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FOUND IN TRANSLATION

Microbial therapeutics: New opportunities for drug delivery

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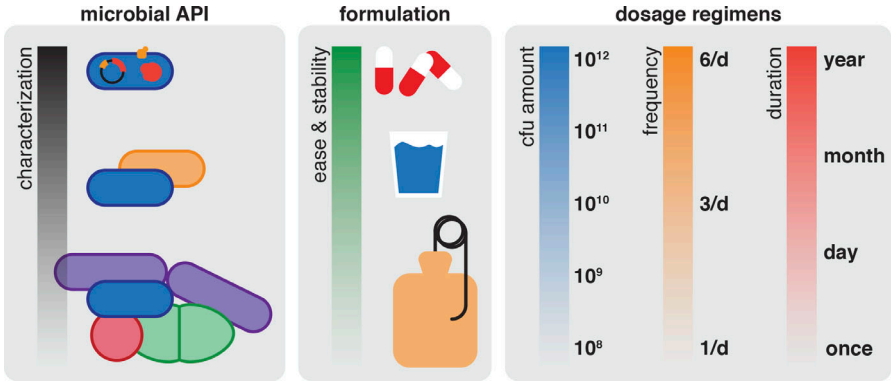
With >40 clinical trials underway, we are nearing the first FDA-approved live microbial therapeutic. Here, Giovanni Traverso, MIT and Harvard Medical School Assistant Professor, and colleagues Miguel Jimenez and Institute Professor Robert Langer from MIT discuss the significant challenges of administering live microorganisms to patients and the opportunities for drug delivery of these new complex therapeutics.

Microorganisms can cure disease

For a majority of modern medical history, germ theory has cast microbes as our enemies; however, it is now abundantly clear that they can be used as therapeutics. In fact, there are now a burgeoning number of companies with product pipelines filled with microbial therapeutic leads in clinical development (Table 1). Nevertheless, significant drug delivery challenges remain for the successful translation of these microbial therapeutics to wide patients populations.

The potential of microbial therapeutics is evident in the few existing live bacterial prophylactic vaccines and more recent work using fecal microbiota transplant (FMT) to cure recurrent *Clostridium difficile* infections (Detmer and Glenting, 2006; Kelly, 2013). While FMT against *C. difficile* has been the most striking initial success of microbial therapies, these procedures remain experimental, and there is much ongoing work to standardize and regulate this approach (Table 1; Food and Drug Administration, 2016b). This early success demonstrates that the targeted use of microorganisms to treat disease is possible. Predating this recent work, the idea of microbiome health and its potential modulation has permeated to the general public, stimulating the commercial development of probiotics marketed as food supplements. However, these food supplements have shown mixed results when applied to a range of conditions (Gareau et al., 2010). In contrast, the new field of microbial drug development aims to generate microorganisms with well-defined, targeted, therapeutic functions.



Microbial APIs range from well-characterized, engineered strains to undefined, fecal-derived microbiota. These APIs are being tested for efficacy in clinical trials using dosage forms that range in ease of use and stability, and with dosage regimens that vary widely (Table 1). Improved drug delivery of microbial APIs will be critical in developing effective microbial therapeutics.

This focused activity has been spurred by our ability to engineer functions more precisely through the advent of synthetic biology tools and to understand the mechanistic impact of microorganism on human health through microbiome research (Mimee et al., 2016). Leveraging these tools, companies are now targeting several therapeutic modalities, such as scavenging of toxic molecules (e.g., Synlogic); in situ production of therapeutic molecules (e.g., ActoBio); intracellular delivery of a genetic payloads (e.g., Adhera); displacing infectious pathogens (e.g., Seres); immune system modulation (e.g., Evelo); and reestablishing a standardized microbiome (e.g., NuBiyota).

Over the last decade, this growing technical expertise has been matched by venture capital funding, enabling many companies to tackle the drug approval process. In 2012, the US Food and Drug Administration (FDA) issued guidance on the use of "live biotherapeutic products" in early clinical trials (Food and Drug Administration, 2016a). Now, as companies move beyond Phase I clinical trials, the FDA has continued this dialog on the appropriate way to manufacture and evaluate this new class of active pharmaceutical ingredient (API; Food and Drug Administration, 2018). The field is starting to tackle the challenges of scaling, standardizing, and formulating these microbial APIs in order to enable large-scale Phase III clinical trials and, eventually, wide use in patient populations.

