# Drug Development & Delivery

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# Bioavailability & Solubility: The Never-Ending Quest

The Science & Business of Pharmaceutical and Biological Drug Development

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p.40

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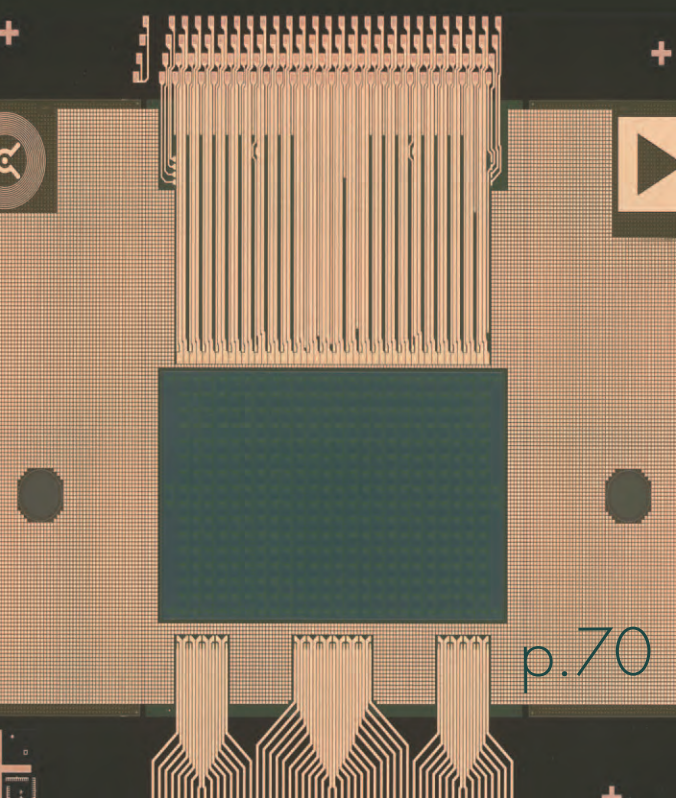


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## Voyager Therapeutics Announces Selection of Gene Therapy Development Candidate for Friedreich's Ataxia in Collaboration With Neurocrine Biosciences, Triggering Milestone Payment

Voyager Therapeutics, Inc. recently announced the joint steering committee with its collaborator Neurocrine Biosciences has selected a lead development candidate in the Friedreich's ataxia (FA) program. The candidate combines a frataxin (FXN) gene replacement payload with an intravenously administered, blood-brain barrier penetrant, novel capsid derived from Voyager's TRACER capsid discovery platform. The companies expect the program to advance into first-in-human clinical trials in 2025.

Selection of the development candidate triggered a $5-million milestone payment to Voyager, which the company expects to receive in the first quarter of 2024. Voyager is eligible to receive additional future development and commercialization milestone payments based on the further advancement of this program.

"The nomination of this development candidate in FA marks an important step in our strategic collaboration with Neurocrine, reflecting the power of combining Voyager's TRACER AAV capsids and payload design capabilities with Neurocrine's expertise in neuroscience and clinical development," said Alfred W. Sandrock, Jr., MD, PhD, Chief Executive Officer of Voyager. "While there has been encouraging recent progress in the treatment of FA, it remains a very challenging and eventually fatal disease for which new therapeutic approaches are needed. We believe our strategy to replace the defective frataxin gene could address the underlying disease etiology of FA. We look forward to progressing this and our other gene therapy programs, including our wholly-owned SOD1 ALS program and our Neurocrine-partnered GBA1 Parkinson's program, towards clinical studies."

The FA program is being developed under the 2019 strategic collaboration agreement between Voyager and Neurocrine Biosciences for research, development, and commercialization of certain AAV gene therapy products for programs targeting Friedreich's ataxia and two other undisclosed targets. Under the terms of the 2019 collaboration agreement, Voyager is eligible to receive up to $1.3 billion in potential development and commercial milestone payments, tiered royalties on net sales, and program funding, and Voyager could exercise an option for 60/40 cost- and profit-sharing (Neurocrine/Voyager) in the US for the FA program following the determination by the joint steering committee of proof of mechanism based on established milestones and metrics.

Voyager's TRACER (Tropism Redirection of AAV by Cell-type-specific Expression of RNA) capsid discovery platform is a broadly applicable, RNA-based screening platform that enables rapid discovery of AAV capsids with robust penetration of the blood-brain barrier and enhanced central nervous system (CNS) tropism in multiple species, including non-human primates (NHPs). TRACER generated capsids have demonstrated superior and widespread gene expression in the CNS compared to conventional AAV capsids as well as cell- and tissue-specific transduction, including to areas of the brain that have been traditionally difficult to reach, while de-targeting the liver and dorsal root ganglia. As part of its external partnership strategy, Voyager has established multiple collaboration agreements providing access to its next-generation TRACER capsids to potentially enable its partners' gene therapy programs to treat a variety of diseases.

## Jubilant HollisterStier Opens Third Manufacturing Line

Jubilant HollisterStier recently celebrated the opening of its third sterile fill finish manufacturing line and broke ground on the next phase of its facility expansion in Spokane, WA.

The expansion includes a new high-speed commercial fill finish line, which is part of a 50k sq. ft. facility expansion, increasing JHS' production capacity by 55 million units per year. At 400 vials per minute, it's equipped with full isolator technology and 100% weight-checking capabilities at production speeds. It also includes an additional three compounding suites with capacity for up to 2000 L bulks and disposable, single-use compounding and filling technologies. The line is also outfitted with two new 300-sq-ft lyophilizers.

JHS is currently scheduling time for client projects on the third manufacturing line and has broken ground on Phase 2 of their facility expansion for a fourth manufacturing line.

This project has been supported in whole or in part with federal funds from the U.S. Department of Health and Human Services, Administration for Strategic Preparedness and Response (ASPR), Biomedical Advanced Research and Development Authority (BARDA), under cooperative agreement award number IDSEP230067.

JHS President and CEO Chris Preti, the Consulate General of India Mr. Prakash Gupta, representatives from the United States Department of Health and Human Services, members of the Spokane City Council, and other key local and state decision makers were in attendance.

The event also featured a video message from State Representative Cathy McMorris Rodgers regarding the positive benefits the JHS' expansion will have on the greater Spokane community, including the addition of 200+ new jobs.

Jubilant HollisterStier LLC, a subsidiary of Jubilant Pharmova Limited, is an integrated contract manufacturer of ophthalmics, sterile injectables, lyophilized products, and sterile ointments. We are committed to providing exceptional manufacturing services to the pharmaceutical and biopharmaceutical industries in North America. With two facilities located in Spokane, Washington and Montreal, Quebec, Canada, we ensure that your project is streamlined with a full range of support and services that it deserves by keeping and training the best talent. By investing in people with cross-functional training and development for critical roles, innovative equipment and predictive technologies, we are able to seize your project's potential for next level performance at every opportunity.


## XOMA Enters Agreement to Acquire Kinnate Biopharma

XOMA Corporation recently announced it has entered an agreement to acquire Kinnate Biopharma Inc. (NASDAQ: KNTE) for (i) a base cash price of $2.3352 per share (the Base Price) and (ii) an additional cash amount of not more than $0.2527 per share (together with the Base Price, the Cash Amount) at the closing of the merger plus a non-transferable contingent value right (CVR), representing the right to receive 85% of the net proceeds from any out license or sale of the Kinnate programs effected within one year of closing of the merger or 100% of the net proceeds from any out license or sale executed prior to the closing.

"This acquisition will further add to our sizable cash balance that resulted from the VABYSMO transaction, as well as potentially add several programs to our royalty portfolio," said Owen Hughes, Chief Executive Officer of XOMA. "Given our successful history of out licensing assets and technologies, we will look to monetize Kinnate's precision oncology programs to the benefit of Kinnate and XOMA shareholders alike."

Following a thorough review process conducted with the assistance of its legal and financial advisors, Kinnate's Board of Directors has determined that the acquisition by XOMA is in the best interests of all Kinnate stockholders and has unanimously approved the Merger Agreement.

Pursuant and subject to the terms of the Merger Agreement, a wholly owned subsidiary of XOMA will commence a tender offer (the "Offer") by March 4, 2024, to acquire all outstanding shares of Kinnate common stock. Closing of the Offer is subject to certain conditions, including the tender of Kinnate common stock representing at least a majority of the total number of outstanding shares; the availability of at least $120.0 million of cash, net of transaction costs, wind-down costs, and other liabilities, at closing, and other customary closing conditions. Immediately following the closing of the tender offer, Kinnate will merge with a subsidiary of XOMA, and all remaining shares not tendered in the offer, other than appraisal shares, will be converted into the right to receive the same cash and CVR consideration per share as is provided in the tender offer.

Kinnate shareholders holding approximately 46% of Kinnate common stock have signed support agreements under which such shareholders agreed to tender their shares in the Offer and support the merger. The acquisition is expected to close in April 2024. XOMA was represented by Gibson, Dunn & Crutcher LLP.

XOMA is a biotechnology royalty aggregator playing a distinctive role in helping biotech companies achieve their goal of improving human health. XOMA acquires the potential future economics associated with pre-commercial therapeutic candidates that have been licensed to pharmaceutical or biotechnology companies. When XOMA acquires the future economics, the seller receives non-dilutive, non-recourse funding they can use to advance their internal drug candidate(s) or for general corporate purposes. The Company has an extensive and growing portfolio of milestone and royalty assets (asset defined as the right to receive potential future economics associated with the advancement of an underlying therapeutic candidate).

## Evonik Partners With University of Mainz to Commercialize New Class of PEG Lipids for Nucleic Acid Delivery

Evonik and the University of Mainz have signed a license agreement to commercialize randomized polyethylene glycols (rPEGs), a new class of PEGs. Evonik intends to use rPEGs for its platform of specialized lipids and commercialize the excipients under the license agreement to meet customer and market needs. Technical-grade rPEG-lipids will be available in the second half of 2024.

As part of the company's Nutrition & Care life sciences division, Evonik's Health Care business has been growing its nucleic acid drug and vaccine delivery portfolio by leveraging its biosolutions, and innovation and co-creation opportunities with life science leaders. By partnering with the Johannes Gutenberg University of Mainz in Germany, where rPEGs were first developed, Evonik is able to offer its customers an expanded toolbox of technologies for nucleic acid-based medicines.

"When scientists at the University of Mainz approached us with their groundbreaking work on rPEGs, we immediately recognized the potential for broader formulation options and the benefits this could bring our customers," said Thomas Riermeier, Head of the Health Care business line at Evonik.

Polyethylene glycols (PEGs) are polymers that have been used in the pharmaceutical industry for more than 30 years to improve the bioavailability, stability, targeting and performance of therapeutics. rPEG polymers have similar properties to PEGs but have a different structure that is intended to offer an improved immunogenicity profile. They are especially suitable for pharmaceutical applications such as in lipids for lipid nanoparticle (LNP) carriers.

"With Evonik, we have found an enthusiastic and experienced partner to bring rPEG-lipids globally to the pharmaceutical industry," said Prof. Dr. Holger Frey, Johannes Gutenberg University of Mainz, who first developed rPEG polymers together with his research group.

Evonik's partnership with the University of Mainz is the latest in a series of strategic steps to meet the transformative needs of the pharmaceutical industry for nucleic acid drug delivery. In 2021, Evonik began a collaboration with Stanford University to scale up the synthesis and formulation of an innovative tissue-specific delivery platform for nucleic acids. Just over a year ago, Evonik opened a new cGMP facility in Hanau, Germany for the development and manufacture of smaller batches of specialized lipids. This was followed by the start of construction of a global-scale production facility for pharmaceutical specialty lipids in Lafayette, Indiana, in partnership with the US Government.

PEG lipids are used today in commercial COVID-19 vaccines. Along with cholesterol and ionizable and structural lipids, PEG lipids form the LNPs needed to deliver nucleic acids, such as mRNA effectively into the cell.

## Vaxxinity's Cholesterol Vaccine Candidate Successfully Lowers LDL-C: Preclinical Data Published

Vaxxinity, Inc. recently announced the publication of data from multiple non-human primate studies demonstrating that VXX-401 reproducibly lowers low-density lipoprotein cholesterol (LDL-C) in non-human primates. The results, which support the continued clinical development of VXX-401 as a candidate for the treatment of hypercholesterolemia and prevention of atherosclerotic cardiovascular disease, were published in the Journal of Lipid Research.

VXX-401 is a synthetic peptide vaccine designed to stimulate the immune system to produce antibodies targeting proprotein convertase subtilisin/kexin type 9 (PCSK9), which reduce circulating LDL-C by inhibiting the breakdown of low density lipoprotein receptor (LDLR). High LDL-C is a major risk factor for coronary heart disease, heart attack, and stroke, and atherosclerosis is the leading cause of disease burden globally. Previous studies have demonstrated that blocking PCSK9 yields lower LDL-C levels and reduces the risk of adverse cardiovascular events.

Across three separate preclinical studies in cynomolgus monkeys, VXX-401 induced a strong and durable antibody response against PCSK9, and robust, sustained reduction of LDL-C over time. Prolonged exposure with VXX-401 resulted in an average of 44% LDL-C reduction. VXX-401 was well tolerated and did not induce any toxicity nor pathology beyond mild injection site reactions. These results suggest that VXX-401 could be a safe and effective anti-PCSK9 immunotherapy.

"Vaxxinity is committed to providing scalable, accessible, game-changing solution for worldwide heart health," said Mei Mei Hu, CEO of Vaxxinity. "Despite multiple approved medications for LDL-C reduction, heart disease remains the number one killer in the world. A cholesterol vaccine like VXX-401 may provide a cost-effective and widely deployable solution that could potentially benefit hundreds of millions of people at risk. A well tolerated intervention that people can start early in life, and remain on for many years, lowering the cholesterol 'area under the curve,' has the potential to help us win the fight against heart disease."

