Microbiome-mediated colonization resistance: defense against enteropathogens and multi-drug resistant organisms

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Chapter 3

Opportunities and Challenges in Development of Live Biotherapeutic Products To Fight Infections

Development of LBPs against infection
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Abstract

Treatment of bacterial infections with broad spectrum antibiotics is a strategy severely limited by the decreased ability of the perturbed resident microbiota to control expansion of antibiotic resistant pathogens. Live Biotherapeutic Products (LBPs) could provide an alternative to antibiotics in infection control by restoring gut colonization resistance and controlling expansion of resistant strains, an important therapeutic need not being addressed with existing anti-infective drug modalities. We review opportunities and challenges in developing LBPs for MDRO colonization and infection control, with a focus on commercial FMT-like products and defined bacterial consortia, and spanning considerations related to availability of models for rational drug candidate selection and dose regimen selection, good manufacturing practice, intellectual property, and commercial viability.

Focus and definitions

FDA defines LBPs as "a biological product that: 1) contains live organisms, such as bacteria; 2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and 3) is not a vaccine" [1]. Within FDA, The Center for Biologics Evaluation and Research (CBER) is responsible for regulating LBPs, and their licensure is obtained by approval of a biologics license application (BLA) [2]. A number of drug modalities currently being advanced meet the definition of LBP, including procedures to transplant fecal microbiota or spore fractions from fecal microbiota, as well as products of defined composition, such as single bacterial strains, engineered bacterial strains, and defined bacterial consortia. Furthermore, LBPs may be administered orally, rectally, topically, or as injectables. This piece focuses on orally and rectally delivered LBPs consisting of natural, unmodified bacteria, which have drawn most interest to date in the context of antimicrobial resistance (AMR), and excludes injectables, topicals, and LBPs consisting of engineered bacterial strains. Development considerations pertinent to engineered LBPs have been reviewed elsewhere [3].

Opportunity for Live Biotherapeutic Products (LBPs) in the context of AMR

The gut is a reservoir for numerous multi-drug resistant organisms (MDRO), including *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella pneumonia*e or *Enterobacter aerogenes*, and Enterococci such as *Enterococcus faecium* and *E. faecalis*. Antibiotic use associated with a range of medical procedures results in collateral damage to the gut microbiota resulting in an increased risk for development of infections, including by *Clostridioides difficile*, and acquired colonization with MDRO [4-6].

The extensive use of antibiotics can also contribute to bacteria developing antibiotic resistance mechanisms. As physicians have become more aware of these threats and antibiotic stewardship programs have been put in place, antibiotic sales volumes have dropped in the US [7], which combined with severe pricing pressure, has led to an exodus of pharmaceutical companies from anti-infective drug development. LBPs could contribute to breaking this vicious cycle in several ways. First, the expansion of resident or acquired MDROs could be kept in check by helping restore the host microbiota after an antibiotic perturbation. This could be particularly useful in vulnerable populations such as patients undergoing hematopoietic stem cell transplantation, intestinal surgeries, organ transplants, chemotherapy, or dialysis. Second, LBPs should not contribute to selecting resistant strains from susceptible populations and therefore no LBP stewardship should be necessary. Supporting this promise, fecal microbiota transplantation (FMT)

has shown high efficacy in prevention of recurrent *Clostridioides difficile* infection (CDI) [8] and defined bacterial consortia have shown promise in rodents models of vancomycin-resistance *Enterococcus* (VRE) infection [9].

Recent work has started to shed light on the mechanisms of post-antibiotic gut microbiome recovery, paving the way for developing targeted prevention strategies [10]. Modes of action through which LBPs may achieve successful eradication or prevent colonization of MDROs include competition for nutrients, production of short-chain fatty acids (SCFA), conversion of primary to secondary bile acids, and production of bacteriocins, among others [11, 12]. There is some limited evidence that administration of an LBP consisting of a single microorganism can help prevent C. *difficile* infection [13], and significantly more evidence that administration of complex bacterial communities such as FMT and defined bacterial consortia [8, 14] can be therapeutically useful. Therefore, we focus here on challenges specific to LBPs consisting of FMT and defined bacterial consortia.