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Microbial APIs present unique but challenging therapeutic opportunities

Live microbial APIs are entirely distinct from biologics and small molecule APIs. This difference is at the core of their potential but presents significant challenges in manufacturing, drug delivery, and evaluation. Unlike purified single molecules, live microbial APIs contain all the molecular machinery of a living cell, allowing for self-renewal, genetically encoded functions, multiple mixed molecular interactions, and adaptive responses.

Self-renewal

Self-renewal holds the potential for developing therapeutics that only need to be taken once yet can have a significant therapeutic effect over a long period. While this ability may hold the key to developing vaccine-like therapeutics for chronic diseases, self-renewal greatly complicates the pharmacokinetics (PK) of microbial APIs. The rate of microbial cell division may be highly dependent on patient-specific factors such as food intake, native microbiome, and disease status. Therefore, self-renewal of microbial APIs may actually introduce significant variability, posing a challenge for establishing effective dosing regimens based on small-scale Phase I clinical trials.

Additionally, self-renewal undermines biocontainment and enables genetic drift of the microbial API over time. Companies like Synlogic have chosen to make their microbial APIs auxotrophic for an essential nutrient only supplied during manufacturing (Kurtz et al., 2019). This prevents replication in patients, simplifying PK models and lowering genetic drift. Others have designed genetic kill switches to cause self-destruction of escaped microbial cells (Lee et al., 2018).

Genetically encoded functions

The genetic basis of microbial APIs allows them to be flexible therapeutic platforms. Drug discovery and lead optimization can take advantage of the myriad of genetic tools for building and screening engineered microorganisms. Companies such as Ginkgo Bioworks are commodifying this DNA-driven build-design-test cycle, giving us an early look at the scale at which microbial APIs may be developed in the future. Complementary to this, companies such as Finch Therapeutics are using next-generation sequencing to define and isolate potential therapeutic microorganisms from patients' own microbiomes.

However, as the ultimate therapeutic functions are encoded in DNA, they are susceptible to mutation and horizontal gene transfer. Methods to limit genetic drift will be critical to ensure genetic stability of microbial APIs (Lee et al., 2018). In addition, whole-genome sequencing throughout the drug development process can be used to understand the genetic failure modes of each microbial API.

Mixed molecular interactions

Microbial APIs present a very large number of molecular interactions simultaneously (i.e., proteins, peptides, carbohydrates, lipids, metabolites, and nucleic acids), each with the potential for a therapeutic effect. For example, the live microbial vaccines and other immune modulating microbial APIs rely on these mixed molecular interactions to function as their own adjuvants.

While promising, such mixed molecular interactions will be present even if they are not designed with a therapeutic effect in mind. This represents a significant possibility for multiple off-target interactions. Even so, since most microbial APIs will be confined to the lumen of the GI tract, many of these interactions are shielded from the broader human biology. Nevertheless, working with such complex APIs poses a manufacturing challenge, as there may be inherent heterogeneity in the cell-to-cell stoichiometry of these interactions. Multiple types of biochemical validation will be necessary to supplement genetic characterization.

Adaptive responses

The ability of microbial APIs to adapt and respond to their environment holds great potential for making closed-loop therapies. Genetically encoded biosensors that detect clinically relevant biomarkers could be used to control therapeutic functions (Mimee et al., 2016). Nevertheless, while advanced, adaptive functions can be designed to work in laboratory environments, it remains to be seen if they will function robustly in the gut. Likely because of this design challenge, the first generation of microbial APIs moving through the approval pipeline are either unmodified or implement single function genetic designs (Table 1). As the field learns more about the impact of the gut environment on these APIs, we expect a second generation of microbial therapeutics to incorporate more advanced adaptive functions.

While difficult, developing such adaptive systems could in turn be used to overcome many of the challenges outlined above, as adaptive microbial APIs could be designed for a consistent therapeutic effect across a heterogeneous patient population. Alternatively, unmodified microbial APIs isolated directly from native microbiomes may already rely on endogenous adaptive systems. Efforts to understand these mechanisms will serve to inform the development of advanced microbial API designs.