VXX-401 is currently in a Phase 1 clinical trial for safety and tolerability. Vaxxinity is on track to report initial topline data in mid-2024. More information about the trial is available at clinicaltrials.gov using Identifier NCT05762276.

VXX-401 was designed using Vaxxinity's proprietary synthetic peptide vaccine platform and is being developed for the treatment of hypercholesterolemia. The platform is designed to harness the immune system to convert the body into its own natural "drug factory," stimulating the production of antibodies. VXX-401 is designed to induce robust, long-acting antibodies against PCSK9 and lower LDL cholesterol to prevent or treat coronary heart disease.

## Aptar CSP Technologies Collaborates With ProAmpac to Launch New Active Material Science Packaging Solution

Aptar CSP Technologies, part of AptarGroup, Inc., recently collaborated with ProAmpac, a leader in material science and flexible packaging, to develop and launch ProActive Intelligence Moisture Protect (MP-1000). This next-generation platform technology combines Aptar CSP's proprietary 3-Phase Activ-Polymer™ technology with ProAmpac's flexible blown film technology to deliver a patent pending moisture adsorbing flexible packaging solution. This is the first in a series of active microclimate management packaging solutions to mitigate degradation risk, maintain potency and improve product performance.

Aptar CSP's 3-Phase Activ-Polymer platform technology is a highly engineered active material science solution trusted by global brands to protect sensitive drug products, probiotics, medical devices, drug delivery systems, and foods. By incorporating the moisture adsorbing Activ-Polymer material into a flexible film structure, MP-1000 delivers high-quality moisture protection without the need for add-on desiccant sachets, thus reducing manufacturing downtime. This solution not only adsorbs excess moisture inside the package, but also shields the contents from moisture exposure that typically occurs when moisture molecules pass through the packaging en route to an add-on desiccant.

This active packaging solution features varying moisture capacities to deliver customized microclimate protection that meets differing product needs. Available in rollstock or pre-made pouches, MP-1000 has excellent seal characteristics and runs on high-speed form-fill-sealing equipment, ensuring product integrity and compatibility with existing flexible packaging equipment.

"We are pleased to unveil this new platform of active material science solutions with ProAmpac. The goal of this collaboration is to transform the way active packaging is delivered and fulfill unmet needs by providing the market with a fully integrated, flexible, multi-layer film solution, powered by CSP's proven Activ-Polymer technology," said Badre Hammond, Vice President of Global Commercial Operations and General Manager APAC for Aptar CSP Technologies. "We look forward to continuing our collaboration with ProAmpac to leverage the current platform to develop new active materials that address a broad range of product stability challenges."

"We are excited to launch the Moisture Protect MP-1000 platforms, the newest addition to the ProActive Intelligence product line. This innovative product has a high moisture-adsorbing capacity and is set to revolutionize moisture protection in flexible packaging. Our extensive material science work and exclusive collaboration with Aptar CSP Technologies has enabled us to bring the latest active packaging products to the market," said Hesam Tabatabaei, Senior Vice President of Global Product Development and Innovation for ProAmpac.

Aptar CSP's Activ-Polymer technology is a trusted solution for protecting high-value drugs, diabetes test strips, continuous glucose monitoring devices, probiotics, drug delivery devices, and even medical devices and implants across the globe. It is custom-engineered to provide a broad spectrum of product-specific protection in a wide range of physical formats. Key capabilities include scavenging oxygen, odors, and VOCs, emitting aromas or antimicrobials, and mitigating risk of mutagenic N-nitrosamine impurity formation and drug degradation.

## Avenue Therapeutics Announces First-In-Class Preclinical Data of BAER-101 in a Translational Model of Absence Epilepsy

Avenue Therapeutics, Inc. recently announced the publication of preclinical in vivo data in Drug Development Research highlighting BAER-101's full suppression of seizure activity using the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model of absence epilepsy. Data published showcase BAER-101's ability to selectively target GABAA α2 and α3 subtypes more than α1 and α5, potentially improving anti-convulsant and anxiolytic activity while minimizing the risk of tolerance and abuse associated with existing treatments in this drug class.

The publication describes the extent of anti-seizure activity of BAER-101 in the GAERS model, a widely used and translationally relevant animal model. The study demonstrated full suppression of seizure activity with a minimal effective dose (MED) of 0.3 mg/kg. The effect of BAER-101 was fast in onset and stable throughout the duration of testing. Results from the testing showed that the number of spike-wave discharges were dose-dependently reduced by BAER-101, and no adverse safety events were observed up to a dose 300x the MED.

BAER-101 is the first clinical candidate which is selective for only α2,3 and not for α1 or α5, a pharmacology consistent with anti-seizure activity that avoids the adverse side effects common to the GABAA positive allosteric modulators (PAM) class. Specifically, the α1-subtype of GABAAR is associated with dizziness and somnolence in both animal and human studies, and the α5-subtype of GABAAR is thought to play a key role in synaptic plasticity, cognition, and memory, suggesting that engagement of α5 risks anti-cognitive effects. The pharmacology of BAER-101 lacks activity at both the α1- and α5-subtypes of GABAAR, and these findings indicate that BAER-101's on-target engagement with a selective subset of synaptic GABAARs is sufficient to suppress absence seizures while avoiding adverse side effects common to the GABAA PAM class.

"Epilepsy remains one of the most prevalent neurological diseases worldwide, with a population of approximately 65 million patients, but there remains a great unmet need for a safe and effective treatment option that suppresses seizure activity without drug resistance or harmful side effects such as sedation, cognitive impairment, ataxia and addiction," said Alexandra MacLean, MD, Chief Executive Officer of Avenue. "The preclinical data published in Drug Development Research demonstrate BAER-101's ability to fully suppress seizures in the GAERS model, a translational animal model for anti-seizure drug development with a documented high predictability of response in humans. Additionally, BAER-101 demonstrated full efficacy with a minimal effective dose of 0.3 mg/kg, indicating that BAER-101 is the most potent compound yet reported in this model, as well as the first to show that a GABA PAM that is selective for the α2 and α3-subtype GABAARs is active in this model. Building on BAER-101's proven safety profile in over 700 patients and healthy human volunteers, these first-in-class preclinical findings support BAER-101's continued development in a Phase 2a trial."

Subject to obtaining the necessary financing, which could be provided through a strategic partnership, Avenue plans to initiate a Phase 2a clinical trial of BAER-101 to further study its anti-seizure properties in patients with common or rare epilepsies.

# Formulation Forum

## Nanoparticle Technologies Used in Ocular Drug Delivery

By: Shaukat Ali, PhD, Sr. Director, Scientific Affairs & Technical Marketing, and Jim Huang, PhD, Founder & CEO, Ascendia Pharmaceuticals Inc.

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### INTRODUCTION

As we continue to discover new and innovative small molecules and therapeutic compounds, over 80% of those candidates in the drug pipeline are challenging to develop due to their poor solubility and bioavailability.[1] That has created a roadblock for bringing in novel drugs to the market across all modalities. Though there are lots of publications and knowledge accumulation in oral and parenteral routes of drug delivery, the topical route of administration, more specifically, ocular delivery has been subject of continued interest in development of innovative drugs due, in part, to poor bioavailability (ca. 5%), and lack of safety and regulatory challenges.[2] The latter requires a thorough understanding of nanotechnologies applicable to innovative formulations for efficient delivery to target tissues in the eyes for treating diseases of the anterior segment.[3]

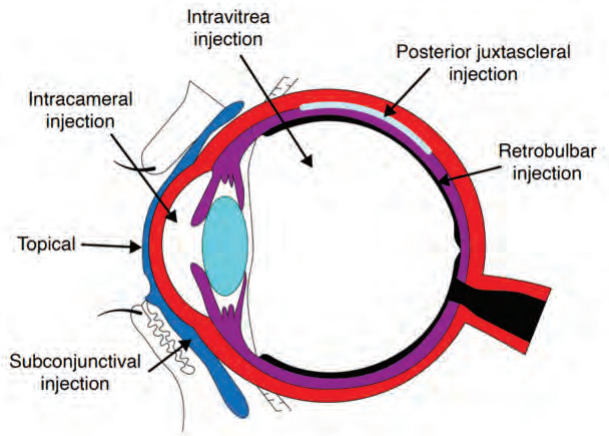
Although many drugs are available for the treatment most of eye diseases, there are still many ocular barriers, such as tear film, corneal, conjunctival, and blood barriers, that cause bioavailability issues.[4] Thus, finding ways to deliver drugs to the specific tissue in front and back of the eyes could be challenging because of a lack of drug solubility and permeation through the corneal membrane or in the interior eyes. Otherwise, drug delivery may require implants through invasive surgery. Currently, most eye drugs still focus on delivery through the topical self-administrative route, and the nanotechnology-based drug delivery system (NODS) remains the acceptable and effective way for topical administration of drugs.[5] NODS is applicable to those entities based on lipid nanoparticles, such as micelles, liposomes, cubosomes, nano- and microemulsions, dendrimers, polymeric hydrogels, among others.[6]

The following will describe the basic understanding of eye diseases, challenges in ocular drug delivery, and the future trends in development of innovative drugs.

### OCULAR DISEASES

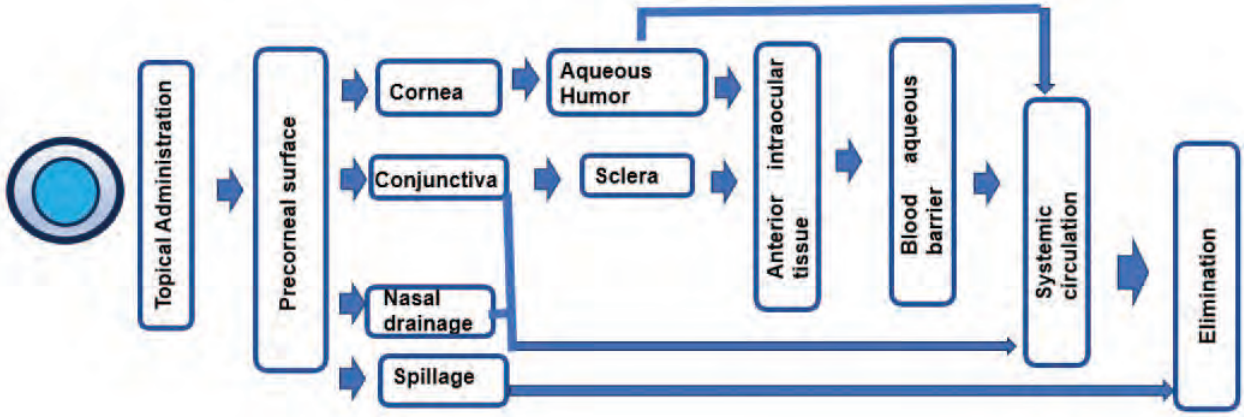
Eye diseases affect over 2.2 billion people globally according to the World Health Organization (WHO), and a majority of these diseases are preventable and treatable. Among the most common and debilitating eye diseases are bacterial and fungal infections, cataracts, age-related macular degenerative (AMD), glaucoma, conjunctivitis, diabetes retinopathy, retinoblastoma, among others. The root cause of some of these diseases however remains elusive.[7] Fungal keratitis, for example, occurs in traumatic cornea and is caused by fungus, such as Candida albicans, Candida glabrata, and others. Diabetes retinopathy results in loss of vision by leakage of blood from the back of the eyes. It is caused by oxidative stress and inflammation upregulated by proinflammatory mediators. Glaucoma is related to increased intraocular pressure, whereas AMD occurs due to irregular angiogenesis in the retinal epithelial that leads to vision loss and blindness if not treated on time.

**FIGURE 1**




**Routes for Ocular Delivery**

**FIGURE 2**




**Topical Route of Administration, Leading Barriers & Clearance**

## OCULAR DELIVERY

Figure 1 shows the route of administration of drug in the eyes. These include topical, subconjunctival injection, intravitreal injection, retrobulbar injection, and intracameral injection. The topical route, however, is non-invasive, widely used, and remains a self-administrative route. The challenges involve the immediate clearance of drugs by spillage and tear drainage that leads to reduced bioavailability.

The topical route of administration is a preferred method for treatment of many ophthalmic diseases. It allows only a limited amount of drug to permeate through the precorneal surface. It helps cross permeation of drugs through the cornea to aqueous by passive transcellular pathway. It is however limited by the membrane tight junctions that allow only 5% of drug absorption.[8] Therefore, solubility, molecular weight, ionic charges, lipophilicity, and pKa are all important properties that enable permeation, absorption, and bioavailability enhancement of drugs. Figure 2 illustrates the topical route of administration and leading pathways for absorption and clearances of drugs.

As indicate in Figure 2, the drug is distributed from the precorneal surface to the anterior segment through the cornea and conjunctiva/sclera pathways, several barrier layers, and eventually leading to systematic circulation and elimination. This is a complex mechanism, but drug distribution via nanoparticles through these barriers depends upon the molecular weight, lipophilicity, and ocular transporters including proteins and melanin binding.[9]

## NANOTECHNOLOGIES FOR OCULAR DELIVERY

Nanoparticles are derived from a range of excipients and polymers suited for encapsulating and delivering drugs to the target tissues. It is important to understand the physico-chemical properties of these excipients and their role played in designing, formulating, loading and carrying, and protecting of drugs.

