EXTENSIVE EXPERIENCE IS CRUCIAL TO SPEED TO THE CLINIC AND MARKET FOR LIVE BIOTHERAPEUTIC PRODUCTS

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As more microbiome-based therapies progress through the clinic toward approval for market launch, concerns over the lack of outsourcing capacity for process development and commercial manufacturing of live biotherapeutic products (LBPs) are increasing. Arranta Bio has more than a decade of experience in the development and tech transfer of LBPs under GMP conditions.

Our actALIVE™ Fast-to-Clinic Program delivers high-yielding, high-viability LBPs for clinical trial use in an accelerated timeline based on Arranta's ALIVE Biotherapeutic Products™ manufacturing platform. With fit-for-purpose cGMP manufacturing capabilities for the supply of clinical trial material and commercial quantities, companies in the microbiome space now have access to the specialized expertise, world-class team and facilities needed to bring novel medicines to patients.

HISTORY OF ARRANTA BIO

Arranta Bio was formed to meet the significant need in the microbiome space for experienced CDMOs with early-stage development expertise through commercial manufacturing of LBPs. Through the acquisition of Captozyme (Gainesville, Florida) in November 2019, Arranta secured extensive capabilities in process development and early clinical scale-up of LBPs and more than a decade of institutionalized knowledge and experience gained developing microbiome products that achieve critical product attributes (such as high final form cell viability, long-lasting stability, and the appropriate administration release profile).

Our unique perspective, which derives from a knowledge base not only in microbiology, but also chemistry and other fields, allows us to evaluate projects with an eye to understanding and defining the process that will provide the optimal conditions for the survival and growth of the microbes. Attention to even seemingly insignificant microbiological details is critical to determining the most efficient processes to cultivate and harvest these unique organisms.

We have worked on more than 135 species of organisms covering 85 genera. Based on our extensive experience, Arranta Bio is able to identify the process conditions most likely to provide optimal performance for a given client program and to avoid pitfalls by using Arranta Bio's ALIVE Biotherapeutic Products™ manufacturing platform.

COMPREHENSIVE DEVELOPMENT PROGRAM

To be successful developing scalable LBP processes, a CDMO must have foundational expertise in process development and manufacturing of microbiome-based products. Traditional approaches to process development and manufacturing are generally not effective, given the unique characteristics and processing requirements of LBPs compared to, say, growing mammalian cells that are then lysed so that a protein can be purified.

It is essential to consider the entire process from the start rather than limiting the focus to just the most proximal stages at a given time. As products are scaled up, many of the critical product attributes become exponentially more important – it is critical that this is understood at the earliest possible stages of development to avoid expensive and time-consuming development rework during the later stages of clinical development. At Arranta Bio, we assess what can be achieved at commercial scale, designing processes for the large-scale initially, and then scaling them down to the lab scale for intensive development.

As a result, Arranta Bio offers customers a comprehensive development and manufacturing program for aerobic, anaerobic, and spore-forming LBPs that considers all stages on the path to commercialization. Our proprietary ALIVE Biotherapeutic Products™ (aLBP) platform has several advantages, as embodied by the ALIVE acronym:

ALIVE™
Biotherapeutic Products

- **A:** Accelerated development
- **L:** Long-lasting stability
- **I:** Immediate release & recovery
- **V:** Viable, high activity
- **E:** Efficient, scalable process

ACCELERATED DEVELOPMENT

Leveraging our more than 10 years of experience and proprietary technology which includes our media and cryopreservative blends (see below), it is possible to take projects from a single strain in a vial (or even consortia of three to five organisms) through development of the master cell bank(s) that enables high-yielding manufacturing processes for the drug substance(s) and drug product(s) resulting in delivery of the first clinical batch in just nine months. For consortia containing 6–15 organisms, the timeline is slightly longer, at 12 months.

LONG-LASTING STABILITY

LBPs are new as therapeutics but have in fact been manufactured for many decades in the food industry, and most recently in the form of probiotic foods and dietary supplements. The food sector, however, is not governed by the same heightened level of quality control and regulatory requirements that exist in the pharmaceutical industry. Complicating the situation is the lack of regulatory guidance. The U.S. Food and Drug Administration and the European Medicine Agency each have published just one guidance document on therapeutic LBPs.

Arranta Bio has taken the approach that the LBPs we produce will have an extended shelf life of at least two years at room temperature. Room-temperature stability avoids the need for cold-chain storage, shipment, and handling. To achieve this goal, we carefully select appropriate excipients, implement tight environmental controls, and employ highly customized primary packaging configurations.