Undefined vs defined LBPs

The two main categories of LBPs that have received attention from drug developers to address AMR are commercial FMT and FMT-like procedures and defined LBPs consisting of single bacteria or consortia of bacteria. FMT and FMT-like procedures consist of full bacterial communities from fecal donor samples or spore fractions of such communities, administered rectally [15] or orally [16, 17]. The focus for these procedures is on standardizing the steps to identify and screen healthy fecal donors, and process, store, ship, and administer the stool formulations. Given the variation of microbiota composition across individuals and over time, the composition of the resulting products varies with each donation and is thus undefined in nature. Regulation of these products is primarily concerned with the process by which they are prepared for transplantation, rather than its undefined contents. Defined LBPs, in contrast, consist of a limited set of bacterial species produced by fermentation from clonal cell banks, resulting in a final product of defined, standardized composition. Regulation of defined LBPs is concerned with both the process as well as the specific components of the product, with an increased scrutiny of characteristics of the component strains such as their genetic identity and their potential for transferring virulence or antibiotic resistance genes to other members of the microbiota.

Challenges to development of LBPs

Biological complexity

The single most formidable challenge for development of LBPs is perhaps the sheer

complexity of the biology being uncovered, and yet to be uncovered, on the role of host microbial communities in human health and disease. The mechanisms by which bacteria influence host phenotypes are often highly pleiotropic, rendering reductionistic potency assays to be of limited value in the development of LBPs. The understanding of the role of host microbial communities in disease is only partial, making selection of optimal patient populations for clinical studies a complex endeavor. The knowledge of the fundamental rules that govern assembly of microbial communities is still in its infancy, making bottom-up approaches to rational construction of drug candidates consisting of bacterial communities rudimentary for now. Perhaps the most salient departure from traditional development of drugs based on small molecules, proteins, or oligonucleotides is that, while transdisciplinary approaches using chemistry, biology, and computational science have been successful to enable these modalities, development of LBPs needs to rely heavily on insights from microbial ecology, a discipline largely ignored by the pharmaceutical industry to date.

Determination of Pharmacokinetic - Pharmacodynamic relationships and dose regimen selection

Pharmacokinetics (PK) is the study of how the host affects the fate of an exogenously administered drug. In the context of LBPs, this should ideally include studying how abundantly and durably the product strains colonize the host, and what proportion of the product strains colonize a given host at a given time. Pharmacodynamics (PD) is the study of how a drug affects the organism, traditionally with a focus on the biochemical and physiologic effects of the drug on the host. This definition is still relevant to LBPs, but study of the PD of an LBP additionally requires understanding the ecological effects of the drug on the host resident microbial community.

It is impossible to fully understand the action of a drug unless the relationship between drug exposure and effect has been reasonably well described. The inherent batch-to-batch variability in the composition of FMT and FMT-like procedures makes it challenging to reasonably describe in a quantitative manner the relationship between PK and PD and thus rationalize clinical successes and clinical failures. In contrast, quantifying with precision the PK of defined LBPs is complex but feasible. It needs to address the non-trivial technical problem of discriminating exogenously administered LBP strains from closely-related resident strains in the host's bacterial community. This has been achieved by culturing the strains in a defined LBP and obtaining high quality, complete genome sequences from which unique genetic markers can be derived and used to track strain-level engraftment from metagenomic sequencing of DNA isolated from fecal material [18, 19]. Recent clinical work following this approach has started to illuminate some basic features of LBP PK that are likely to be generalizable, specifically showing that higher dose, more frequent administration, and pretreatment with short

courses of antibiotics to create a niche for the LBP to engraft can significantly improve the abundance and durability of LBP strain colonization, as well as the proportion of LBP strains that colonize [18].