### Range of Excipients/Polymers Include

**Lipids:** DSPC, DPPC, DMPC, DOPC, DPPE, Soy lipids, DSPE-mPEG 2000; Cationic lipids (DOTAP), among others

**Oils/Glycerides/Surfactants:** Mono-, di- and triglycerides, fatty acids; castor oil, polyoxyl 40 hydrogenated castor oil, polyoxyl 35 castor oil, polyoxyl 15 hydroxystearate, polysorbate 80, vitamin E-TPGS, Poloxamer 407, Poloxamer 188, Transcutol, among others

**Polymers:** PLGA, PCL, PVA, HPMC, HPC, povidone, copovidone, Soluplus, carboxymethyl sodium, chitosan, hyaluronic acid, alginate, gellan gum, among others

### Lipid Nanoparticles as Vehicles for Ocular Delivery

Saturated lipid nanoparticles, nanostructured lipid carriers, liposomes, cubosomes, micro- and nanoemulsions; nanosuspensions, dendrimers, micelles, among others are a range of carriers for ocular or ophthalmic delivery. These assemblies ranging in size between 10 nm and 1000 nm are widely utilized for delivery of a range of therapeutics to overcome the physiological barriers and to target specific ocular tissues. As stated earlier, these are derived from a range of pharmaceutically accepted and listed FDA inactive ingredients, including excipients, polymers, and co-solvents and surfactants. Thus, for designing appropriate safe and efficacious formulations by selecting one or more ingredients, the list of approved ingredients further facilitates the regulatory process. For example, hyaluronic acid, alginate, and/or cellulosic approved excipients are commonly preferred for enhancing the residence time in the cornea and conjunctiva.[10] Tatke et al investigated triamcinolone nanoparticles composed of glyceryl monostearate and Compritol 888 to

**TABLE 1**

| Drug | Nanocarrier | Indication | Formulation | Reference |
|---|---|---|---|---|
| Cyclosporine A | Micelles | Dry eye | Vitamin E, Vitamin E-TPGS, polyoxyl 40 hydrogenated castor oil | 17 |
| | Nanoemulsions | | mPEG-PLA | 18 |
| | Nanoparticles | | Chitosan, Transcutol P, oleic acid, Tween 20 | 19 |
| Ciprofloxacin | Nanoparticles | Antibacterial | Compritol 888, Tween 80, chitosan, poly (2- ethyl-2-oxazoline) | 20 |
| | Cubosomes | Conjunctivitis | Phytantriol, Poloxamer 407, chitosan, b-glycerophosphate 127 | 21 |
| Azithromycin | Lipid Nanoparticles | Antibiotics | PLGA, Poloxamer 407, PVA | 22 |
| | Liposomes | Dry eye | Cholesteryl hemisuccinate, MCT, lecithin, DSPE-PEG 2000, Vitamin E | 14 |
| Tacrolimus | Ionotropic gellation | Corneal allograft rejection | Poloxamer 188, soy lecithin, cholesterol, hyaluronic acid | 23 |
| Tacrolimus Cerium Oxide | Nanosuspensions | Dry eye | Gellan gum, aluminum chloride, glycol chitosan | 24 |
| Travoprost | Lipid Nanoparticles | Glaucoma | Twin 80, isopropyl alcohol | 25 |
| Acetazolamide | Lipid Nanoparticles | Glaucoma | Phosphatidylcholine, cholesterol, vitamin E, trehalose, erythritol, borate, HPMC | 26 |
| Vancomycin | Nanoemulsions | Ocular bacterial Infection | Glyceryl tripalmitate, Eudragit RS100, polyoxyl 15 hydroxystearate, oleic acid, sod. alginate | 27 |
| Dexamethasone | Microemulsions | Dry eye | Cholesterol, Labrafac, Tween 80, phenyl boronic acid, 3-amino-phenyl boronic acid, chondroitin sulfate, Preciol ATO 5, Compritol 888 ATO, Miglyol 812 N, Kolliphor HS15 and EL, CTAB | 28 |
| | Nanoparticles | Dry eye | Compritol 888 ATO, Miglyol 812 N, Kolliphor HS15, CTAB, Kolliphor EL | 29 |
| Amphotericin B | Nanostructured lipid carriers | Fungal keratitis | Compritol 888 ATO, lecithin, soybean oil, Poloxamer 188, chitosan | 30 |

**Recent Developments in Nanotechnologies for Development of Drugs in Ocular Delivery[14, 17-30]**

enhance the trans-corneal permeation of drug.[11] Kalam, et al evaluated the solubility and ocular bioavailability of tedizolid phosphate nanocrystals using stearyl amine and benzalkonium chloride and found the drug was stable over 180 days at 25°C and 37°C and showed extended release over 12 hours.[12] Liu, et al evaluated hydrophilic moxifloxacin encapsulated lipid-polymer nanoparticles containing egg phospholipid, DSPE, cholesterol, and chitosan, which was surface modified with hyaluronic acid for targeting. It was observed it improved permeation and bioavailability.[13] Ren, et al used cholesterol hemisuccinate with medium chain triglycerides to improve the solubility, loading, and stability of azithromycin in the liposomes.[14] In a study demonstrating the use of SLN, NLC, and eye drop formulations of brimodinine, El Salamouni, et al observed NLC enhanced 1.3-fold higher permeability compared to SLN due to stronger affinity with cell membrane, leading to sustained release and greater impact on lowering the intraocular pressure compared to SLP and eye drops.[15] Morsi, et al investigated acetazolamide nanoemulsions composed of polysorbate 80, polyxyl 35 castor oil, Transcutol, and peanut oil in combination with xanthan gum, HPMC, or Carbopol gel and found stability and efficacy of drug were improved compared to eye drops and tablets.[16] Table 1 cites a few examples of NPs in ocular delivery.

## CHARACTERIZATION OF NANOPARTICLES

Nanoparticles for ocular delivery, like any other applications, require a thorough characterization due in part to particle size uniformity, polydispersity, and homogeneity of the formulations. The visual appearance for identifying the insoluble particulates, transparency vs. translucency, or milky white are important critical quality attributes and must be optimized.[31]

Monitoring the morphological changes, stability, zeta potential, surface tension, refractive index, and rheological properties are equally important for maintaining the product quality. For instance, a zeta potential around ±20 mV is appropriate for electrostatic attachment with the corneal surface, and higher values between ±20-40 mV is also desired for preventing aggregation and maintaining the stability of these particulates. Citing a cubosomal formulation with zeta potential of -30.2 mV, for instance, it improved the bioavailability and activity as compared to Alphagan® eye drops.[32]

Dynamic light scattering (DLS) is typically used to characterize the particle size and polydispersity index (PDI) using a Malvern or Coulter counter analyzer. While PDI closer to 0 indicates a unimodal distribution, the values closer to 1 indicate a heterogeneous system. For ocular delivery, smaller PS and PDI particles are preferred to enhance the corneal permeation and bioavailability. Ocular formulations with low viscosity ranging between 2 mPas and 3 mPas allows better patient compliance;

**TABLE 2**

| Brand Rx Name | Formulation | Chemical Name | Therapeutic Indication | Year Approved |
|---|---|---|---|---|
| Visudyne® | Liposome | Verteporfin | Macular degeneration | 2000 |
| Restasis® | Nanoemulsion | Cyclosporine A | Dry eye | 2002 |
| Retisert® | Nanoemulsion | Fluclrolone | Uveitis and macular edema | 2005 |
| Durezol® | Nanoemulsion | Difluprednate | Postoperative ocular inflammation | 2008 |
| Besivance® | Nanosuspension | Besifloxacin | Ocular bacterial infection | 2009 |
| Tobradex ST® | Nanosuspension | Tobramycin Dexamethasone | Ocular inflammation and bacterial infection | 2009 |
| Prolensa® | Solution | Bromfenac | Postoperative inflammation and pain | 2013 |
| Cequa® | Nanomicelles | Cyclosporine A | Dry eye | 2018 |
| Xelpros® | Microemulsion | Latanoprost | Glaucoma or ocular hypertension. | 2018 |
| Inveltys® | Nanosuspension | Loteprednol etabonate | Postoperative ocular inflammation and pain | 2018 |
| Eysuvis® | Nanosuspension | Loteprednol etabonate | Dry eye | 2020 |
| Verkazia® | Nanoemulsion | Cyclosporine A | Vernal keratoconjunctivitis | 2021 |

**Ocular Nanoparticle Drugs Approved by the FDA**

however, the higher viscosity allows for sustained release, less frequent dosing, and enhanced bioavailability.[31]

Surface tension of ocular formulations is critical for patient compliance and retention of drug to the corneal surface. An ocular dose is typically 5-15 mL with few exceptions for commercial eye drops. Ocular formulations with surface tension of < 35 mN/m can lead to discomfort in the eye, but with surface tensions of 40-50 mN/m, they can provide more relief and comfort to the eye.[33] Likewise, pH and osmolality ranging between 4 and 8 and 231 and 446 mOsm/kg, respectively, can lead to improved stability and significant permeation of drug through the eye.[7]

## FDA APPROVED DRUGS

As nanoparticle technologies continue to address the challenges with poorly soluble molecules across all the dosages with the aim to increase solubility and enhance bioavailability, the industry is weighing options for identifying and adapting new excipient technologies for bringing innovative drugs faster to market. That has driven the sense of urgency for finding technologies for unmet medial needs in ocular formulations. Consequently, several drugs have been approved employing nanoparticle technologies for the treatment of diseases affecting the anterior segment of the eye. Table 2 lists a number of approved ocular drugs to market over the years.

## SUMMARY & FUTURE PERSPECTIVES

Ocular drug delivery is challenging due to many anatomical barriers. Nanoparticle technologies in ocular delivery, however, have demonstrated wide utilities across all molecules, small and large. As shown in Table 2, several of the approved drugs and many more molecules are undergoing clinical development. Citing a few examples, NCT03001466 is undergoing Phase 2 and is composed of urea loaded in Poloxamer 407 nanoparticles for dry eye syndrome, while NCT02420834 is composed of a liposomal spray used for artificial tears for dry eye. Clinical trial (NCT02845674) involving Cyclosporine A is undergoing Phase 3 as a 0.09% micellar solution for dry eye syndrome.[34] With the acceptance of nanotechnologies in the recent past, we believe nanoparticle technologies will pick up the steam in ocular delivery for the launch of new molecules to market with more innovative polymeric and lipid assemblies like cubosomes.[35,36]

Ascendia Pharma with its enabling platform technologies, NanoSol® (nanosuspension), EmulSol® (nanoemulsion) and LipidSol® (lipid nanoparticles) are primed to address the challenges with poorly soluble drugs. For example, using EmulSol® technology, Ascendia can address the particle size with its top down/or bottom-up approach with FDA-approved excipients in nanoparticle formulations composed of 0.05% Cyclosporine A with a particle size <50 nm and compossed of soy bean oil, lecithin, and polysorbate 80.[37]

With its cGMP sterile manufacturing capabilities, Ascendia is poised to tackle the challenges of formulation development and manufacturing of poorly soluble molecules to address the unmet medical needs to design better and smarter nanoparticle ocular formulations in the future. ◆

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# DRUG DELIVERY

# EUDRACAP® Select - Examining a Case From Development to Clinical Trial

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## INTRODUCTION

Formulating live biotherapeutics and accelerating drug development time can be challenging. The following study focuses on the development of a customized functional coated capsule, EUDRACAP® Select, for the delivery of live biotherapeutics and demonstrates its effectiveness in the oral delivery of a sensitive proprietary microbiome ecosystem while simplifying the drug development process.

Growing scientific evidence strongly suggests the microbiome plays a crucial role in various diseases, impacting everything from immune function and inflammation to metabolism and mental health. Understanding these implications could lead to groundbreaking advancements in disease prevention, diagnosis, and treatment. Certain diseases or medical treatments can disrupt the microbiota, leading to an imbalance known as dysbiosis. Restoring the complete gut microbiota ecosystem is a promising therapeutic tool to improve clinical outcomes in patients.

Cancer and its treatments can disrupt the gut microbiota, impair gut epithelial repair mechanisms, and compromise immune homeostasis and responsiveness. Microbiome therapy can prevent the decay of the gut ecosystem, preserve immune homeostasis, and optimize gut function. To achieve the desired clinical outcome, a robust and targeted delivery system is crucial.

Microorganisms are normally sensitive to acidic conditions and therefore require an acid-resistant delivery system. At the same time, they should not be exposed to the high moisture and temperatures of standard enteric coating processes, which could lead to a reduction in the number of viable microorganisms. The use of a customized, empty, ready-to-fill, modified-release coated capsule is a viable alternative for this type of therapy.

The study discussed here focuses on an oral formulation developed in a collaboration between Evonik and MaaT Pharma, with Evonik providing functional ready-to-fill EUDRACAP Select capsules and MaaT Pharma providing stable, pooled, full ecosystem microbiota.

## OBJECTIVE & SOLUTION: DEVELOPING A SUITABLE ORAL DELIVERY SYSTEM

The objective of this study was to develop a suitable oral delivery system for the full ecosystem fecal microbiota and test its safety and tolerability in acute myeloid leukemia (AML) patients exposed to intensive rounds of chemotherapy and antibiotics in a Phase 1b clinical trial sponsored by MaaT Pharma (identifier NCT04150393). EUDRACAP Select functional capsules were developed to address the above challenges.

