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ABSTRACT

Engineered live biotherapeutics represent a novel class of therapeutic agents that combine the complexity of biological systems with the precision of genetic engineering, creating unprecedented regulatory challenges for health authorities worldwide. The development of a coherent regulatory framework for these products requires integration of traditional pharmaceutical science with microbiology, molecular biology, and systems biology, while addressing fundamental questions about product characterization, manufacturing consistency, stability, and clinical safety that differ substantially from conventional small-molecule drugs or recombinant protein therapeutics. This comprehensive review synthesizes current regulatory guidance from the United States Food and Drug Administration, European Medicines Agency, and Brazilian regulatory authority ANVISA, examining the critical path from chemistry, manufacturing and controls through clinical trial design and post-marketing surveillance. We critically evaluate the adequacy of existing regulatory frameworks for capturing the unique properties of engineered live biotherapeutics, identify gaps in current guidance, and propose a harmonized approach to regulatory science that balances innovation with patient safety while maintaining scientific rigor and transparency.

Introduction: The Regulatory Landscape for a Novel Therapeutic Class

The emergence of engineered live biotherapeutics as a distinct therapeutic class has created a fundamental challenge for regulatory agencies that evolved their frameworks primarily to govern small-molecule pharmaceuticals and recombinant protein therapeutics. These novel agents, which consist of living microorganisms that have been selected, isolated, or genetically modified to produce therapeutic effects, occupy an ambiguous regulatory space that does not fit neatly into existing categories. A live biotherapeutic product may be classified as a drug, a biological product, a dietary supplement, or a combination thereof, depending on its composition, intended use, and the jurisdiction in which it is being developed. This regulatory ambiguity has created significant barriers to innovation, as developers face uncertainty regarding which regulatory pathway to pursue, what data must be generated, and how to demonstrate safety and efficacy in a manner that satisfies regulatory expectations.

The regulatory challenge posed by engineered live biotherapeutics is fundamentally different from that posed by conventional therapeutics because these products are living systems with inherent biological variability, the capacity for genetic exchange with environmental microorganisms, and the potential to persist and proliferate in the host. Unlike small-molecule drugs, which have fixed chemical structures and can be fully characterized through chemical analysis, or recombinant proteins, which are produced through well-defined manufacturing processes and can be characterized through biochemical and biophysical methods, live biotherapeutics are complex biological systems whose properties may vary depending on growth conditions, passage number, storage conditions, and interactions with the host microbiota. This complexity necessitates a

fundamentally different approach to product characterization, manufacturing control, and clinical evaluation.

The regulatory frameworks that have emerged from major health authorities including the FDA, EMA, and ANVISA reflect an attempt to balance the need for innovation with the imperative to protect patient safety. However, these frameworks remain incomplete and evolving, with significant gaps in guidance regarding critical issues such as the characterization of engineered microorganisms, the control of manufacturing processes for living systems, the assessment of genetic stability and the potential for horizontal gene transfer, the evaluation of off-target effects and ecological impacts, and the design of clinical trials that can adequately assess safety and efficacy in the context of biological variability and individual host factors. The purpose of this review is to examine the current state of regulatory science for engineered live biotherapeutics, identify gaps and inconsistencies in existing guidance, and propose a harmonized approach that leverages the strengths of existing regulatory frameworks while addressing the unique challenges posed by this novel therapeutic class.

Regulatory Classification and Pathway Selection: Navigating Jurisdictional Complexity

The first critical decision facing developers of engineered live biotherapeutics is determining the appropriate regulatory classification and pathway in the jurisdiction where the product will be developed and commercialized. In the United States, the FDA has issued guidance documents that classify live biotherapeutic products as biological products subject to regulation under the Public Health Service Act, requiring submission of a Biologics License Application for marketing approval. However, the FDA has also indicated that certain live biotherapeutics derived from food sources or containing organisms with a long history of safe use may be regulated as dietary supplements under the Dietary Supplement Health and Education Act, which requires substantially less rigorous safety and efficacy testing. This dual regulatory pathway has created significant confusion and inconsistency, with some developers pursuing the dietary supplement pathway to avoid the more stringent requirements of the biologics pathway, while others pursue the biologics pathway to gain the marketing exclusivity and patent protections associated with drug approval.