Quantifying the PK of a defined LBP is thus already possible. *Predicting* the PK of an LBP however, remains a significant challenge. Successful colonization of LBPs will likely be a function of a combination of features of the LBP, the host resident microbiota, the host, and other environmental factors. Key features of the LBP that influence colonization include dose, dose frequency, and species traits that may help with engraftment in the gut (e.g. a shared evolutionary history with the host). Features of the resident bacterial community that influence the success of invasion by an exogenous LBP may include bacterial density [20], diversity, and community structure, among others. Features of the host that may influence colonization include disease status, age, the host immune system, and host genetics. Finally, other environmental factors including diet and previous or concomitant drug use may have particularly salient effects on PK. Among drug-LBP interactions, interactions with broad spectrum antibiotics represent a case of particular medical interest in the context of AMR. Antibiotic perturbation can significantly lower the bacterial density and diversity of the resident gut community [10], thus freeing up resources for invaders and creating a niche for LBP engraftment.

The factors outlined above, combined with the host specificity of bacterial communities, render use of rodent animal models of limited value in the selection of dose and dose regimens for human studies. Healthy volunteer studies, controlled human infection models (CHIM), or ultimately dose-ranging studies in patients provide a more representative, albeit expensive alternative to determining PK-PD relationships. Early clinical efforts in the microbiome field omitted dose ranging exploration altogether before advancing drug candidates to late stage efficacy studies, and this may have been a factor contributing to clinical failures [21].

Exploration of pharmacodynamic effects of LBPs on the resident host microbiota is complicated by the myriad community features revealed by metagenomics, metabolomics and proteomics analyses. This work could be significantly aided by the use of standardized indices of gut microbiota health or disease susceptibility. Such indices may rely on measures of community structure that correlate with clinical outcomes. For example, oligodomination by certain opportunistic pathogens has been strongly associated to risk of infection [22]. In the context of AMR, such indices may support the development of LBPs by quantifying in a simple, easy to comprehend manner, the risk that a given patient may become infected and/or dominated by a pathogen, and by serving as a surrogate measure of the contribution of an LBP towards outcomes such as lowering infection risk for that patient [23, 24].

Intellectual Property

Obtaining patent protection is an essential component of successful drug development. An important element in obtaining patent protection for LBPs in the US has been navigating requirements codified in the United States Code (USC) as 35 USC § 101 (utility). This requirement defines the boundaries of what is patent eligible, to the exclusion of "natural phenomena". The US Supreme Court in *Mayo* [25] and *Myriad* [26] limited the scope of patent eligibility for natural products, making it more difficult to obtain composition of matter claims covering such products. Nevertheless, several applicants have obtained composition of matter claims on defined LBPs [27] by arguing successfully that their claims combine additional elements that result in new functional properties that yield something that is "significantly more" than what exists in nature [28]. Obtaining broad patent coverage can be more challenging for FMT products, which have not received broad composition of matter claims due to a combination of factors including lack of differentiation from what exists in nature, lack of novelty over prior art, and inability to sufficiently describe the composition of the FMT preparation. Instead, applicants have resorted to pursuing narrower method of use claims highlighting unique modifications to the stool preparation process such as filtration, lyophilization, or encapsulation steps [28].

Challenging marketplace for anti-infectives

The last decade has seen a massive exodus of pharmaceutical companies from anti-infective drug development due to structural economic issues that ultimately result in the inability to make meaningful profits from selling antibiotics. A first, salient issue with the economic marketplace is that US hospitals are strongly incentivized to use cheaper antibiotics whenever possible, unless there is absolute clinical need for more expensive antibiotics, because US insurers pay for in-patient antibiotics as part of a lump sum to hospitals, and thus cheap antibiotics increase hospital profit margins [29]. For example, fidaxomicin has been proven superior to vancomycin, a cheap generic, in sustained cure of CDI, but its uptake has been limited due to pricing concerns [30]. Most drugs are not paid for like this. Oral LBPs which do not require administration in a hospital setting and can instead be taken at home may partially circumvent this issue, but ultimately only proposed reforms currently before Congress like the DISARM Act can fix this structural issue. A second issue with the marketplace is that stewardship programs aimed at limiting spread of AMR put downwards pressure on sales volumes of new antibiotics, the use of which is left as a last resort. This has led to calls for new regulation delinking antibiotic sales volume from return on investment through prizes or insurance-like models [31]. The current framework for AMR stewardship is based on small molecule antibiotics and focuses on limiting the selection of resistant strains as a result of antibiotic use. The mechanisms of action by which LBPs help restore the gut microbiota and its colonization resistance against pathogens are highly unlikely to elicit selection of resistant strains, and in fact could help limit the expansion of host-resident

resistant strains which could otherwise thrive in a perturbed microbiota. As a result, we predict that there should not be downwards pressure on sales volumes of a hypothetical successful LBP anti-infective.