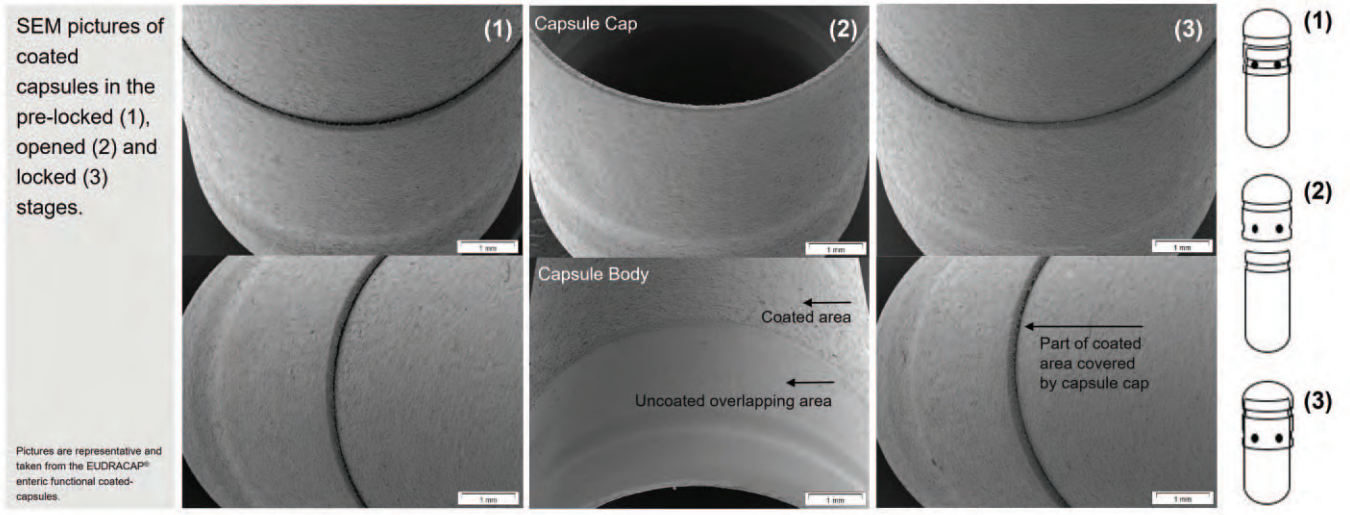
## DEVELOPMENT OF CUSTOMIZED EUDRACAP SELECT CAPSULES

Pre-locked, hard HPMC capsules were used for this study. Empty capsules were coated with a proprietary combination of EUDRAGIT® polymers to a specific weight gain based on their surface area. These ready-to-fill, enteric capsules were compatible with standard filling equipment and required no banding or additional downstream processing.

Coating performance was evaluated via a three-stage dissolution test. Caffeine:lactose blends were filled into enteric-coated size 0 capsules. Dissolution tests were performed in USP Type II apparatus at 37°C, with a basket speed of 75 rpm; filled capsules were exposed to 0.1 N HCl media for 2 hours followed by pH 6.8 potassium phosphate monobasic buffer for 1 hour and pH 7.2 buffer for 2 hours. The developed capsules were also subjected to a biorelevant dissolution test described further.

For the Phase 1b clinical study, the capsules developed were filled with a specific amount of MaaT's proprietary standardized, high-richness, high-diverse microbiome ecosystem, containing a group of bacterial species, the Butycore®, known to produce anti-

## FIGURE 1




*Microscope: JEOL JSM IT300, Acc: 10 kV, El.Mag: 20 x, Detector: SED*

**SEM pictures of coated capsules in the pre-locked (1), opened (2), and locked (3) stages.**

inflammatory short-chain-fatty acids. The study was performed according to the protocol described further (identifier of the study: NCT04150393).

For illustrative purposes, Figure 1 shows capsules coated in the pre-locked (1), opened (2), and locked (3) stages; these capsules were produced by a process similar to that described here. Part of the capsule body is not coated as shown in Figure 1. This part of the body is fully covered when the capsule is in the final locked stage, as observed in Figure 1. In the final locked stage, the cap also covers part of the coated area of the body, this helps to form a hermetic seal between the cap and the body of the capsule.

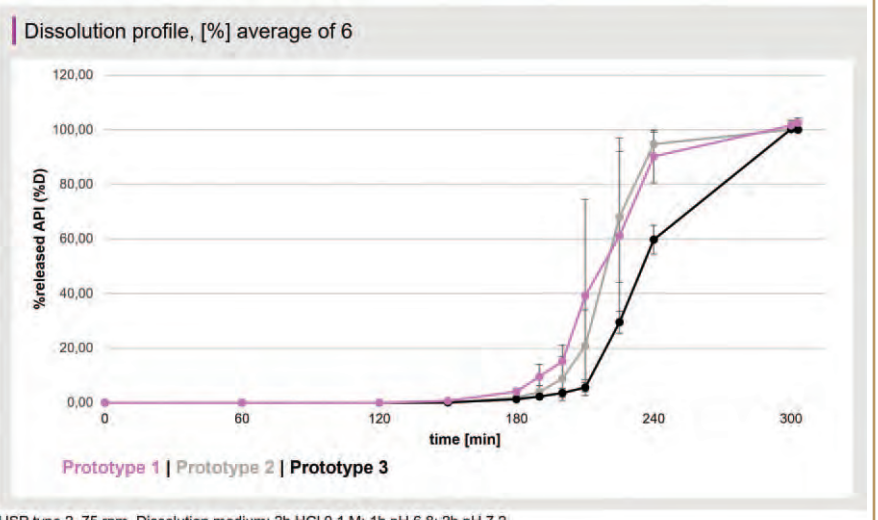
## PROTOTYPES MATCHING THE TARGETED PROFILE

During the development phase, different formulations and weight gains were tested to target the lower end of the gut. The targeted release is expected to occur at the ileocecal junction. For this purpose, three prototypes were tested to find the optimal amount of coating and combination of polymers. Figure 2 shows Prototype 1 is not robust enough and has a considerable level of standard deviation. On the other hand, Prototype 3, despite being more robust, delays the release tested *in vitro*. This behavior could indicate the amount of polymer applied to the capsule is higher than necessary and could possibly delay the release *in vivo*. Therefore, Prototypes 1 and 3 were not considered for further steps. Prototype 2 showed full protection in HCl 0.1 M up to 120 min and also for a further 60 minutes in pH 6.8, after which its contents were rapidly released in pH 7.2. Therefore, Prototype 2 was selected as the most promising formulation and was then submitted for a stability study.

## EMPTY CAPSULES TESTED FOR STABILITY

To test the stability of the capsules, a stability program was established with four different conditions: (a) long-term, (b) intermediate, (c) accelerated, and (d) long-term in refrigerated conditions. The latter was included in the stability study program

## FIGURE 2




USP type 2, 75 rpm, Dissolution medium: 2h HCl 0.1 M; 1h pH 6.8; 2h pH 7.2

**Prototypes matching the targeted profile.**

to ensure lower temperatures would not cause the capsule to become brittle at some point in time, which could be a risk factor for the final product formulation. The capsules included in the stability study were sampled from a representative scaled-up technical batch. The capsules were stable under all the different conditions tested, including accelerated and refrigerated. Considering the higher importance for this project, in which the final pooled full ecosystem microbiota needs to be stored under refrigerated conditions, only the results of this condition are shown in Figure 3. The 6-month stability study carried out so far has yielded positive results for all conditions tested, ensuring a robust functionally coated capsule has been achieved. The long-term stability study is still ongoing.

## *IN VITRO* PREDICTION OF CAPSULE BEHAVIOR & ROBUSTNESS

The cost of clinical trials in drug development has been increasing in recent years and has a significant influence on the overall development costs. Therefore, it is advisable to stress the developed drug as much as possible in *in vitro* tests to reduce the risk of failure in later clinical studies. Dissolution of modified-release products can be significantly influenced by physical stress of biorelevant magnitude in the human GI tract, such as high pressure, low buffering capacity, and jet-like propulsions, and this is not easy to predict by *in vitro* studies.[1]

To simulate the physicochemical conditions of the GI tract, the EUDRACAP Select capsules were subjected to a dissolution stress test performed by a specific device capable of simulating the levels of physiological mechanical stresses that occur during the passage of a solid dosage form through the GI tract. The dosage form is subjected to sequences of agitation, including movement and pressure fluctuations, alternating with static phases, as observed *in vivo*. The device also allows simulation of intermittent contact of the dosage form with the dissolution medium. In addition, the intestinal pH profiles, characteristic of fasting intake conditions, were simulated with a biorelevant medium.

Figure 4 shows a schematic of the test set-up. Movements and pressures are applied according to a defined protocol as detailed in Figure 4 (B), which also shows there is no release of capsule contents for up to 2 hours. During simulated gastric emptying (1), the capsules yielded no deformation and signs of leakage. Low- intensity mechanical stress simulated at 1 hour (2) and 2 hours (3) did not affect drug release. The mechanical agitation simulated at 3 hours (4) triggered fast dissolution of part of the tested capsules. Ileocecal passage at 4 hours (5) triggered fast drug release (deformation and perforation) from the capsules.

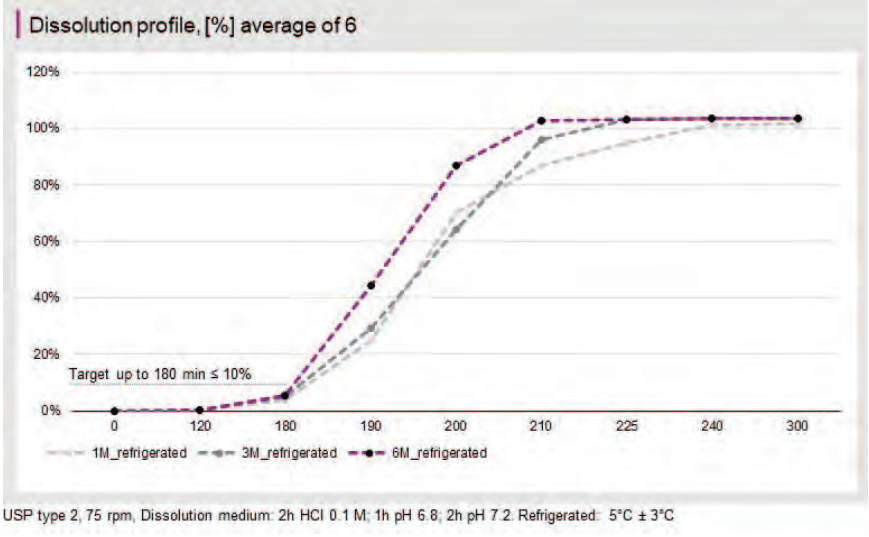
The results indicate Prototype 2 capsules are capable of delivering live biotherapeutics to the distal small intestine and proximal colon, making them suitable for first-in-human trials.

## ENCAPSULATED FULL-ECOSYSTEM MICROBIOTA WAS TESTED IN PHASE 1B CLINICAL TRIAL

AML treatment combines intensive chemotherapy (IC) with broad-spectrum antibiotics (ATB) that induces a strong gut microbiota dysbiosis, promoting pathological conditions and increasing incidence of complications. Growing evidence suggests loss of diversity in the gut microbiota due to conditioning regimen, chemotherapy, antibiotics, and reduced dietary intake promotes the development of Graft-versus-Host disease (GvHD) and impact negatively overall survival of patients receiving allogenic hematopoietic stem cell transplantation.[2]

The developed EUDRACAP Select capsule containing lyophilized pooled full ecosystem fecal microbiota drug candidate (MaaT033) was tested for tolerability,

**FIGURE 3**




**Stability tests for empty capsules.**

safety, and efficacy in a Phase 1b clinical trial. The study was an open-label, single-arm with 21 patients divided into 5 different cohorts. This study, sponsored by MaaT Pharma, took place at six investigational sites in France. The dose was scaled and administered according to Table 1. The objectives of the study were to test tolerability, dose regimen (safety and activity, engraftment), and patient compliance, and to select the dose for Phase 2 study.

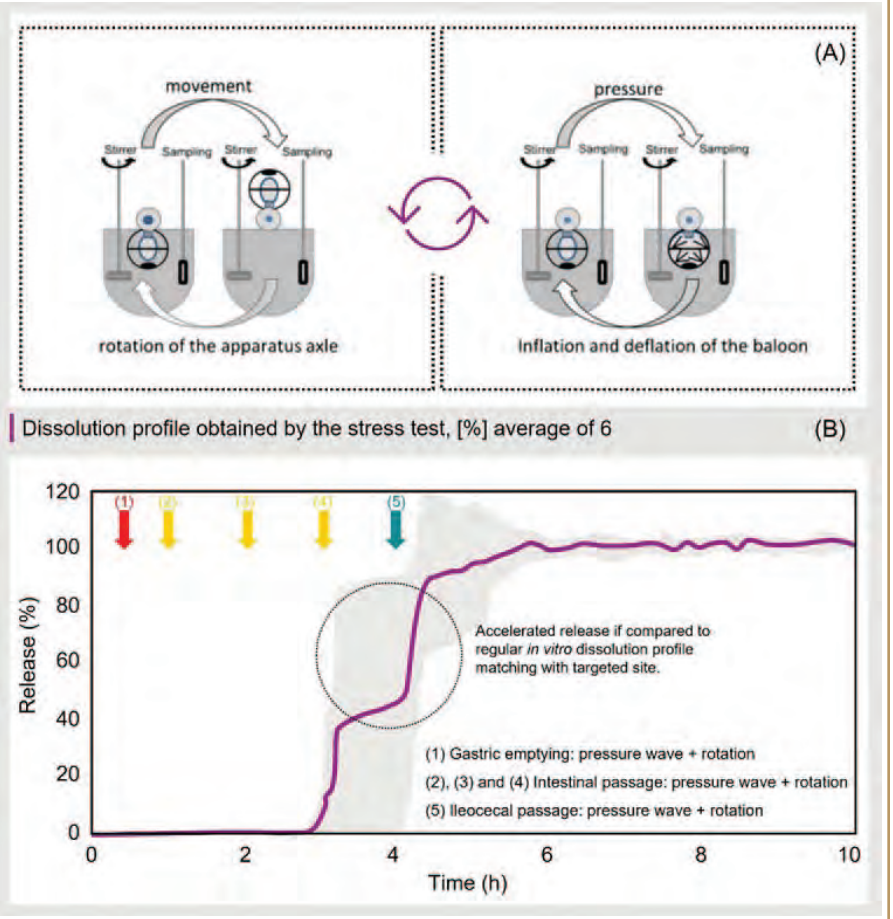
Cohort 5 was not performed because sufficient data were obtained from cohorts 1 to 4. Engraftment and richness in operational taxonomic units were examined on day 0 (baseline) of the treatment, and then on days 7 (±2) (V2), 19 (±5) (V3), and 44 (±10) (V4).

