as anti–PD-L1 therapy (3). In addition, mouse MCA205 sarcomas were only responsive to anti–CTLA4 therapy when *Bifidobacterium spp*. were present in the gut microbiome (3). Supplementation with probiotics restores sensitivity to immunotherapy and clears tumors in antibiotic-treated and germ-free mice. Patients often decide to use probiotics to treat intestinal side effects of chemotherapy and immune checkpoint inhibitors. However, the impact of probiotics on both the side effects and efficacy of the therapy remains unclear or deserves more rigorous investigation. Their importance is demonstrated by recent surprising results. *Bifidobacterium longum*- and *Lactobacillus rhamnosus GG*–based formulations showed an inverse relationship with the efficacy of immunotherapy in mice colonized with microbiota from patients who responded positively to anti–PD-1 therapy (4). Thus, even if generally believed beneficial and harmless, probiotics can still impair response to immunotherapy and should be discussed between patients and clinicians. Moreover, current probiotics are unable to engraft or demonstrate long-term benefits. Like FMT, they can yield variable results, and the inability to detect the administered bacteria in fecal samples suggests that most probiotics do not survive in the luminal tract, likely due to niche unavailability and competition with luminal microflora as well as immunologic and peristaltic defenses against non-native bacteria. For these reasons, microbiome researchers have focused on bacteria that can target tumors and survive long enough to mediate a therapeutic response.

Naturally Tumor-Colonizing Bacteria

A novel approach to detect and treat malignancies is by using a specific type of probiotic, tumor-colonizing bacteria. Microbes may preferentially colonize tumors over healthy tissues due a niche preference for a hypoxic environment, change in oxygen gradient, mucin production, or specific carbon sources for energy, particular metabolite availability, and/or a suppressed immune system. Anaerobic bacteria can naturally engraft tumors and not other hypoxic or inflamed tissues, which confers tumor specificity when administered intravenously. *Salmonella, Listeria*, and *Clostridium* spp. also have the innate ability to induce apoptosis and necrosis in tumor cells. While some bacteria can directly kill tumor cells, others activate the immune system, which is one of the main mechanisms by which tumor-colonizing bacteria can potentially treat cancer. This is often achieved through bacterial structural components and products of bacterial metabolism. For example, lipopolysaccharide, a constituent of the bacterial outer membrane, acts as an adjuvant by triggering toll-like receptor-4 and the production of proinflammatory and antitumor cytokines in dendritic cells. *Listeria* spp. instead infect myeloid derived suppressor cells and activate production of IL12, which is associated with antitumor T cell and natural killer cell responses (5). To achieve their antitumor activity, these bacteria need first to engraft and colonize the tumor but this is not always possible, especially if the target is an immune-excluded tissue or a non-mucosal tissue.

Engineered Therapeutic Bacteria

Because the natural tendency of bacteria to accumulate in tumors is not enough to affect their progression, synthetic biology tools can increase their efficacy and reduce off-target effects (6). The integrin αvβ3 is expressed in several cancer types and represents an appealing molecule for pan-tumor targeting. Moreover, its ligand (i.e., the Arg-Gly-Asp [RGD] peptide) and binding site are known. *Salmonella typhimurium* engineered to express the RGD peptide has a >1,000-fold specificity and antitumor activity for αvβ3-expressing glioma and melanoma mouse xenografts compared with the control strain (7). Tumor antigens have also been used for cancer targeting. Membrane expression of antibody fragments against the carcinoembryonic antigen and CD20 allowed bacteria accumulation in colorectal cancer and lymphoma, respectively, and reduced colonization of liver and spleen (8). Other strategies have focused on specific tumor antigens but may cause tumor adaptation and antigen mutation, ultimately resulting in resistance to the therapy.

Moreover, scientists have engineered bacteria to limit their toxicity and off-target effects. While *Salmonella, Listeria*, and *Clostridium* spp. naturally present tumor-killing properties, these may be attributed to virulence factors, that cause potential side effects and complications. Due to these safety concerns, major virulence genes may be knocked out without impairing tumor targeting and killing.

While chemotherapy remains the primary method of treating tumors, its strong and severe side effects limit its full application, tolerability, and patient quality of life. Instead, tumor-colonizing bacteria can be engineered to deliver chemotherapies in a more specific manner or to a more specific region under specific conditions. For instance, *Bifidobacterium infantis* ectopically expressing cytosine deaminase converted 5-fluorocytosine into the cytotoxic 5-fluorouracil in the tumor and significantly inhibited tumor growth in mice (9). Similarly, other bacteria have been engineered to target tumor vasculature and to express and release chemokines, cytokines, and antibodies to recruit and activate T cells into the tumor and prevent lymphocyte exhaustion. Though these proof-of-concept studies showed the ability of engineered bacteria to suppress tumorigenesis with cytotoxic genes, they also demonstrate the need for better regulatory control that will only express these genes when the bacteria are interacting with cancer tissue.