Microbial APIs require rethinking drug delivery

Microbial APIs pose unique challenges and opportunities for drug delivery and manufacturing. Since these APIs are living cells, the standard pharmacological processes (absorption, distribution, metabolism, and excretion, also known as ADME) are significantly different. For microbial APIs intended to be confined to the gastrointestinal tract, we may instead consider gastrointestinal distribution, attachment, replication, and shedding (giDARS). This changes how we consider modulation of PK through formulation and dosage form design. Furthermore, pharmaceutical manufacturing must be adapted to accommodate this sensitive micron-sized API.

Preservation methods used for probiotics and by microbial strain banks have provided starting points for current clinical trials. This means dosing patients with freshly thawed liquid microbial suspensions or refrigerated gelatin capsules filled with freeze-dried microbial biomass. These methods provide an immediate route for testing microbial APIs in patients. However, they are not scalable and leave little room for modulating the giDARS to target specific disease profiles. The challenge for the field will be to adapt current drug delivery approaches to enable scalable dosing, long-term stability, and precise targeting of microbial APIs.

Dosing

Current clinical trials provide an initial look at the required doses and dosing regimens of microbial APIs. For immune-modulation, which likely requires lower doses, patients are dosed with 10^9–10^{10} CFU once to twice a

Table 1. **Current company-run clinical trials of microbial APIs administered to the gastrointestinal tract stratified by API type as posted on ClinicalTrials.gov**

Company	Strain (engineered function)	API ID	Disease	Ph.	Trial ID (start year, status)	Dose (CFU)	Freq.	Len.	Form.
Engineered microorganisms									
Adhera	*Escherichia coli* (β-catenin shRNA delivery)	CEQ508	FAP	1	START-FAP (2010, comp.)	10^8–10^9	1/d	28 d	-
Synlogic	*E. coli* (NH_3 scavenging)	SYNB1020	UCD	1	NCT03179878 (2017, comp.)	2×10^9–2×10^{12}	1–3/d	1–14 d	Susp.
			Cirrhosis	1/2	NCT03447730 (2018, rec.)	5×10^{11}	3/d	6 d	
	E. coli (Phe scavenging)	SYNB1618	PKU	1/2	NCT03516487 (2018, rec.)	10^{10}–5×10^{11}	3/d	1–7 d	
ActoBio	*Lactococcus lactis* (proinsulin and IL-10 secretion)	AG019/tep	T1D	1/2	NCT03751007 (2018, rec.)	2–6 cap.	1/d	1 d–8 wk	Cap.
Oragenics	*L. lactis* (hTFF1 secretion)	AG013	OM	1	NCT00938080 (2009, comp.)	2×10^{11}–1.2×10^{12}	1–6/d	14 d	Susp.
				2	NCT03234465 (2017, rec.)	2×10^{11}	3/d	7–9 wk	
Single defined microorganisms									
Osel	*Clostridium butyricum*	CBM588	CDI	2	NCT01077245 (2010, with.)	2 g	2/d	42 d	Gran.
IBT	*Lactobacillus reuteri*	IBP-9414	NEC	2	NCT02472769 (2015, comp.)	10^8–10^9	1/d	14 d	Susp.
Evelo	Commensal strain	EDP1815	Psoriasis and eczema	1	NCT03733353 (2018, rec.)	55 mg–2.76 g	1/d	15 d	Cap.
		EDP1066		1	NCT03542994 (2018, rec.)	66 mg–3.3 g	1/d	15 d	
		EDP1503/pem	CRC, TNBC, ICI relapse	1/2	NCT03775850 (2018, rec.)	7.5×10^{10}	2/d	14 d	
			Melanoma	2	NCT03595683 (2018, rec.)	7.5×10^{10}	2/d	14 d	
4D Pharma	*Bacteroides thetaiotaomicron*	Thetanix	CD	-	NCT02704728 (2016, comp.)	3 cap.	2/d	7.5 d	Cap.
	Enterococcus gallinarum	MRx0518/pem	Solid tumor	1/2	NCT03637803 (2018, rec.)	1 cap.	2/d	2 yr	
	Blautia hydrogenotrophica	Blautix	IBS	2	NCT03721107 (2018, rec.)	$>10^{10}$ (2 cap.)	2/d	8 wk	
	Bifidobacterium breve	MRx0004	Asthma	1/2	NCT03851250 (2019, n.rec.)	10^9–10^{10} (2 cap.)	2/d	12 wk	
Multiple defined microorganisms									
Leadiant	*Lactobacillus acidophilus* and *Bifidobacterium lactis*	STP-206	NEC	1	NCT00922324 (2009, comp.)	-	1/d	1–7 d	-
				1/2	NCT01954017 (2013, com.)	-	1/d	2–11 wk	-
Seres	Firmicute spores	SER-262	CDI	1	NCT02830542 (2016, active)	10^4–10^8	1/d	1 d	C.susp.
Vedanta	Eight strains	VE303	CDI	2	NCT03788434 (2018, rec.)	2–10 cps	1/d	14 d	Cap.
Siolta	Consortium	STMC-103H	IgE-AD	1	NCT03819881 (2019, rec.)	-	2/d	28 d	-
NuBiyota	∼30 commensal strains	MET-2	CDI	1	NCT02865616 (2016, rec.)	1.5–10 g (3–20 cap.)	1/d	2–10 d	Cap.
			UC	1	NCT03832400 (2019, rec.)		1/d	4–14 d	
		MET-3	Obesity	e1	NCT03660748 (2018, rec.)	-	-	-	
		MET-4	LA-OPSCC	-	NCT03838601 (2019, n.rec.)	-	-	2 d–4 wk	
		MET-4/ICI	Solid tumor	e1	NCT03686202 (2018, rec.)	-	1/d	>2 d	