## STRONG ENGRAFTMENT & INCREASED MICROBIOTA RICHNESS OBSERVED

The results of the Phase 1b clinical trial were first reported at the 64th edition of the American Society of Hematology.[3] Figure 5 (A) shows strong and sustained engraftment was observed in all four cohorts, even stronger for cohort 3 and cohort 4, in which three capsules per day were administered. The engraftment level refers to the ratio of operational taxonomic units (OTU) that were not present in the patient at baseline, but were present in MaaT033 and were found in the patient following treatment, ie, the treatment-induced related engraftment. For this analysis, shared OTUs between MaaT033 and patients at baseline were excluded (values starting from zero). Persistent engraftment can be observed by relatively stable OTU levels at V4, following consolidation of chemotherapy and about 4 weeks following the treatment with MaaT033 was finished. It was also observed MaaT033 bacterial engraftment is inversely correlated with patients' baseline microbiota richness (data not shown).

The richness of the microbiota was also evaluated in terms of variety of engrafted OTUs. Similar to the engraftment results, an increase in the number of OTUs was induced by MaaT033, which was also persistent, especially for cohort 2, cohort 3, and cohort 4, as shown in Figure 5 (B).

**FIGURE 4**



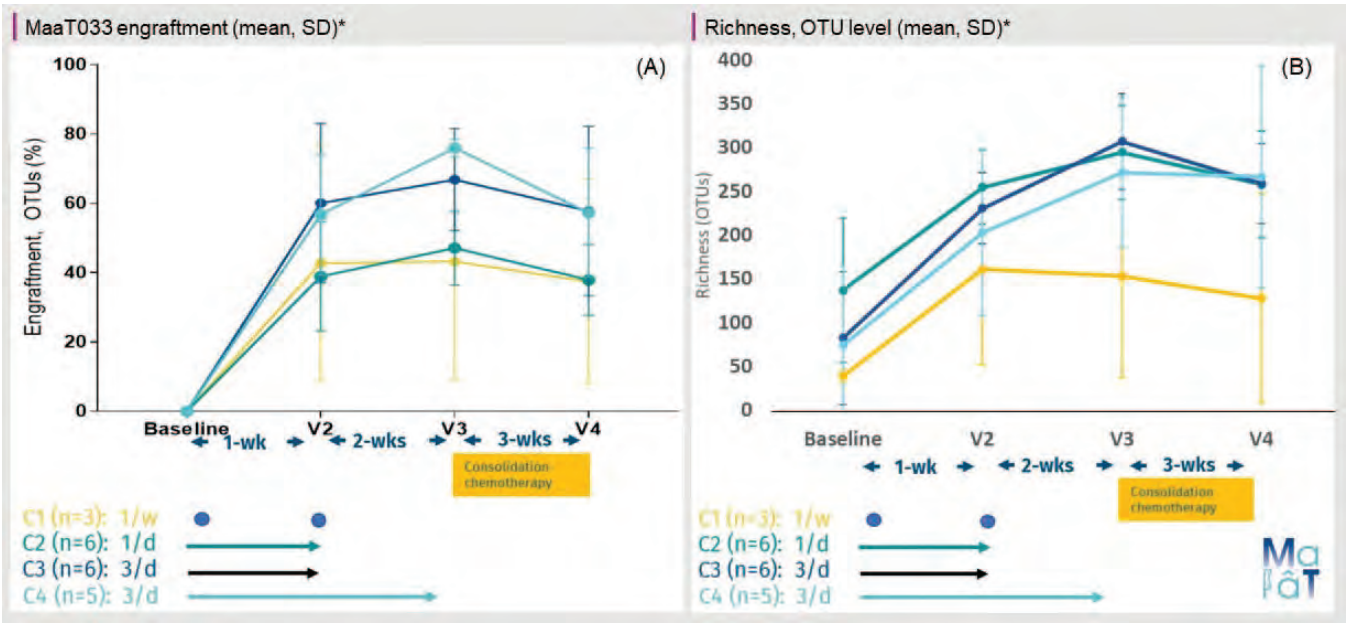

**Stress test apparatus to simulate high pressures and velocity of the GIT (A); and dissolution profile obtained by the stress test (B).**

**TABLE 1**

| Cohort | Description |
|---|---|
| 1 | 1 capsule per week for 2 weeks |
| 2 | 1 capsule per day for 1 week |
| 3 | 3 capsules per day for 1 week |
| 4 | 3 capsules per day for 2 weeks |
| 5 | Not performed, sufficient data from cohort 1-4 |

**Cohort & Dose Regimen**

## FIGURE 5




**Engraftment at different timepoints of the study (A); and richness, OTU level (B) - data MaaT Pharma - Phase 1b trial.**

During the study, four serious adverse events were reported in four patients, but only one was considered as possibly related by the investigator. This event was an infectious diarrhea with enteropathogenic *E. coli* that started 3 days following MaaT033 treatment initiation. Genome sequencing was performed, and it was concluded the *E. coli* that caused the reported event was not found in the MaaT033 or in the patient before the treatment started, so although the association between the event and MaaT033 is highly unlikely, it cannot be formally excluded. Other events were not reported as serious or potentially related to MaaT033.

## CONCLUSION

*In vitro* testing has demonstrated the efficacy of the developed EUDRACAP Select used in the MaaT033 formulation, even under stressful conditions. MaaT033 formulated with EUDRACAP Select appears to be safe and effective in restoring gut microbiota in AML patients receiving IC and ATB. Three MaaT033 capsules per day for 1 week induce an increase in microbiota richness and an effective and persistent engraftment in AML patients. A Phase 2b trial is underway to evaluate MaaT033 as an adjunctive in patients with hematological malignancies receiving allogeneic hematopoietic stem cell transplantation.

Evonik has provided a capsule that is effective and robust enough to deliver even sensitive molecules. The capsule is ideal for powders, pellets, granules, and other dosage forms and is compatible with high-speed capsule filling machines. Using EUDRACAP capsules saves developers time in process scale-up and validation, and has the benefit of using EUDRAGIT polymers, which have been around for 70 years. ◆

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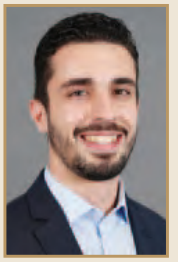
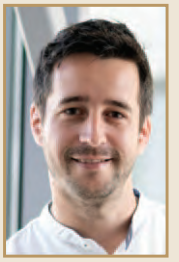


**Lucas Cusin** is a pharmacist with more than 10 years of experience in the field of oral excipients. He currently works at Evonik as a Strategic Project Manager, with a focus on oral drug delivery.



**Dr. Steven Smith** earned his PhD in Chemistry for his research on covalent and covalent-reversible kinase inhibitors and currently works at Evonik as Head of Customer Projects focusing on formulation development for oral dosage forms.

**Dr. Kamlesh Oza** earned his PhD from The Ohio State University and at Evonik, heads Technical & Formulations and Application Services for North America.

Drug Development
EXECUTIVE

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Thermo Fisher Scientific

ThermoFisher SCIENTIFIC

# Thermo Fisher Scientific: What to Expect From the Next Wave of RNA-Based Therapeutics

The emergence of mRNA vaccines during the COVID-19 pandemic propelled this innovative technology to the forefront of medical advancement. As we usher in the next era of RNA-based therapeutics, RNA's potential extends far beyond COVID-19, promising groundbreaking treatments for cancer, metabolic diseases, and a range of other infectious diseases. To meet growing demand and to bring new RNA treatments successfully to market at scale, developers and manufacturers must embrace best practices to overcome potential obstacles.

*Drug Development & Delivery* recently had a discussion with Dr. Dale Patterson, Vice President and General Manager, Molecular Biology at Thermo Fisher Scientific, to delve into the world of RNA therapies and understand their next act beyond COVID-19.

**Q: While mRNA garnered significant public attention during the COVID-19 pandemic, researchers have been exploring the clinical use of RNA for years. What does the current landscape for RNA-based vaccines and therapeutics look like?**

**A:** mRNA (messenger ribonucleic acid) became a household name during the COVID-19 pandemic, and for good reason. Innovative mRNA-based vaccines developed with unprecedented speed played a key role in curbing the spread of the virus and protecting individuals from severe infection. However, these vaccines are only the tip of the iceberg when it comes to the many clinical possibilities for mRNA and other categories of RNA therapeutics, including RNA interference (RNAi), RNA aptamers, and other therapies, such as antisense oligonucleotides (ASOs), which

have the highest number of currently approved treatments. RNA-based therapeutics are unique in they address disease at the source, opening the door for improved, safer, and more effective treatment options for diseases ranging from cancer to chronic conditions like diabetes.

Drug developers are rapidly taking notice of the many clinical possibilities for RNA-based therapies and vaccines. According to GlobalData, the market for mRNA-based oncology therapies is expected to reach $2 billion by 2029. Today, there are 90+ mRNA-based drug candidates in clinical trials with at least a dozen drugs in development using other types of RNA. What's exciting is how quickly we are seeing mRNA and other RNA-based therapeutics make it into the clinical phase compared to the timeline we've seen for other biotherapeutics.

**Q: Can you explain how mRNA vaccine development differs from the approach taken with traditional vaccines?**

**A:** Given the eradication of diseases like smallpox and polio, the impact of traditional vaccines on human health cannot be minimized. Still, mRNA-based vaccines hold significant promise and have been shown to have greater specificity and efficacy than traditional vaccines. The world saw one of the most obvious differences between mRNA and traditional vaccines firsthand during the COVID-19 pandemic. As they do not contain a weakened or inactivated virus and can be developed with a platform process that does not require the use of cells, mRNA-based vaccines can be developed and manufactured much more quickly. This makes them an ideal defense against new infectious diseases or variants. In addition to speed, the process to develop mRNA vaccines is overall much simpler and more cost-effective with fewer steps required. Furthermore, the footprint required to scale production and make a significant amount of mRNA vaccines is far less than what would be required with traditional vaccines.

**Q: What should developers keep in mind during the early stages of mRNA vaccine or therapeutic development?**

**A:** Regardless of how new they are to the mRNA space, there are some best practices developers should keep in mind as they set out to develop new mRNA vaccines or therapeutics. First, developers should be mindful about choosing high-quality raw materials optimized for the needs of their project. This is one of the earliest steps in the mRNA development process, and choosing right can pay dividends down the line. Selecting materials solely based on cost without being mindful of future quality and regulatory requirements could impact your long-term success and limit the scalability of your product later. The raw materials you select should meet your project's needs for quality and documentation, regulatory support, and scalability as you move closer to commercialization.

**Q: How can developers work to ensure the scalability of their mRNA-based vaccines and therapeutics?**

**A:** As previously discussed, choosing the right raw materials at the start is a key factor in ensuring the scalability of your mRNA products. Additionally, developers should do their due diligence when selecting suppliers. When it comes to scalability, it's important to select an experienced supplier that can scale with your project. Remember to ask key questions, such as whether they have an established track record of partnering with developers who have commercialized new therapies or if they have a contingency plan in case of shortages.

For this next wave of mRNA-based vaccines and therapeutics, it's important to not just be able to scale up but to be able to scale down. For example, personalized cancer vaccines using mRNA are in development now for incredibly small patient populations. That means manufacturing these vaccines won't need to be scaled nearly to the extent that the COVID-19 vaccines were. In fact, being able to scale down manufacturing and potentially make these vaccines on-demand will be most cost-effective. At Thermo Fisher, one of our offerings is technology that essentially allows these therapeutics to be manufactured at the same quality as they would be in a large bioreactor but at a smaller scale. Overall, when it comes to scalability, developers should think about what will be needed to manufacture these new drugs for smaller populations to ensure they are accessible.

**Q: Now that the spotlight is on mRNA, what are your predictions for the future of this field?**

**A:** With so many mRNA therapeutics currently in development, the next 3 to 5 years will likely see an explosion in the number of mRNA drugs approved, including therapeutics and vaccines for some of the most difficult-to-treat or intractable diseases and conditions. mRNA has capabilities that other types of therapeutics lack and can target and attack disease at the

cellular level, unlocking new potential strategies for treating diseases like cancer or rare, genetic conditions. Additionally, as mRNA therapeutics and vaccines are approved throughout the next few years, this may help speed up the approval process for other drugs in the pipeline by offering new pathways and precedents for regulators, such as the US FDA. We are at a turning point for the use of mRNA as a new, flexible way to treat so many different diseases. It will be exciting to see other developers join the mRNA wave and explore new and broader applications.

**Q: Thermo Fisher and Moderna announced a 15-year mRNA manufacturing deal in February 2022. What can you tell us about Thermo Fisher's approach to collaborating with companies in this space?**

**A:** As we saw during the COVID-19 pandemic, the speed at which mRNA vaccines were brought to the public was due in part to strong collaborations between developers and manufacturers. BioNTech, a leading player in the mRNA market, partnered with Pfizer – a large pharmaceutical company with plenty of experience bringing vaccines to market at scale. Moderna, on the other hand, worked with various CDMOs, including Thermo Fisher, to scale its manufacturing process to ensure its vaccine could reach the global community.