Good Manufacturing Practice

Manufacturing of FMT-like products and defined LBPs intended for oral or rectal administration (and thus, likely based on anaerobic organisms) has a few shared challenges. These include minimizing exposure to oxygen, in particular in steps of the process where the organisms are metabolically active and preserving the viability of bacterial cells during processing and storage. A variety of factors influence the viability of bacteria during the manufacturing process and subsequent storage, including oxygen exposure, growth media, shearing, composition of the buffer solutions used to suspend the bacteria before freezing or freeze-drying, cooling rate, and freeze-thaw cycles, among others. The problem of maintaining cell viability during freezing and particularly during freeze-drying for long term storage deserves special attention, as it is one of the most technically challenging steps of manufacturing an LBP. During freezing and freeze-drying, the bacterial cell wall is exposed to mechanical forces due to formation of ice crystals inside and outside the cell, which can disrupt the membrane and kill the cell. During freeze-drying, furthermore, the process of removing water by sublimation generates osmotic pressures that can damage the cell membrane. Optimization of freeze-drying cooling cycles and development of buffer solutions containing cryoprotectants or lyoprotectants is therefore an important step to ensure the long-term preservation of LBPs. While preservation conditions for a number of aerobes and some facultative anaerobes such as E. *coli*, and Lactobacillus and Lactococcus species has been described in the literature, there is very little published on the topic of preservation of anaerobic gut commensals [32]. Further complicating the matter of long-term preservation of LBPs, the efficiency of cooling regimes and cryoprotectant and lyoprotectant substances can be highly bacterial species-specific.

There are certain manufacturing considerations that are unique to FMT-like products. Feces are a heterogenous substance composed of bacteria, viruses, fungi, food, and host secretions, which does not naturally yield itself to precise characterization. Consequently, manufacturing considerations emphasize rigorous donor screening and processing of stool donations, and relatively de-emphasize in-depth characterization of the composition, which would vary with every donation. FMT is performed using suspensions made of donor stool from carefully selected and screened healthy individuals. Donors undergo extensive health questionnaires and their blood and stool samples are analyzed for a list of known pathogenic viruses, bacteria, and parasites before being accepted. Recently, some amendments have been introduced to this process as a result of FDA's issuance of a series of safety alerts on the potential risks of life-threatening infections with the

use of FMT [33, 34] and on the risk of transmission of Sars-CoV-2 with FMT, leading to a halting of FMT studies in the US during 2020. Processing of stool donations varies depending on whether the final formulation is intended for oral or rectal administration. Stool samples may undergo a series of steps to filter the non-microbial components of stool, or the non-spore forming bacterial components of stool, depending on the product. FMT drug product may be released after meeting a specification of potency consisting of an estimate of the total aggregate of viable organisms present in the product, and the same assays may be used to demonstrate the FMT product stability for the planned duration of the clinical studies in which it is being used.