However, these live biotherapeutic products are generally tested under non-colonizing conditions (i.e., gnotobiotic or antibiotic-treated mice) and have failed to induce functional changes in hosts with an intact microbiome, including humans. There are multiple barriers to the survival of an engineered probiotic in the luminal environment, including innate and adaptive immunity, competition with other native microorganisms, and niche availability. To address the inability of engineered bacteria to colonize the host, several strategies have been outlined, including the development of tools to manipulate bifidobacteria, bacteroides, lactococci, and lactobacilli to increase engraftment in the intestinal lumen. To fill this gap, we recently developed a technique that consists of engineering native undomesticated *Escherichia coli* (*E. coli*), isolated from a conventional host, to express a function of interest prior to reintroducing them into the same or a new host (10). Native bacteria are already adapted to the luminal microenvironment and thus perpetually colonized 100% of the transplanted host after a single administration. This advance in the field has

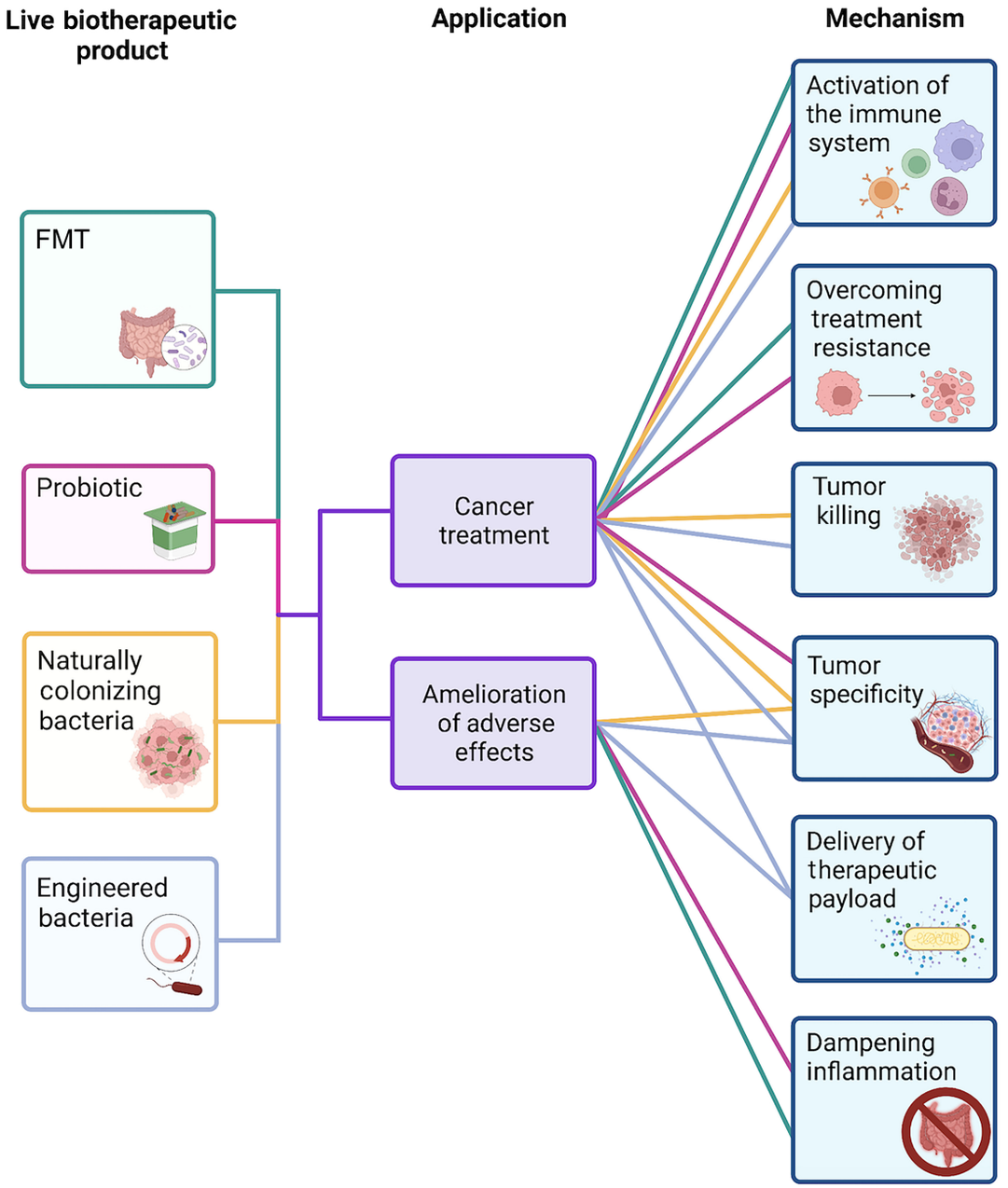

Figure 1.
Applications of live therapeutic products to cancer treatment. Live biotherapeutic products such as FMT, probiotics, naturally colonizing bacteria, and engineered bacteria treat cancer and ameliorate side effects related to the therapy. These products can be laboratory strains or obtained from healthy donors or patients who have a desired response to therapy. Their mechanisms of action are not fully understood but they may act by targeting the immune system or be cytotoxic to the tumor itself. In addition, tumor-colonizing and engineered bacteria can deliver therapeutic payloads to the tumor and limit side effects and toxicity. The diagram connects the applications and mechanisms of action of each of these live therapeutic products using different colored lines. Green, FMT; pink, probiotics; yellow, naturally colonizing bacteria; blue, engineered bacteria. (Created with BioRender.com.)

promising applicability for the treatment of both intestinal and extraintestinal diseases. Furthermore, engineered native bacteria are engraftable and, thus, can be exploited as a preventive treatment for subjects with genetic predisposition and family history of cancer that otherwise will be on watch-and-wait or an increased surveillance approach.

Challenges to Clinical Translation

Contrary to probiotics, which are considered dietary supplements and loosely regulated, live biotherapeutic products are live organisms designed and developed to treat, cure, or prevent a disease or condition (https://www.fda.gov/media/115730/download), and therefore their