At Thermo Fisher, we have a unique approach to collaboration and offer developers pathways to build or buy. We have a complete end-to-end product portfolio, and developers can choose to collaborate with us at just one stage of the development or manufacturing process or from beginning to end. Additionally, we offer services to support developers from early stage emerging startups to large pharmaceutical companies. When it comes to this new era for RNA-based therapeutics, we are committed to working closely with developers and sharing knowledge to bring these innovative treatments to patients more quickly. ◆


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# PBPK MODELLING

## Critical Parameters for Simulating Oral Absorption Using PBPK Models

By: Deanna Mudie, PhD

### INTRODUCTION

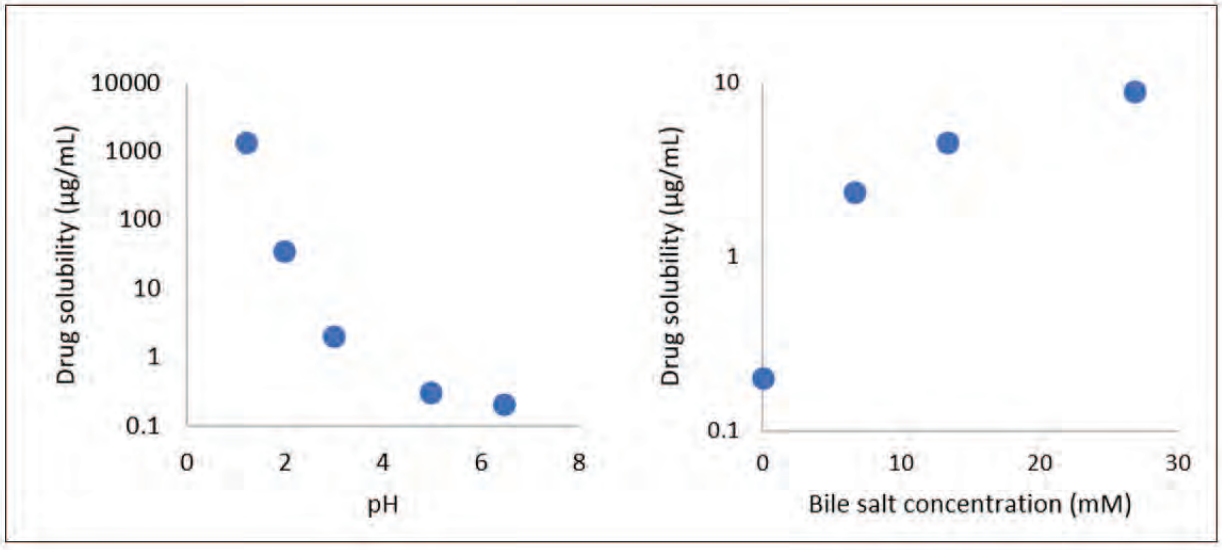
Physiologically based pharmacokinetic (PBPK) modeling, along with its subset, physiologically based pharmaceutics modeling (PBBM), is a powerful tool for predicting drug behavior in vivo, including exposure and interactions with other drugs. Computer models are combined with in vitro assays to generate predictions to inform drug development activities, speeding development, and avoiding potential problems before they arise.

Identifying formulations to mitigate absorption risks is one application. Many drug candidates have characteristics, such as poor solubility or permeability, that jeopardize achieving target oral exposure. Using PBPK software to identify absorption risks requires knowledge of key drug formulation properties, the physiological properties of the gastrointestinal (GI) tract, and how the two interact. What are the properties, how can they be calculated or measured *in vitro*, and then used in PBPK simulations of *in vivo* performance?

### SOLUBILITY

Dissolved drug concentration is a fundamental driver of drug bioperformance.[1] It is determined by the solubility or by the activity of a metastable drug form. It drives the rate and extent of dissolution and precipitation in GI fluids, and the permeation rate across the GI membrane. Dissolved drug concentration is influenced by the interplay among the properties of the drug, excipients, and GI fluid. Key physiological parameters impacting dissolved drug concentration include GI fluid pH and composi-

**FIGURE 1**




***In vitro* solubility of weakly basic drug posaconazole. Left: pH-solubility profile (0 mM bile salts). Right: solubility with and without bile salts (pH 6.5).**

tion, while key drug and excipient properties include solid form type, pH, lipophilicity, and melting temperature.[2]

Representing solubility in PBPK software is not as simple as entering a single number. Regional differences in GI fluid properties along the length of the GI tract make the situation dynamic, as does the potential for solid form conversions when a metastable solid form is present. Regional differences in solubility can be explained by differences in fluid pH between GI tract compartments, and in concentrations of mixed lipidic aggregates, such as bile salt micelles.

Fluid pH can alter the degree of drug ionization of weakly acidic and basic drugs. For example, a weakly basic drug is typically highly ionized at the acidic pH of the fasted human stomach, but mostly non-ionized at the moderate pH of the small intestine, giving higher solubility in the stomach than the intestine. This pH dependence makes it important to measure the pH-solubility profile of a drug as an input to allow simulation of the degree of ionization and therefore solubility in different GI tract compartments.

Drug solubilization by bile salt micelles present in some GI fluids makes it important to estimate solubility in the presence of bile through in vitro measurements of solubility in GI fluid surrogates, such as Fasted Simulated Intestinal Fluid (FaSSIF), which contains the solubilizing agents sodium taurocholate and lecithin. Measuring drug solubility as a function of FaSSIF concentration allows determination of a bile salt micelle partition coefficient, allowing PBPK software to calculate drug solubilization levels in each GI compartment (Figure 1).

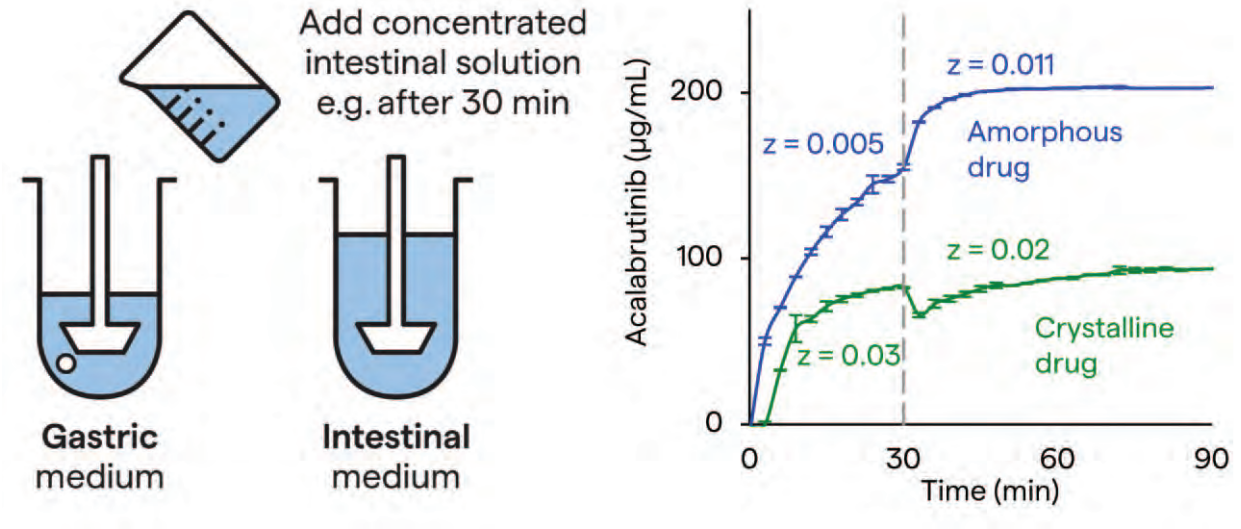
As metastable solid forms can convert, it is important to include the solubilities of each relevant form in PBPK software. For example, in modeling an amorphous solid dispersion, the pH-solubility profile and solubilization ratio of both the amorphous form, as well as of any crystalline forms to which the drug is likely to precipitate in vivo, are required inputs to account for the lower activity and dissolution rate of the latter forms.

## DISSOLUTION

Solid oral dosage forms must dissolve in GI fluids before they can absorb across the GI membrane and enter the bloodstream. The rate and extent of dissolution is thus an important input.[1] Like solubility, the interplay between drug substance, excipient, and physiological properties influences *in vivo* drug dissolution.[3] Solubility is a key driving force for dissolution, together with other key factors such as the solid surface area, concentration of drug in solution, and fluid hydrodynamics. To improve reliability when mathematically inputting dissolution into PBPK software, one must consider these important variables.

*In vitro* dissolution testing is the gold standard for forecasting *in vivo* drug product bioperformance. It can be used to generate inputs to PBPK software, such as the rate and extent of dissolution and precipitation as a function of properties, such as pH and bile salt concentrations. It is helpful to tailor the methodology to the

**FIGURE 2**




***In vitro* dissolution of weakly basic drug acalabrutinib. Left: USP 2 transfer test schematic. Right: *In vitro* drug concentration vs. time with calculated z-factor values.**

target population/physiology and drug product problem statement by using a variety of customized, fit-for-purpose dissolution apparatuses.[4]

Some examples of simple *in vitro* dissolution apparatuses include the small-scale Pion μDiss Profiler™ for dry powders and suspensions, and the larger-scale United States Pharmacopeia (USP) 1 and 2 apparatuses for dosage forms, including tablets and capsules. These tests can be run using a single dissolution medium targeting dissolution in a particular region of the GI tract. Alternatively, they can be run as transfer-tests, in which the drug formulation is added to a simulated gastric medium, and a concentrated simulated intestinal buffer is subsequently added to create a simulated intestinal medium.[4]

For either single medium or transfer *in vitro* dissolution tests, the rate and extent of dissolution are monitored by measuring the dissolved drug versus time and incorporated into PBPK simulations. Ideally, a mechanistic or semi-mechanistic dissolution equation is selected to translate *in vitro* dissolution to predictions of *in vivo* dissolution. Such equations calculate dissolution as a function of fluid properties (eg, pH, concentration of bile salts), dose, and dissolved drug concentration, properties that may differ between the *in vitro* test(s) and simulation parameters. Examples of semi-mechanistic dissolution equations include the z-factor, Wang-Flanagan, Johnson, and product particle size (P-PSD) models (Figure 2).[5]

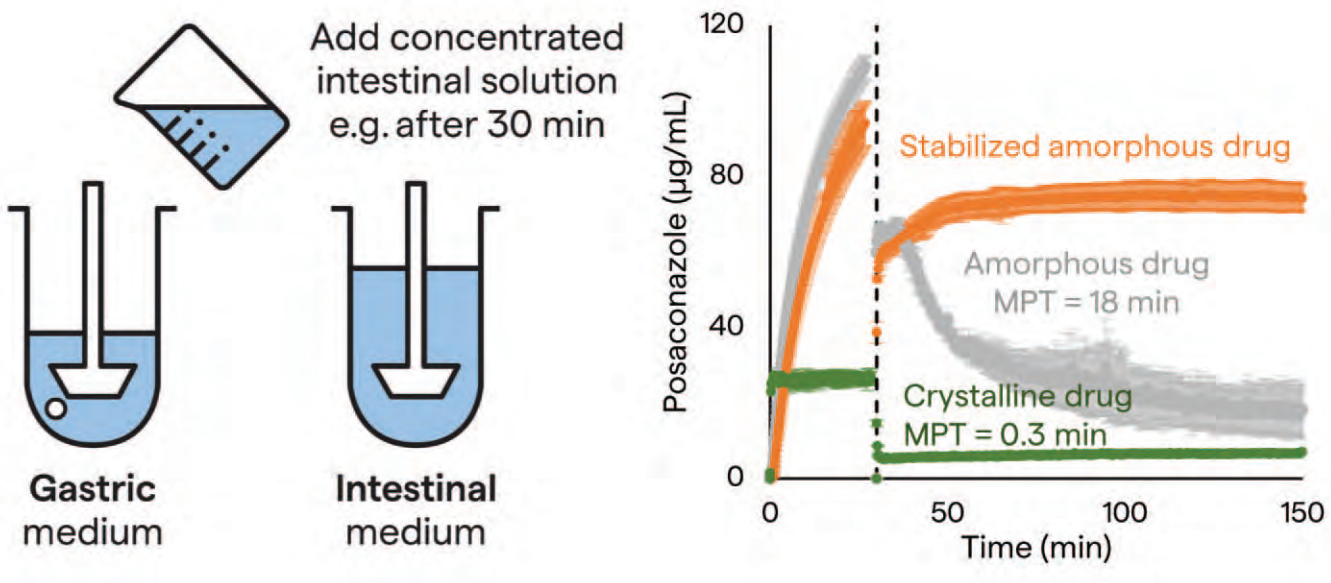
## PRECIPITATION

Drug molecules can precipitate in gastrointestinal fluids to form solid drug particles when they are dissolved at concentrations exceeding their thermodynamic solubility. Precipitation undermines absorption as it can reduce the driving force for permeation across the GI membrane. Supersaturated concentrations can occur when dissolved drug is introduced into the GI tract above its thermodynamic solubility in that region. For example, a weakly basic drug with high solubility in an acidic stomach may transit to the small intestine at a supersaturated concentration.[6] Further, a drug formulated as an amorphous solid dispersion, salt form, or metastable polymorph might dissolve to the solubility of that enabled form, which is supersaturated with respect to the solubility of the thermodynamically stable form. In all these cases, precipitation can occur.

Not all drugs precipitate in vivo, even under supersaturated conditions, and this depends upon factors such as solubility, degree of supersaturation, drug properties, GI fluid composition, and formulation components.[6] *In vitro* methods are often used to determine the tendency of a drug to precipitate from a formulation and quantify the rate of precipitation for input into PBPK software.