Table 1. **Current company-run clinical trials of microbial APIs administered to the gastrointestinal tract stratified by API type as posted on ClinicalTrials.gov (*Continued*)**

Company	Strain (engineered function)	API ID	Disease	Ph.	Trial ID (start year, status)	Dose (CFU)	Freq.	Len.	Form.
Purified fecal microbiota									
Seres	Firmicute spores	SER-287	UC	1	NCT02618187 (2015, comp.)	-	1/d–1/wk	8 wk	C.susp.
				2	NCT03759041 (2018, rec.)	-	1/d	10 wk	
		SER-109	CDI	2	NCT02437487 (2015, comp.)	10^8 (4 cap.)	1/d	1 d	
				3	NCT03183128 (2017, rec.)	-	4/d	3 d	
Fecal microbiota									
MaaT	Donor derived	MaaT013	GVHD	2	NCT03359980 (2017, rec.)	10^{12}	1/d	3×/16 d	Enema
		FMT	ICU patients	2	NCT03350178 (2017, term.)	-	-	-	
	Autologous	FMT	AML	2	NCT02928523 (2016, comp.)	10^{12}	1/d	2 d	
Finch	Donor derived	CP101	CDI	2	NCT03110133 (2017, rec.)	-	1/d	1 d	Cap.
			ASD	2	NCT03829878 (2019, n.rec.)	-	1/d	1 d	
		oral FMT	CRE	e1	NCT03527056 (2018, n.rec.)	-	1/d	1 d	
Rebiotix	Donor derived	RBX2660	CDI	2	NCT01925417 (2013, comp.)	>1.5 × 10^9	1/d	1 d	Enema
				2	NCT02299570 (2014, comp.)	-	1/d	2×/7 d	
				2	NCT02589847 (2015, active)	-	1/d	2×/7 d	
				3	NCT03244644 (2017, rec.)	-	-	-	
			UTI	1/2	NCT03367910 (2017, inv.)	-	1/d	1 d	
			HE	1/2	NCT03439982 (2018, rec.)	-	-	-	
		RBX7455	CDI	1	NCT02981316 (2016, active)	1–4 cap.	2/d	2–4 d	Cap.