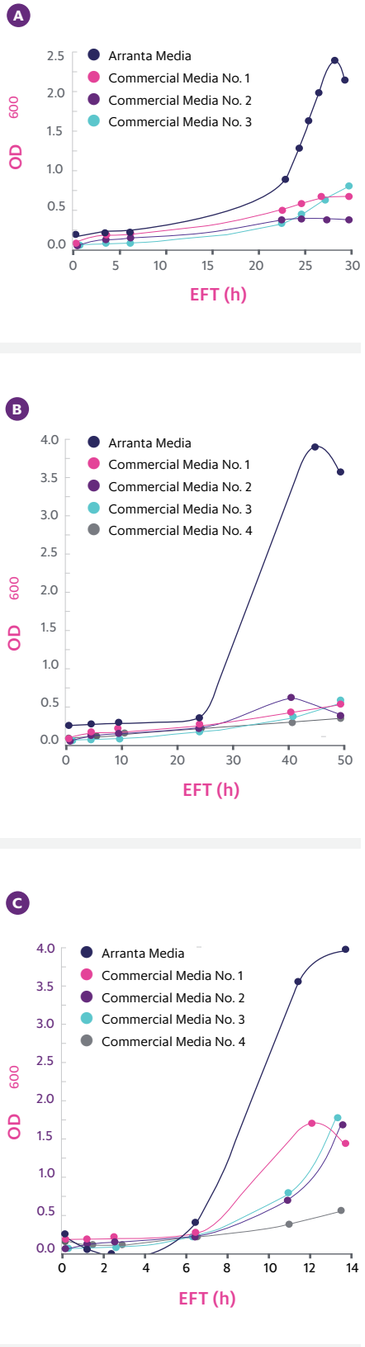
Excipients are used in the final formulation to influence the cellular size and structure and to help stabilize LBPs during cryopreservation and/or to preferentially bind moisture to ensure maintenance of viability and activity of the product once it is in the package.

IMMEDIATE RELEASE & RECOVERY

Although colony forming units (CFUs) per capsule is the predominant measure of activity for probiotic supplements, this value does not provide any information on how quickly the LBP will grow and recover once ingested. If an LBP is intended to treat a gastrointestinal tract disease, it will not matter if a billion CFUs are administered if they are passed through the stool before they move from the dormant to the active state.

Many LBP therapies are being developed for disorders of the small intestine, such as Crohn's disease (CD) and ulcerative colitis (UC). The transit time into the small intestine from the mouth is approximately 4–6 hours. During that time, the capsule needs to pass through the stomach intact in order to reach the upper part of the small intestine and release the bacteria, which then must become hydrated and replicate

FIGURE 2 Representative Comparison Plots for Arranta Bio Media Blends (AMBTM)



so they can graft in the small intestine and exhibit efficacy.

Effective drug development at Arranta Bio, therefore, considers many factors relevant to achieving recovery and release, which we define as critical quality attributes for LBPs. Those factors include the capsule composition, which much be selected to ensure that the dormant bacteria are released in the right location, and the lag time for the bacteria to "awaken" so that they become active in the target organ.

It is equally important to understand the conditions within the target organ to ensure that the LBP is formulated in a manner that will encourage engraftment of the microorganism. The microbiome is present in all parts of the body, and conditions vary extensively, from pH to the presence of oxygen and other biochemicals.

HIGH VIABILITY, HIGH ACTIVITY

Clearly, LBPs by definition must contain living organisms that exhibit some type of activity. The ultimate goal during process development is to maximize the number of cells that stay alive (viable) throughout the entire production process. Factors influencing viability range from the general fermentation conditions to the harvest technique, excipient/cryopreservative selection, and lyophilization conditions. Development of an optimum cell bank is also crucial.

At Arranta Bio, we have a minimum target viability for all LBPs of 40% and often achieve significantly higher viabilities.

EFFICIENT, SCALABLE PROCESS

Because most of the organisms that comprise LBPs tend to be unstable, it is essential to minimize process times in order to minimize organism die-off. Effective small-scale processes that take much longer to complete when performed at large scale are thus not practical for commercial LBP manufacturing.

Arranta Bio ensures that the processes we develop at small scale will work at later stages and for commercial production in terms of timing and engineering aspects by employing downsized versions of equipment and systems used at large scale. As a result, scale-up occurs seamlessly from 1 L all the way to 4000 L.

In addition, the importance of cell bank quality is too often underestimated. Arranta Bio focuses on optimizing the master cell bank to ensure that the organism being produced is optimally healthy, exhibits the right performance behaviors, and is generated in the maximum yield.

Comprehensive characterization of LBP strains is also essential for successful process development. While most people developing LBP therapeutics understand the clinical impact of their products, they do not typically know why those particular organisms provide those results, largely because the available analytics are outdated. Knowledge of critical parameters and acceptable ranges – e.g., redox and osmolality – and how to control them is necessary to achieve and maintain the proper functionality throughout the production process.

PROPRIETARY SOLUTIONS

The team at our development and early clinical supply Gainesville site has developed a set of proprietary Arranta Media Blends (AMB™) and proprietary Arranta CryoPreservatives (ACP™) that are used to rapidly screen client microorganisms to identify optimal process conditions. Using Arranta's proprietary blends, which only contain plant-derived components, it is possible to obtain results in a matter of weeks compared with months needed to develop new blends, dramatically shortening project timelines.

MEDIA FORMULATIONS

Many of the organisms developed as microbiome-based therapies tend to form aggregates with many organisms organized in long chains. When this "hair" forms, analysis becomes complicated, because as many as 100 cells may only be detected as one, leading to much lower CFU results. There are limited commercially available media designed for LBPs, and most contain blood or other animal-derived components, forcing LBP developers to select sub-optimal options.