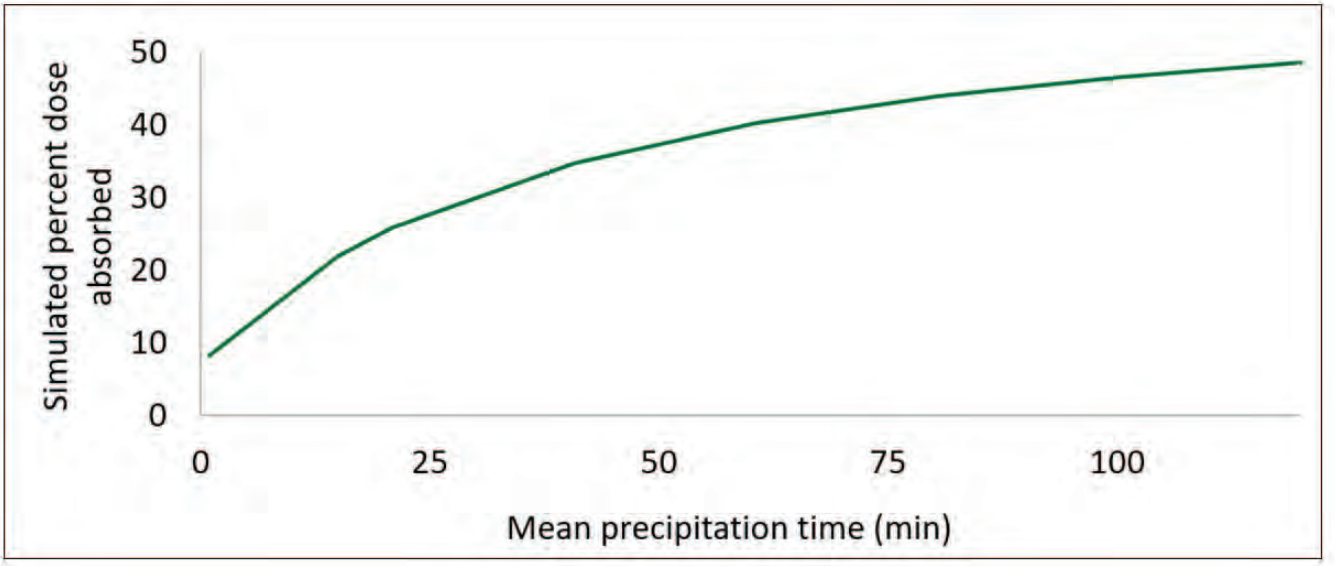
A useful *in vitro* method for estimating precipitation rate is the transfer test previ-

**FIGURE 3**




***In vitro* precipitation of weakly basic drug, posaconazole. Left: USP 2 transfer test schematic. Right: *In vitro* concentration vs. time for precipitating formulations (amorphous drug & crystalline drug) and non-precipitating formulation (stabilized amorphous drug).**

## FIGURE 4




**Parameter sensitivity analysis. Simulated percent dose absorbed for weakly basic drug posaconazole demonstrates sensitivity to MPT.**

ously described. *In vitro* transfer tests should incorporate physiological GI fluid properties and composition, and a relevant drug dose. For example, the relevant *in vitro* dose concentration might be selected by dividing the *in vivo* dose by the estimated GI fluid volume. Ideally, multiple concentrations would be tested to explore the impact of dose-to-volume on degree of supersaturation and the potential for precipitation. Alternatively, there are more sophisticated *in vitro* tests that allow for more physiologically relevant supersaturation ratios, perhaps by including fluid transit and/or an absorption compartment, that can provide greater insight into precipitation.[7]

Drug precipitation rate can be tracked by monitoring the decline in drug concentration versus time. Precipitation rate can be translated to predictions of *in vivo* precipitation by, for example, a first-order fit to the data to determine a mean precipitation time (MPT).[5] MPT can be input into PBPK software, allowing drug precipitation to be simulated when drug concentration exceeds the solubility. Multiple values for MPT can be defined to account for differences in precipitation rate for different GI compartment or pH values (Figure 3).

## PERMEATION

To access the bloodstream, drug permeates from GI fluids across the GI membrane by passive diffusion and/or multiple active transport processes. Key parameters impacting the rate and extent of permeation include regional effective permeability across the intestinal wall, surface area available for absorption, and drug species type and concentration at the intestinal wall.[8] These parameters depend on formulated drug properties, including lipophilicity, size, and charge, and GI fluid properties such as intestinal wall composition and fluid hydrodynamics.

Human effective permeability ($P_{eff}$) is a key input to PBPK software used to simulate *in vivo* permeation. $P_{eff}$ can be predicted using quantitative structure-activity relationship models, measured using *in vitro* models of permeability, such as through human intestinal epithelial cells, originated from Madin-Darby canine kidney (MDCK) cells or human Caco-2 cells, or using *in situ* animal models.[8] No single method is a reliable surrogate for in vivo $P_{eff}$, but they are particularly useful when reference compounds with known $P_{eff}$ are used.

When inputting effective permeability derived from cell monolayers or *in situ* animal models, PBPK software can use built-in correlations to convert these data into $P_{eff}$ in humans or in animal species such as rat or dog. To simulate the rate of drug permeation, PBPK software uses $P_{eff}$ in addition to variables accounting for the effective permeation surface area in a given GI compartment, fluid flow rate, and drug concentration.

## PUTTING PBPK VARIABLES INTO CONTEXT

Solubility, dissolution, precipitation, and permeation are integral to predicting the rate and extent of oral drug absorp-

tion. Their relative importance depends upon the drug, formulation, and physiological parameters, such as species and prandial state. Determining input parameter importance, or sensitivity, can be done within PBPK software by conducting parameter sensitivity analyses (PSAs) to determine which parameters significantly impact simulation outputs (Figure 4).

Careful attention should be paid to highly sensitive parameters, as small differences in their values cause large differences in simulation outputs. When sensitive parameters are identified, one should evaluate the robustness of that input and determine if more *in vitro* and/or *in vivo* experimentation is needed to gain confidence in the input value.

With an increasing number of drugs posing absorption challenges, the ability to predict how a new molecule will behave *in vivo* is important. Being able to predict and mitigate absorption problems before they arise should help the project progress with fewer delays.

PBPK is a useful tool for predicting absorption challenges and guiding formulation selection early in development when used with *in vitro* test methods tailored to the drug, formulation, and product properties. As more *in vitro* and *in vivo* data are gathered throughout the drug development process, early PBPK predictions can be refined and validated. This "learn and confirm" process facilitates informed decision-making throughout the drug lifecycle, aiding important drug development activities, such as setting clinically relevant specifications, developing biopredictive dissolution methods, food effect evaluation, and pH-dependent drug-drug interaction assessment.[9,10] ◆

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## BIOGRAPHY


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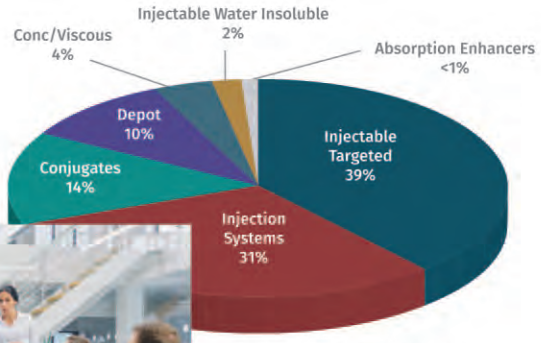
| Excipient vs Strength | 375 mg telaprevir |
|---|---|
| HYPROMELLOSE ACETATE SUCCINATE 12070923 (3 MM2/S) **(Core/Content)** | 375 mg |
| SODIUM LAURYL SULPHATE (Core/Content) | 7.58 mg |
| DIBASIC CALCIUM PHOSPHATE ANHYDROUS **(Core/Content)** | 75.76 mg |
| CROSCARMELLOSE SODIUM **(Core/Content)** | 30.3 mg |
| MICROCRYSTALLINE CELLULOSE **(Core/Content)** | 75.76 mg |
| SODIUM STEARYL FUMARATE **(Core/Content)** | 29.29 mg |
| COLLOIDAL SILICON DIOXIDE **(Core/Content)** | 7.58 mg |
| POLYVINYL ALCOHOL, UNSPECIFIED **(Tablet/Capsule coat)** | 11.72 mg |
| POLYETHYLENE GLYCOL **(Tablet/Capsule coat)** | 5.92 mg |
| TALC **(Tablet/Capsule coat)** | 4.33 mg |
| FERRIC OXIDE YELLOW **(Tablet/Capsule coat)** | 0.32 mg |
| TITANIUM DIOXIDE **(Tablet/Capsule coat)** | 7 mg |
| FD&C RED NO. 40 **(Tablet/Capsule coat)** | |
| FD&C BLUE NO. 2 **(Tablet/Capsule coat)** | |

## Evaluate New and Promising Technologies

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# PREFILLED SYRINGES

## Making a Prefilled Syringe Smart: Technological Solutions to Advance Patient Care & Clinical Trial Outcomes

By: Salvatore Forte, MS

### INTRODUCTION

A prefilled syringe (PFS) is widely employed in healthcare. With the raising prevalence of injectable biological products, the PFS is becoming the primary mode of drug administration. The global PFS market is projected to reach $13.33 billion by 2028, with a compound annual growth rate (CAGR) of 11.11%.[1] PFS is growing in preference over conventional needle and vial delivery systems because it preserves drug sterility, limits drug waste due to overfilling, improves needlestick safety, simplifies the administration process, and reduces the likelihood of dosing errors. A PFS has particularly found two major fields of application: clinical trials and chronic disease therapy. Adherence to the dosing regimen, as well as compliance to the injection protocol, are essential for treatment success in both these use-cases. Hence, the request for a connected, smart PFS is growing just as much to tackle poor medication adherence and compliance issues.

### MARKET NEEDS: CLINICAL TRIALS & CHRONIC DISEASE MANAGEMENT

In a clinical trial, one of the major hurdles for pharma companies is the lack of objective data on treatment adherence based on real measurements rather than informal patient feedback. The latter represents the only thing pharma companies can rely upon still to date. Non-adherence to the dosing regimen can largely affect the assessment of drug efficacy.[2] When study participants do not perform injections as prescribed, this can result in underestimated drug efficacy, which compromises the outcome of the clinical trial and ultimately delays the drug time-to-market.

In chronic disease management, adherence and compliance errors are equally frequent. A patient is usually required to perform self-injection weekly, without receiving assistance from a healthcare professional (HCP). This is when a connected PFS can come to the rescue: it allows to automatically keep record of injections by possibly fostering patient engagement.

A connected PFS provides a reliable means to solve the challenge of monitoring treatment compliance and adherence in both clinical trials and home-based therapies. Nonetheless, there is still a technology gap to fill in the drug delivery industry. The market is certainly populated with connected pens and autoinjectors, but where the industry is yet lacking is in the integration of sensors and connectivity features on a PFS. In recent years, Flex has investigated several variants of a smart PFS implementation to address different market requirements and usability scenarios. The following provides a review of technological proposals for a smart PFS reference design platform, including the premises and the technical aspects associated to the implementation of it.

### DESIGN & DEVELOPMENT STRATEGY ON SMART PFS

This section reports the strategy, the rationales, and the conclusions that led the Flex team to investigate multiple smart PFS variants, based upon different connectivity and architectural options. Major criteria that were set to guide the design and development study were:

- The addition of digital features shall not require a modification of the PFS
- The addition of digital features shall have no (or least) impact on the overall form-factor

Based on this, two concept ideas have been explored for the implementation of a smart PFS, mostly relating to the section of the PFS delivery system where the digital features should be fitted in (Figure 1)

- Separate electronic module to mount on the flange of the plunger rod as add-on
- Custom plunger rod which incorporates electronics features

Other meaningful criteria that were assessed to narrow the concepts down to those finally selected and fully developed were:

- Quantity of info: it describes the added value that the technological solution is providing in terms of data that can be tracked.
- User experience: it describes the impact the added technology is having on the usability of the PFS, and the overall injection experience.
- Ease of implementation: it describes the degree of complexity of the technological solution in terms of expected engineering effort, and estimated time-to-market.
- Cost: it describes the additional cost of technology being added to the PFS.

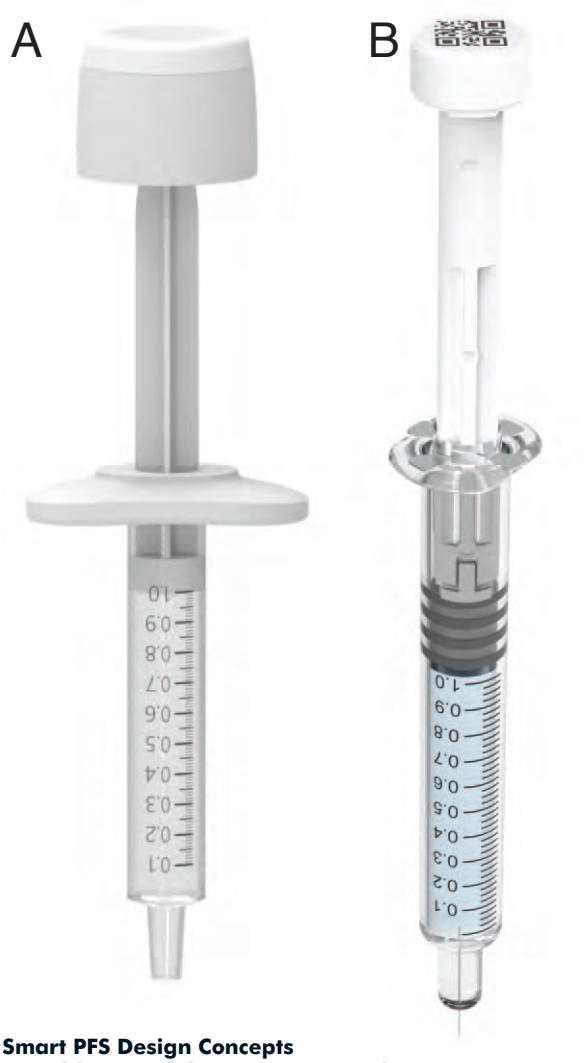
The Flex team brainstormed a shortlisted set of info to collect from the PFS, selecting those that appeared to be most relevant to the chronic disease management and/or clinical trial applications. The choice leaned toward:

- Drug product info (digitally encoded into the syringe)
- Start and end of injection
- Force applied to extrude the drug
- Volume of injected drug

On the connectivity end, it was decided to investigate both Near-Field Communication (NFC) and Bluetooth Low Energy (BLE) based devices to address two different types of market:

- NFC as a very affordable, more eco-friendly (due to the absence of batteries) and easier to implement solution, which however requires a voluntarily action on the user side (ie, tap) in order to transfer data from the PFS to an NFC-enabled smartphone.
- BLE as a more premium solution that offers a smoother user experience, and more sensing capabilities, with the downside of a higher price-point and the need of integrating batteries (which in principle results in a higher environmental impact).

**FIGURE 1**




**Smart PFS Design Concepts**
**(A) add-on module vs. (B) custom plunger**

## SMART PFS IMPLEMENTATION: A) ADD-ON MODULE VS. B) CUSTOM PLUNGER

### Add-On Module

One possible device embodiment is an electronic module to install as add-on to the PFS's plunger rod. The add-on can be assembled immediately after the drug filling. For the sensor part, in its simplest implementation, the device can enable the sensing of the injection completion. For the connectivity part, two alternative concepts for the add-on supporting either NFC or BLE connectivity are possible.