Ph., clinical trial phase; Freq., dosing frequency; Len., dosing length; Form., formulation; hTFF1, human trefoil factor 1; tep, teplizumab; pem, pembrolizumab; FAP, familial adenomatous polyposis; UCD, urea cycle disorders; PKU, phenylketonuria; T1D, diabetes mellitus, type 1; OM, oral mucositis; CDI, *C. difficile* infection; NEC, necrotizing enterocolitis; CRC, colorectal cancer; TNBC, triple-negative breast cancer; ICI, immune checkpoint inhibitors; CD, Crohn's disease; IBS, irritable bowel syndrome; IgE-AD, atopic immunoglobulin E-mediated allergic disorder; UC, ulcerative colitis; LA-OPSCC, head/neck squamous cell carcinoma; GVHD, graft vs. host disease; ICU, intensive care unit; AML, acute myeloid leukemia; ASD, autism spectrum disorders; CRE, carbapenem-resistant Enterobacteriaceae; UTI, urinary tract infection; HE, hepatic encephalopathy; e, early; comp., completed; rec., recruiting; with., withdrawn; n.rec., not yet recruiting; ter., terminated; inv., enrollment by invitation; susp., suspension; cap., capsule; gran., granules; c.susp., encapsulated suspension.

day for 14 to 28 d (e.g., Evelo and Adhera). This is in line with the dose of live, oral microbial vaccines (4×10^8–10^{10}), which are dosed only one to four times over 1 wk (Food and Drug Administration, 2013, 2016c). For scavenging or protein delivery applications, which likely require higher doses, patients are dosed with 10^{11}–10^{12} CFU once to thrice a day for 14 d to 9 wk (e.g., Synlogic and Oragenics; Kurtz et al., 2019). Aligned with this, Seres recently pointed to a low dose of 10^8 as a likely cause of poor efficacy in a Phase II clinical trial (Henn et al., 2018).

Can these doses and regimens be improved through better drug delivery to enhance stability and targeting of the microbial API?

Stability

Like current biologics, microbial APIs also suffer from heat and chemical denaturation. However, microbial APIs also contain delicate lipid membranes. This fragility presents a stability challenge during manufacturing, storage, and administration.

Current manufacturing processes expose APIs to harsh solvents, temperatures, and pressures. Will these processes be adaptable for microbial APIs? Large-scale production of viable yeast biomass for food production has shown that this is possible; however, yeast is known to have intrinsic stress resistance (Attfield, 1997). Furthermore, current biomanufacturing processes already produce large quantities of microbial biomass for protein production; however, little focus is placed on cell viability (Huang et al., 2012).

In terms of storage stability, current microbial APIs require cold-chain storage to maintain potency. In some cases, this even means storage at cryogenic temperatures (Kurtz et al., 2019). Will patients adhere to these strict requirements or will they be required to visit dedicated dosing facilities? Can we learn from food-grade yeast sachets that are stable at room temperature?

Once administered, microbial APIs must contend with the low pH of the stomach and high bile salt content of the duodenum. Current live microbial vaccines use enteric-coated gelatin capsules or buffers to overcome some of these challenges (Food and Drug Administration, 2013, 2016c). Can other formulation approaches to be adapted to protect microbial APIs from bile acids?

Targeting

Current microbial formulations provide little targeting beyond the intrinsic distribution dictated by the microorganism's biology. To enhance the efficacy of microbial therapeutics, targeting the microbial API to the site of disease will be critical. Targeting may entail localization to a specific anatomical location (e.g., jejunum, ilium, or colon) or subcompartment (e.g., lumen, mucus layer, or epithelium). Additionally, targeting may involve delivery to a specific ecological niche (Lemon et al., 2012).

Targeted delivery may be approached through the use of materials with enteric, colon-targeting, omniphobic, mucoadhesive, or mucus-penetrating properties. Ecological targeting may be achieved through the co-administration of selective antibiotics to open a target niche. Alternatively, genetically encoded features of the microbial API can be used to modify its intrinsic anatomical and ecological distribution (Mimee et al., 2016).

Conclusions

It is clear that we are just years away from the first FDA-approved microbial therapeutic. A handful of companies are paving the way by tackling the complex but necessary process of drug approval. In so doing, these efforts have raised critical questions regarding the manufacturing and drug delivery of microorganisms as APIs. As the field pushes forward, new methods and approaches will be necessary to overcome these challenges. Novel formulations and dosage forms will complement the genetic design of microbial APIs to enhance the efficacy of microbial therapeutics.

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