manufacturing, safety, consistency and therapeutic effect are strictly regulated. Concerns about the translation in clinics include live bacteria's ability to proliferate in the tumor and thus the potential to disseminate and cause infection and sepsis. Indeed, oncologic patients are often immunosuppressed because of chemotherapy and are more susceptible to infections and bacteria overgrowth. Engraftment, colonization and expression of the therapeutic function are fundamental characteristics of the ideal chassis. Biocontainment is also necessary to prevent the horizontal transmission of potentially harmful engineered genes (e.g., antibiotic resistance) to the host microbiome and environment. Furthermore, "kill switch" genetic circuits to terminate the engineered bacteria are needed when their function will not be necessary anymore, or in the need of premature and sudden suspension of the therapy. Some of these issues have been already addressed in chimeric antigen receptor immunotherapy and can be applied to bacteria, or have been demonstrated in reduced community *in vivo* models. Suicide genes activated by the administration of drugs or by particular molecules in the tissues (e.g., excess of inflammatory mediators or damage) are an example. Alternatively, conditional activation (i.e., sense-and-control) of the function of interest by factors at the site of interest only (e.g., oxygen level, pH, nutrients availability) and tumor targeting could improve safety and efficacy of engineered live biotherapeutic products.

Future Directions and Concluding Remarks

Live biotherapeutic products have great potential as a cancer treatment (**Fig. 1**). While most engineered approaches have focused on expressing eukaryotic genes in bacteria, ectopic expression of prokaryotic genes for therapeutic purposes can be therapeutic as well. For example, we engineered the bile salt hydrolase (BSH) into gut native *E. coli* to deconjugate bile acids and to restore insulin sensitivity in the *ob/ob* mouse model of type 2 diabetes for months after a single treatment (10). Lack of bacterial deconjugation of bile acids can hinder the farnesoid X receptor (FXR) and contribute to the progression of colorectal cancer in mice. BSH-expressing bacteria may counteract colon cancer by disrupting bile acid metabolism and thus, increase FXR activity with a single treatment, potentially yielding long-lasting results. Such a treatment, if successful, could be used to suppress tumor formation in some high-risk populations, such as those with hereditary cancers or inflammatory bowel disease.

Altogether, this preclinical evidence demonstrated that we could express several prokaryotic and eukaryotic genes in bacteria, and the possibilities of further engineering are endless. Regardless of the progress made, the translation of these therapies to the clinic will be challenging and the clinical benefit of these engineered therapies is still to be proven. A milestone in the field will be the successful application of the first engineered bacteria as cancer treatment, and thus demonstrate the establishment of a new armament of therapeutic products in fight against cancer.

Authors' Disclosures

A. Brevi reports a patent for EP3886881A2 pending. A. Zarrinpar reports grants from NCI, VA BLR&D, NIBIB, NHLBI, NIDDK, NIAAA, and NCATS. A. Zarrinpar has a patent for PCT/US18/27998 pending and licensed to Endure Biotherapeutics. A. Zarrinpar holds equity and is the acting Chief Medical Officer of Endure Biotherapeutics.

Disclaimer

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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