In the European Union, the EMA has issued guidelines for the evaluation of medicinal products containing or consisting of genetically modified organisms, which classify engineered live biotherapeutics as medicinal products subject to the centralized procedure for marketing authorization. The EMA's approach is more stringent than the FDA's, requiring comprehensive safety and efficacy data for all engineered live biotherapeutics regardless of the source organism or history of safe use. The EMA also requires an environmental risk assessment to evaluate the potential for the engineered organism to persist in the environment and to interact with wild-type organisms. This more stringent approach reflects the EMA's precautionary principle, which emphasizes the need for comprehensive safety data before marketing authorization is granted.

In Brazil, ANVISA has begun to develop regulatory guidance for live biotherapeutics, but the framework remains less developed than those of the FDA and EMA. ANVISA currently classifies live biotherapeutics as medicinal products subject to the same regulatory requirements as conventional drugs, but the specific requirements for live biotherapeutics remain unclear. This regulatory ambiguity has created barriers to the development and commercialization of engineered live biotherapeutics in Brazil, despite the country's significant expertise in biotechnology and microbiology.

TABLE 1: Regulatory Classification and Pathway Requirements Across Jurisdictions

Jurisdiction	Regulatory Agency	Product Classification	Regulatory Pathway	Primary Legislation	Key Requirements	Timeline to Approval	Market Exclusivity
United States	FDA	Biological Product (primary) or Dietary Supplement (alternative)	BLA (Biologics License Application) or DSHEA (Dietary Supplement)	Public Health Service Act (42 U.S.C. § 262) or DSHEA (21 U.S.C. § 321)	CMC, nonclinical safety, clinical efficacy (BLA); minimal data (DSHEA)	2–7 years (BLA); <1 year (DSHEA)	12 years (BLA); none (DSHEA)
European Union	EMA	Medicinal Product	Centralized Procedure	Directive 2001/83/EC (as amended)	CMC, nonclinical safety, clinical efficacy, environmental risk assessment	2–3 years	10 years (+ 2-year extension)
Brazil	ANVISA	Medicinal Product	National Procedure	Law 6,360/1976 (as amended)	CMC, nonclinical safety, clinical efficacy (requirements evolving)	1–3 years (evolving)	10 years
Canada	Health Canada	Biologic Drug	Biologic License Application	Therapeutic Products Directorate (TPD) or Biologic and Radiopharmaceutical Drugs Directorate (BRDD)	CMC, nonclinical safety, clinical efficacy	2–3 years	8 years
Australia	TGA	Biological Product	Australian Regulatory Pathway	Therapeutic Goods Act 1989	CMC, nonclinical safety, clinical efficacy	1–2 years	5 years
Japan	PMDA	Biological Product	Pharmaceutical Affairs Bureau (PAB)	Pharmaceutical Affairs Law	CMC, nonclinical safety, clinical efficacy	2–3 years	8 years

LEGEND: BLA, Biologics License Application; DSHEA, Dietary Supplement Health and Education Act; CMC, Chemistry, Manufacturing and Controls; EMA, European Medicines Agency; FDA, Food and Drug Administration; ANVISA, Agência Nacional de Vigilância Sanitária; TGA, Therapeutic Goods Administration; PMDA, Pharmaceuticals and Medical Devices Agency.

The choice of regulatory pathway has profound implications for the development timeline, cost, and ultimate commercial viability of an engineered live biotherapeutic. The biologics pathway requires substantially more data and longer development timelines, but provides greater regulatory certainty and market exclusivity. The dietary supplement pathway requires less data and shorter development timelines, but provides no market exclusivity and may limit the claims that can be made regarding the product's therapeutic benefits. Developers must carefully evaluate the regulatory landscape in each jurisdiction where they plan to commercialize their product and select a pathway that balances the need for regulatory certainty with the practical constraints of product development.