Defined LBP manufacturing considerations, by virtue of the composition being known and standardized, can put increased emphasis on the characterization of the component strains and less emphasis on an in-depth understanding of the donor from whom the strains were originally isolated. FDA expects a description of the drug substance including the biological name of each of the strains and strain designations, the original source of each of the strains, their passage history, and a description of the phenotype and genotype of the product strains [1]. Furthermore, sponsors are expected to characterize their LBPs using assays that assure the identity, purity, and potency of the drug substance and final drug product, and to apply these same assays over time as part of a stability program to ensure the product remains within specification for the duration of the proposed clinical studies. Identity tests are expected to detect each of the bacterial strains that compose the LPB, and to discriminate among LBP component strains. High quality genome sequences for each strain can provide an authoritative identification of each organism and enable comprehensive identification of potentially undesirable safety traits such as antibiotic resistance genes or virulence factors. A further assessment of the risk of transmission of such genes to relevant microbial flora (for example, based on proximity to mobile elements) is of particular interest. Sponsors are also expected to determine the antibiotic resistance phenotypes of the LBP strains, with a particular focus towards identifying clinically relevant antibiotics that can be used as rescue therapies in the event of an infection suspected to be caused by LBP strains. Purity tests are expected to show the absence of contaminating bacteria or yeast above acceptable limits. Potency tests commonly used for LBPs assess the product viability, for example in terms of viable CFUs per dose.

Defined LBPs are manufactured starting from clonal cell banks via fermentation, which may require optimization of growth media and physiological parameters like mixing, temperature, pH, retention time, and redox potential. After fermentation, bacteria are harvested by downstream steps such as filtration, which may require selection of appropriate filtration membranes and optimization of process variables such as transmembrane pressure and flow rates to minimize shear-induced damage to the

bacterial cell. A further challenge inherent to multi-strain defined LBPs manufactured as monocultures is that the number of banking campaigns, production runs, and characterization assays required scales linearly with the number of strains in the product. Taken together, these considerations impose a significant burden on drug developers but also create an opportunity to innovate: a non-trivial amount of the advances made by LBP developers will originate in their process development and GMP manufacturing activities.

Preclinical and clinical models to discover LBPs and study their pharmacology

While not strictly required by FDA, use of in vivo and in vitro models to test the efficacy and characterize the mechanism of action of LBP candidates prior to use in humans can be a sensible business decision. A challenge in use of animal models to study efficacy of microbiome drugs is that it is not always clear what microbiome endpoints are the most relevant surrogates of therapeutic efficacy. For example, pinpointing a specific microbiome endpoint most predictive of efficacy in treating immune or metabolic disease is not straightforward. An advantage of designing LBPs for use in AMR is the relative clarity of the microbiome endpoints used to quantify efficacy and their relation to the therapeutic goal: the microbiome endpoint of an animal model used for efficacy testing may be reduction or elimination of MDRO carriage in the gut (e.g., carbapenem-resistant *Enterobacteriaceae* [CRE], extended spectrum beta-lactamase [ESBL], or VRE), and the therapeutic goal may be to prevent infection outcomes with that same MDRO. Rodents, for example, have been colonized (at least temporarily) with pathogenic MDRO strains that infect humans, without resorting to surrogate mouse pathogens [14], and used to rationally select defined bacterial consortia that reduce intestinal colonization. Whether the surrogate endpoints of decolonization models truly predict clinical outcomes of LBPs will have to be demonstrated in future clinical studies.

A challenge in measuring efficacy of LBPs in AMR applications is the difficulty in anticipating which patients will be exposed to the pathogen, become colonized, and develop disease, which complicates execution of clinical studies powered on the basis of disease outcome endpoints. CHIM, where carefully selected human volunteers are deliberately infected with well-characterized infectious agents in a controlled setting can be an effective way of measuring the efficacy of a drug agent in these circumstances. CHIM have the advantage of decreasing the number of patients needed to detect efficacy in phase 2 and 3 trials, and have been used for testing vaccines in early in clinical development, dating back to 1900 [35]. CHIM offer the opportunity to study the physiological, immunological and metabolic changes that occur upon infection, including potentially assessing the role of the gut microbiome in transmission of antibiotic resistance and virulence genes.

Conclusion

Identification of commensal bacteria that can restore gut colonization resistance after antibiotics in high-risk patients is an important new strategy to prevent infection and transmission of MDROs. Use of LBPs as anti-infectives could circumvent a key limitation of antibiotics, namely the need for stewardship driven by selective pressure on resistant strains, while providing a potentially safe and convenient way of restoring the microbiota after antibiotic use in high risk patient populations.

Notes

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Conflicts of interest
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