We have found that certain media formulations prevent aggregation regardless of the type of organism, and optimum processes can be rapidly developed by screening against them. Our proprietary AMB™ blends have been shown to significantly outperform commercially available media formulations suitable for GMP manufacturing. We obtain not only higher yields, but more stable organisms with shorter lag phases.

We have conducted media screening using multiple commercial and literature-derived media formulations identified by the source as optimal for the genus being examined (**Figure 2**). The Arranta Bio proprietary media are clearly vastly superior to the representative commercial and literature-derived media blends. The benefits of Arranta Bio's media are numerous: (1) yields are substantially greater, (2) lag phases are shorter, and (3) growth rates are significantly higher (more exponential growth curves). Indirect benefits have also been identified downstream, including higher resulting viability and shorter lag phases through freeze-drying, which equates to higher product quality.

CRYOPRESERVATIVE BLENDS

Mixes of cryopreservatives are added to the cell slurry obtained following harvest of the LBP after fermentation to maintain viability through the harsh freezing and drying process, which can be damaging to live microorganisms.

Some cryopreservatives penetrate the cells, while others do not. They influence viscosity, osmolality, surface tension, and other properties. The goal is to choose the right mixture that provides the optimal conditions for each specific organism to survive during cryopreservation. As with media for LBPs, there are only a limited number of commercially available cryopreservative blends.

The proprietary Arranta CryoPreservatives (ACP™) are blends that enable us to achieve Arranta Bio's 40% minimum viability target nearly 100% of the time.

As can be seen in **Figure 3**, all of Arranta's proprietary ACP™ blends provided higher viability levels than the two representative commercial blends. The percent viability for the commercial blends was less than 3%, while all of Arranta Bio's proprietary ACP™ blends maintained greater than 40% viability through freeze drying.

*ACT*ALIVE™ FAST TO CLINIC PROGRAM

Arranta Bio offers an accelerated development program to deliver microbiome clinical material for first-in-human (FIH) trials using Arranta's proprietary platform process incorporating our optimized AMB™ media and ACP™ cryopreservatives to achieve >40% viability in filled capsules.

Clinical trial materials for consortia of five organisms or fewer can be produced in nine months, and for consortium of 6–15 organisms in 12 months from the start of the program. Drug substance is produced at the 50-L fermentation scale and then freeze-dried. Drug product is manufactured in batch sizes up to 10,000 capsules.

As important as the accelerated timeline, is Arranta's understanding of the significant rolled throughput yield (RTY) jeopardy at every step of the production process associated with LBP microbiome products. Accordingly, a carefully planned and executed quality by design (QbD) approach at the earliest stages of development for every unit of operation in the manufacturing process – from cell bank to encapsulation – is absolutely critical to give the product the best opportunity for success in clinical trials and beyond. Arranta's actALIVE™ Fast-to-Clinic Program therefore ensures the rapid production of high-yielding, high-viability LBPs with increased stability.

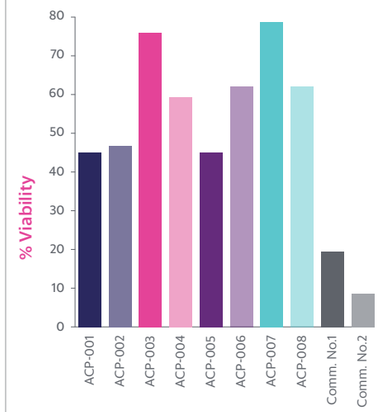


FIGURE 3 Representative Comparison Plot for Selected Arranta Bio CryoPreservatives (ACPTM)

Arranta Bio is also uniquely positioned within the microbiome space to facilitate the entire life cycle from bench to market. Moreover, the technical team that performs the basic scientific work early on is involved and actually on hand to support and troubleshoot the launch from our Watertown commercial-ready facility. The result is further compressed timelines and reduced cost and risk.

CONCLUSION

The future of microbiome-based therapy is certainly exciting. The bounty of evidence linking the microbiome to many aspects of human health is rapidly expanding. Once the first products receive approval for commercial launch, funding will almost certainly accelerate into the microbiome space and validate the activity that is already occurring. The first therapies that will reach the market will target gut-related diseases. But it is the microbiome-based therapies already in the clinical development pipeline for other indications – from oncology to diabetes to autism – that are likely to have the greatest impact on human health and to further propel sector growth. P

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Dr. Aaron B. Cowley is the Chief Scientific Officer at Arranta Bio, having been a co-founder and the Chief Technical Officer of Captozyme, which served the microbiome CDMO market for 10 years since 2009 and merged with Arranta Bio in November 2019. He was the recipient of the prestigious Ruth L. Kirschstein National Research Service Award for his post-doctoral work at the University of Georgia. He holds a bachelor's degree in biochemistry from Benedictine College, a Ph.D. in inorganic chemistry from the University of Kansas, and an MBA from the University of Florida.

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