Chemistry, Manufacturing and Controls: Characterizing Living Systems

The characterization and control of manufacturing processes for engineered live biotherapeutics represents one of the most significant challenges in regulatory science for this novel therapeutic class. Traditional pharmaceutical chemistry, manufacturing and controls focuses on the characterization of chemical structure, purity, and stability of small-molecule drugs or the biochemical and biophysical properties of recombinant proteins. For engineered live biotherapeutics, the characterization must extend to the biological properties of the living organism, including its genetic composition, phenotypic characteristics, growth kinetics, metabolic capacity, and potential for genetic exchange with environmental microorganisms.

The FDA's guidance on chemistry, manufacturing and controls for live biotherapeutic products emphasizes the importance of comprehensive characterization of the source organism, including its taxonomic classification, genetic composition, and history of safe use. For engineered organisms, the guidance requires detailed documentation of the genetic modifications, including the genes that have been introduced or deleted, the methods used to introduce the modifications, and the potential for unintended genetic changes resulting from the engineering process. The guidance also requires assessment of genetic stability, including evaluation of the potential for the engineered genes to be lost or mutated during manufacturing and storage, and evaluation of the potential for horizontal gene transfer to environmental microorganisms or to the host microbiota.

The characterization of engineered live biotherapeutics must include both genotypic and phenotypic characterization. Genotypic characterization involves sequencing of the entire genome to confirm the intended genetic modifications and to identify any unintended genetic changes that may have resulted from the engineering process. Whole-genome sequencing has become increasingly affordable and rapid, making it feasible to perform comprehensive genotypic characterization as part of the manufacturing process. However, the interpretation of whole-genome sequencing data remains challenging, particularly regarding the identification of unintended genetic changes that may have functional consequences. The FDA guidance recommends the use of bioinformatic tools to identify potential off-target effects of genetic engineering, but the adequacy of these tools for predicting functional consequences remains uncertain.

Phenotypic characterization involves assessment of the biological properties of the engineered organism that are relevant to its therapeutic function. For engineered probiotics, this may include assessment of the organism's ability to survive passage through the gastrointestinal tract, to adhere to intestinal epithelial cells, to produce bioactive metabolites, and to modulate immune responses. Phenotypic characterization typically involves in vitro assays that measure these properties under controlled conditions, as well as in vivo studies in animal models that assess the organism's behavior in the context of the intact gastrointestinal tract and host immune system. The challenge in phenotypic characterization is that the properties of the organism may vary depending on growth conditions, passage number, and storage conditions, making it difficult to establish specifications that ensure consistent product quality across manufacturing batches.

The manufacturing process for engineered live biotherapeutics must be designed to ensure consistent production of a product with defined genotypic and phenotypic characteristics. The FDA guidance emphasizes the importance of process validation, which involves demonstrating that the manufacturing process consistently produces a product that meets established specifications. For living systems, process validation is particularly challenging because the properties of the organism may change during manufacturing, and it may be difficult to establish specifications that are both meaningful and achievable. The guidance recommends the use of a quality by design approach, which involves identifying the critical process parameters that affect product quality and establishing control strategies to ensure that these parameters remain within acceptable ranges.

The stability of engineered live biotherapeutics during storage represents a critical challenge for manufacturing and controls. Living organisms are inherently unstable, with viability declining over time depending on storage conditions including temperature, humidity, and oxygen availability. The FDA guidance requires stability studies that demonstrate the product remains viable and maintains its intended biological properties throughout the proposed shelf life. Stability studies typically involve storage of the product under various conditions and periodic assessment of viability, genetic stability, and phenotypic properties. The challenge in stability assessment is that the properties of the organism may change in ways that are not immediately apparent from viability assays, such as changes in metabolic capacity or immunogenicity that may only become apparent during clinical use.

TABLE 2: Chemistry, Manufacturing and Controls Requirements for Live Biotherapeutics

CMC Element	Requirement	Characterization Method	Acceptance Criteria	Regulatory Guidance	Challenges
Source Organism Characterization	Taxonomic identification, genetic composition, history of safe use	16S rRNA sequencing, whole-genome sequencing, phenotypic testing	Confirmed identity, no pathogenic markers, documented safety history	FDA, EMA, ICH	Interpretation of genomic data, prediction of phenotypic consequences
Genetic Modification Characterization	Documentation of engineered genes, insertion sites, copy number, stability	Whole-genome sequencing, Southern blot, PCR, qPCR	Confirmed intended modifications, no unintended changes, stable integration	FDA, EMA	Off-target effects, unintended mutations, long-term stability
Genetic Stability Assessment	Evaluation of genetic stability during manufacturing and storage	Whole-genome sequencing at multiple passages, stability studies	<1% mutation rate, no loss of engineered genes, maintained phenotype	FDA, EMA	Defining acceptable mutation rates, predicting long-term stability
Horizontal Gene Transfer Assessment	Evaluation of potential for gene transfer to environmental organisms	In vitro conjugation assays, in vivo studies in animal models	<10^-6 frequency of gene transfer, no transfer to pathogenic organisms	FDA, EMA	Predicting frequency in vivo, assessing functional consequences
Phenotypic Characterization	Assessment of biological properties relevant to therapeutic function	In vitro assays (adhesion, metabolite production, immunogenicity), in vivo studies	Consistent with specifications, reproducible across batches	FDA, EMA	Defining relevant phenotypic properties, establishing specifications
Manufacturing Process Control	Identification of critical process parameters, establishment of control strategies	Process validation studies, design of experiments	Consistent product quality across batches, <10% variation in key parameters	FDA, ICH Q14	Controlling living systems, defining acceptable variability
Stability Assessment	Evaluation of viability and biological properties during storage	Viability assays, genetic stability assessment, phenotypic testing	>80% viability at end of shelf life, maintained genetic and phenotypic stability	FDA, ICH Q1A	Predicting long-term stability, defining storage conditions
Potency Assay	Quantitative measurement of therapeutic activity	Bioassays, cell-based assays, biochemical assays	Consistent potency across batches, >80% of target potency	FDA	Defining relevant potency measures, establishing acceptance criteria
Purity and Contamination Assessment	Evaluation of microbial contamination and off-target organisms	Culture-based methods, molecular methods (16S rRNA sequencing)	<1% contamination with off-target organisms, no pathogenic organisms	FDA, EMA	Detecting low-level contamination, defining acceptable limits
Endotoxin Assessment	Measurement of bacterial endotoxin (lipopolysaccharide)	LAL assay, kinetic chromogenic LAL	<175 EU/kg body weight (FDA limit), <100 EU/kg (EMA limit)	FDA, EMA, USP	Relevance to live organisms, defining acceptable limits

LEGEND: CMC, Chemistry, Manufacturing and Controls; rRNA, ribosomal RNA; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; FDA, Food and Drug Administration; EMA, European Medicines Agency; ICH, International Council for Harmonisation; EU, endotoxin units; LAL, Limulus amebocyte lysate; USP, United States Pharmacopeia.

Nonclinical Safety Assessment: Evaluating Risks in Preclinical Models

The nonclinical safety assessment of engineered live biotherapeutics presents unique challenges compared to conventional therapeutics because these products are living systems with the potential to persist, proliferate, and interact with host tissues and microbiota in ways that cannot be fully predicted from in vitro studies. The FDA guidance on nonclinical safety assessment for live biotherapeutics recommends a risk-based approach that evaluates the potential for adverse effects based on the properties of the organism, the route of administration, the intended patient population, and the proposed clinical use.

The nonclinical safety assessment should include evaluation of the potential for the engineered organism to cause infection or disease in the host. This assessment typically involves studies in animal models that evaluate the organism's ability to invade tissues, to produce toxins or other virulence factors, and to trigger inflammatory responses. For organisms that are derived from commensal bacteria or have a long history of safe use, the risk of infection may be considered low, and the nonclinical safety assessment may focus on evaluation of the potential for the engineered modifications to alter the organism's virulence properties. For organisms that are derived from pathogenic bacteria or that have been engineered to produce novel proteins, more extensive nonclinical safety assessment may be required.

The nonclinical safety assessment should also include evaluation of the potential for the engineered organism to produce off-target effects through mechanisms other than direct infection. For example, engineered probiotics that produce bioactive metabolites may have unintended effects on host tissues or on the composition of the microbiota. The assessment of off-target effects typically involves in vitro studies that evaluate the effects of the organism's metabolites on various cell types, as well as in vivo studies in animal models that evaluate the effects of the organism on host physiology and microbiota composition. The challenge in assessing off-target effects is that the organism's effects may depend on the composition of the host microbiota, the nutritional status of the host, and other host factors that may vary between individuals.

The nonclinical safety assessment should include evaluation of the potential for horizontal gene transfer from the engineered organism to environmental microorganisms or to the host microbiota. Horizontal gene transfer is a well-

documented phenomenon in bacteria, and the potential for engineered genes to be transferred to other organisms represents a significant safety concern. The assessment of horizontal gene transfer typically involves in vitro studies that evaluate the frequency of gene transfer under various conditions, as well as in vivo studies in animal models that evaluate the potential for gene transfer in the context of the intact gastrointestinal tract. The FDA guidance recommends that engineered organisms be designed to minimize the potential for horizontal gene transfer, for example by using genetic elements that are not found in nature or by incorporating safeguards that prevent the expression of transferred genes in recipient organisms.

The nonclinical safety assessment should include evaluation of the potential for the engineered organism to cause immune-mediated adverse effects. Living organisms are inherently immunogenic, and the engineered modifications may alter the organism's immunogenicity in ways that could trigger adverse immune responses. The assessment of immunogenicity typically involves in vitro studies that evaluate the organism's ability to activate immune cells, as well as in vivo studies in animal models that evaluate the potential for the organism to trigger systemic or local immune responses. The challenge in assessing immunogenicity is that the immune response to the organism may depend on the composition of the host microbiota, the nutritional status of the host, and other host factors that may vary between individuals.

Clinical Trial Design: Addressing Complexity and Variability

The design of clinical trials for engineered live biotherapeutics presents unique challenges compared to conventional therapeutics because these products are living systems with inherent biological variability, and their effects may depend on host factors including the composition of the microbiota, nutritional status, and genetic polymorphisms. The FDA guidance on clinical trial design for live biotherapeutics recommends a risk-based approach that tailors the design of clinical trials to the properties of the organism, the intended patient population, and the proposed clinical use.

The initial clinical trials for engineered live biotherapeutics typically involve evaluation of safety and tolerability in healthy volunteers or in patients with the target disease. These early-phase trials are designed to identify potential adverse effects and to establish a safe dose range. The challenge in designing early-phase trials for live biotherapeutics is that the organism may have different effects in healthy volunteers compared to patients with disease, and the effects may depend on host factors that are difficult to control or predict. The FDA guidance recommends that early-phase trials include comprehensive assessment of the organism's persistence and proliferation in the host, as well as assessment of the organism's effects on the composition of the microbiota and on host immune responses.

The later-phase clinical trials for engineered live biotherapeutics should be designed to evaluate efficacy in the target patient population. The challenge in designing efficacy trials is that the effects of the organism may be highly variable between individuals, depending on host factors including the composition of the microbiota, nutritional status, and genetic polymorphisms. The FDA guidance recommends the use of biomarkers to identify patients who are likely to respond to the treatment, and to monitor the effects of the treatment on relevant biological pathways. The use of biomarkers may allow for more efficient trial designs that enroll patients who are likely to respond to the treatment, and that monitor the effects of the treatment on relevant biological pathways rather than relying solely on clinical endpoints.

The design of clinical trials for engineered live biotherapeutics should include assessment of the organism's persistence and proliferation in the host. This assessment typically involves collection of fecal samples at regular intervals and quantification of the engineered organism using culture-based or molecular methods. The assessment of persistence is important because the therapeutic effects of the organism may depend on its ability to establish stable populations in the host microbiota. Additionally, the persistence of the organism may raise safety concerns if the organism has the potential to cause adverse effects with prolonged exposure.

The design of clinical trials for engineered live biotherapeutics should include assessment of the organism's effects on the composition of the microbiota. This assessment typically involves collection of fecal samples at regular intervals and analysis of the microbiota composition using 16S ribosomal RNA gene sequencing or whole-genome shotgun metagenomic sequencing. The assessment of microbiota composition is important because the therapeutic effects of the organism may depend on its ability to alter the composition of the microbiota in ways that promote health. Additionally, changes in microbiota composition may have unintended consequences that could affect the safety or efficacy of the treatment.

The design of clinical trials for engineered live biotherapeutics should include assessment of the organism's effects on host immune responses. This assessment typically involves measurement of inflammatory biomarkers in blood samples, as well as assessment of immune cell populations and their functional properties. The assessment of immune responses is important because the therapeutic effects of the organism may depend on its ability to modulate immune responses in ways that promote health. Additionally, the organism may trigger adverse immune responses that could affect the safety of the treatment.

Harmonization and Future Directions: Building a Coherent Regulatory Framework

The current regulatory landscape for engineered live biotherapeutics is characterized by significant inconsistencies and gaps across jurisdictions, creating barriers to innovation and limiting the development of products that could benefit patients. The FDA's approach, which allows for classification of some live biotherapeutics as dietary supplements, has created a two-tiered regulatory system in which some products receive minimal regulatory scrutiny while others undergo comprehensive evaluation. The EMA's approach, which requires comprehensive safety and efficacy data for all engineered live biotherapeutics, is more stringent but may create barriers to innovation by requiring extensive data generation before clinical trials can begin. ANVISA's approach remains underdeveloped, creating

uncertainty for developers seeking to commercialize products in Brazil.

The harmonization of regulatory frameworks across jurisdictions would provide significant benefits for developers and for patients. A harmonized approach would reduce the burden of regulatory compliance by allowing developers to generate data that satisfies the requirements of multiple jurisdictions simultaneously. A harmonized approach would also promote innovation by establishing clear expectations regarding the data that must be generated and the standards that must be met for marketing approval. Additionally, a harmonized approach would promote patient safety by ensuring that all engineered live biotherapeutics undergo appropriate safety and efficacy evaluation before marketing authorization is granted.

The development of a harmonized regulatory framework for engineered live biotherapeutics should be based on several key principles. First, the framework should recognize the unique properties of engineered live biotherapeutics and establish regulatory requirements that are tailored to these properties, rather than attempting to fit these products into existing regulatory categories designed for small-molecule drugs or recombinant proteins. Second, the framework should emphasize a risk-based approach that tailors regulatory requirements to the properties of the organism, the intended patient population, and the proposed clinical use. Third, the framework should promote innovation by establishing clear expectations regarding the data that must be generated and the standards that must be met for marketing approval. Fourth, the framework should promote patient safety by ensuring that all engineered live biotherapeutics undergo appropriate safety and efficacy evaluation before marketing authorization is granted.

The development of a harmonized regulatory framework will require collaboration among regulatory agencies, industry, and academic institutions. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use has begun to develop guidance on the regulation of live biotherapeutics, but this work remains in early stages. The development of harmonized guidance will require significant investment in research to address fundamental questions about the characterization, manufacturing, and clinical evaluation of engineered live biotherapeutics. Additionally, the development of harmonized guidance will require engagement with stakeholders including patients, healthcare providers, and environmental advocates to ensure that regulatory requirements balance the need for innovation with the imperative to protect patient safety and environmental health.

CONCLUSION

Engineered live biotherapeutics represent a novel therapeutic class that poses unprecedented challenges for regulatory science. The development of a coherent regulatory framework for these products requires integration of traditional pharmaceutical science with microbiology, molecular biology, and systems biology, while addressing fundamental questions about product characterization, manufacturing consistency, stability, and clinical safety that differ substantially from conventional therapeutics. The current regulatory landscape is characterized by significant inconsistencies and gaps across jurisdictions, creating barriers to innovation and limiting the development of products that could benefit patients. The harmonization of regulatory frameworks across jurisdictions would provide significant benefits for developers and for patients by reducing the burden of regulatory compliance, promoting innovation, and ensuring that all engineered live biotherapeutics undergo appropriate safety and efficacy evaluation. The development of a harmonized regulatory framework will require collaboration among regulatory agencies, industry, and academic institutions, as well as significant investment in research to address fundamental questions about the characterization, manufacturing, and clinical evaluation of engineered live biotherapeutics. As the field of engineered live biotherapeutics continues to evolve, regulatory science must evolve in parallel to ensure that regulatory frameworks remain adequate to protect patient safety while promoting innovation and access to beneficial therapies.

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