The NFC version can incorporate a printed NFC inlay, with an NFC tag connecting to a printed sensor switch for detecting the end-of-injection event. The NFC add-on does not incorporate any battery, with the tag chipset that gets powered by the NFC field of the reader (eg, NFC-enabled smartphone) as soon as it comes in close proximity with the PFS. The user can tap the PFS

with the smartphone before executing the injection to verify the authenticity of the drug (ie, product info), and after the injection to log the end-of-injection event together with a timestamp.

The BLE version allows for more sophisticated sensor implementations. The sensing of the injection completion can be accomplished in multiple ways, either through mechanical, magnetic, or capacitive technologies. To streamline cost and engineering simplicity, the add-on could implement a miniaturized switch, which activates when the plunger reaches the stopping point. The embedded software is then able to associate this trigger event to the indication that the injection is completed. In contrast to NFC, the BLE add-on incorporates a coin-cell battery, and connects to a BLE mobile app for data transmission.

A more advanced implementation of the BLE add-on could feature a miniaturized force sensor to measure the drug delivery force applied throughout the injection (up until the stopping point). This is becoming particularly relevant considering new therapies are often relying upon injections of highly viscous biological formulations. Force measurements can be accomplished with a variable capacitor sensor. The add-on discussed herein is based on a sensor principle that leverages a spring-activated sheet-metal plate facing the battery holder to form a parallel-plate capacitor subsystem as shown in Figure 2.

The whole sensing structure is extremely small. The use of a battery holder as second electrode reduces the parts count, expected to lower the cost of the bill of material, and the assembly time.

### Custom Plunger Rod

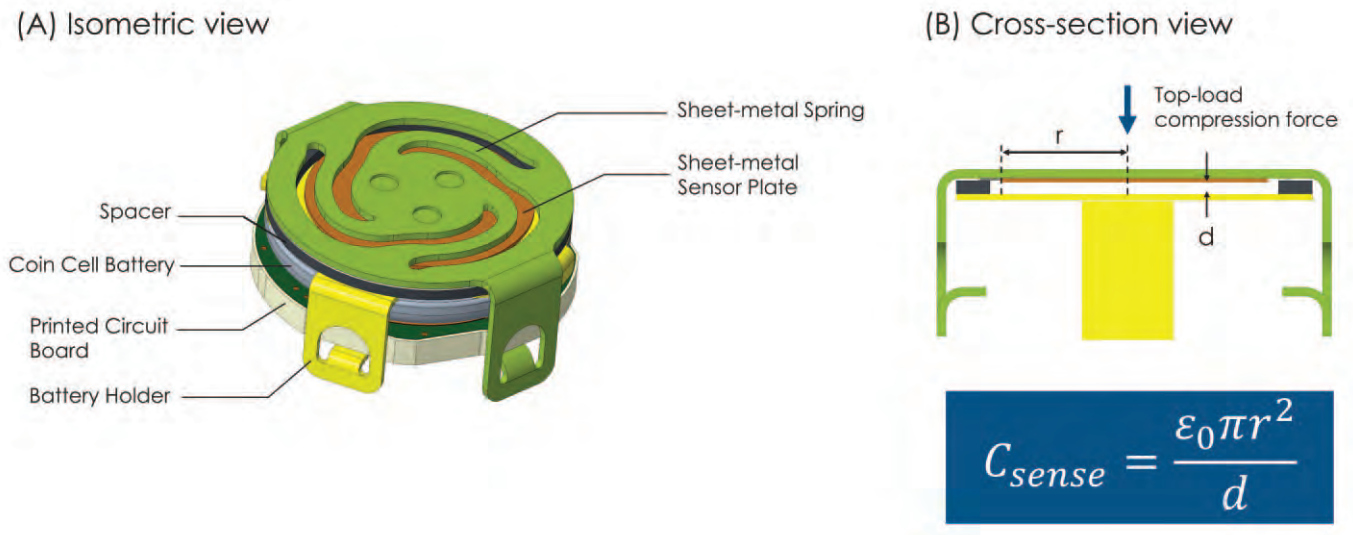
Another possible smart PFS embodiment is a custom-made plunger rod, which incorporates electronics features. The connected plunger is meant to replace a purely mechanical plunger rod: compatibility with commercially available PFS must be guaranteed. Two alternative concepts based on either NFC or BLE are possible also in this case. Regardless of the connectivity type, the smart plunger rod discussed herein was specifically designed to be fully compatible with 1-mL PFS, and it has the same length and diameter of existing plungers.

The NFC version could be implemented as simple as having an NFC tag encapsulated into the flange of the plunger rod. The tag's memory could be programmed at factory level with product information, such as the drug's name, the drug's manufacturing or filling date, and the expiry date. Then, the information encoded into the smart plunger can be read as usual by an NFC-enabled smartphone. This is a passive NFC solution, with no sensing capabilities associated to it. Again, no battery is necessary for an NFC-based smart plunger.

The BLE version could be a more feature-rich solution, with the plunger that could integrate a printed circuit board (PCB) with sensors and BLE connectivity. The BLE-enabled plunger incorporates the PCB and a coin-cell battery into the inner volume of the rod as illustrated in Figure 3.

For the sensor part, the circuit board could implement a positional sensor to measure continuously the exact drug volume that has been delivered as the user presses the plunger rod. Besides the start and end of injection, the device can record even partial dosing (Figure 4), which aids to identify compliance errors.

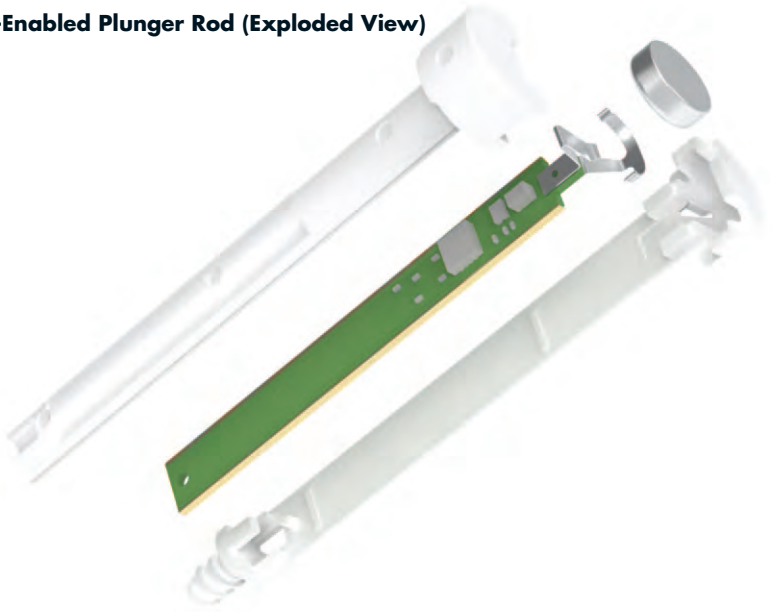
**FIGURE 2**




**Force sensor system. Capacitance measurement is converted in force value upon sensor calibration.**

## FIGURE 3

**BLE-Enabled Plunger Rod (Exploded View)**



## COST ANALYSIS

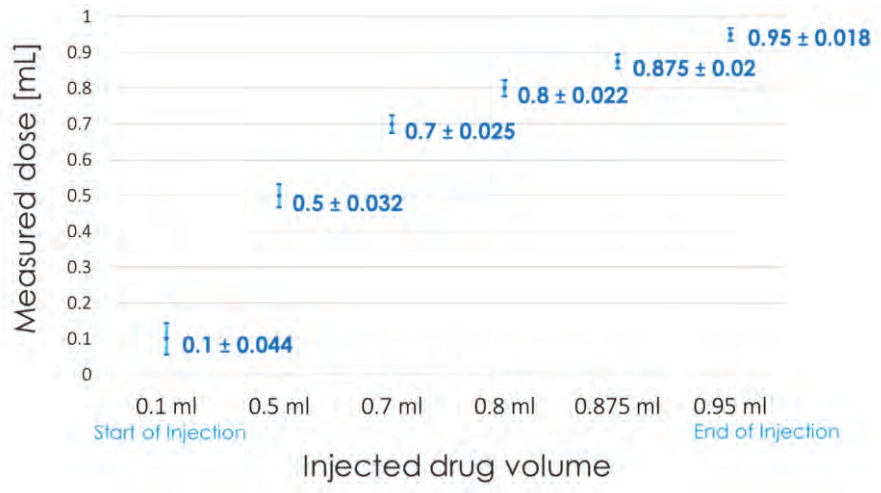
The acceptance of smart features into a PFS has been limited so far by the relatively high cost that is required to incorporate such features. From the design and development standpoint, there is yet an effort to lower the system cost to overcome the market entry barrier for an electronically assisted PFS. For the electronic part, reducing the cost of connectivity is essential. With this push toward disposable devices, silicon vendors are delivering chipsets with a simplified silicon architecture, which translates in a very low price-point. This holds particularly true for BLE, which is usually a more expensive technology compared to NFC. A custom-designed PCB antenna is also a sound decision to further reduce the system cost. The design of a 2.4-GHz antenna on such small devices is not trivial. It is crucial to predict the field loading effect that is generated by all the elements surrounding the antenna, such as the battery, the plastic enclosure, as well as the human body (eg, user's hand) acting as absorbers from the radio-frequency (RF) standpoint. All these elements will have a strong effect in detuning, and overall lowering the electromagnetic radiation of the antenna. The antenna can be designed with the aid of RF simulation tools, such as Ansys HFSS.[3] This is a powerful tool that allows to build an accurate 3D digital model of the smart PFS to streamline the design of the antenna to achieve optimal radiation efficiency.

## CONNECTIVITY TO CLOUD: DATA SECURITY

The smart PFS must be an integral part of an end-to-end digital ecosystem. This combines the device with software and cloud services that can ingest PFS's data to elaborate meaningful insights about the therapy. Data collected by a smart PFS are sent to a companion mobile app (via NFC or BLE), with the smartphone that then bridges the data to a back-end cloud system over the Internet, as shown in Figure 5.

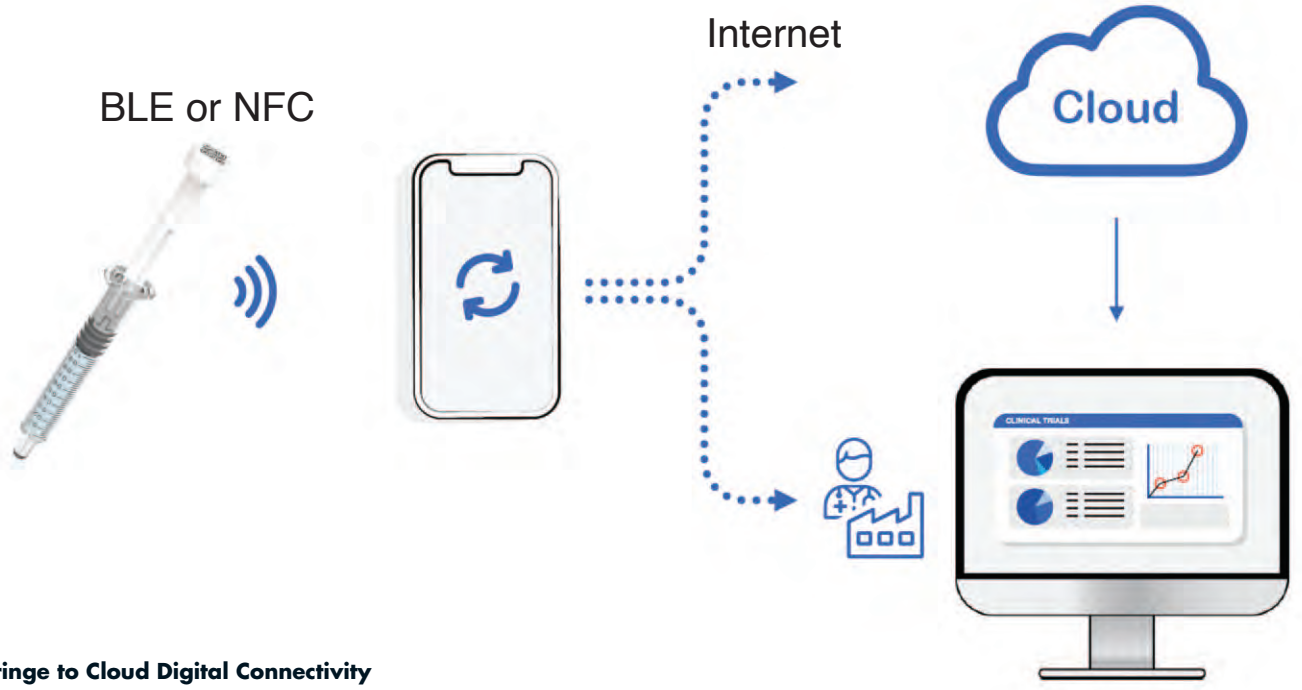
For the simple fact that the device is operating into the network, it becomes potentially exposed to cyber-attacks. It is mandatory to secure thoroughly the communication channel up to the cloud's services that will use the data. However, adding security to a smart PFS presents some technical challenges. There is the need to implement advanced security protocols, activate data encryption, and store digital certificates securely. Careful consideration shall be paid to the selection of the silicon itself. It is important to rely on a chipset that embeds the appropriate security support, including encryption engine

## FIGURE 4




**Measurement of delivered dose. Non-linear scale to increase measurement accuracy towards the end-of-injection.**

FIGURE 5




**Syringe to Cloud Digital Connectivity**

and secure storage of crypto keys. Regardless of the type of implementation (as described in previous sections), the smart PFS can implement industry-standard AES128-EAX scheme, which provides both mutual authentication with the cloud and data encryption to protect the transmitted data.

## DESIGN FOR ENVIRONMENT

The benefit of using a smart PFS must not come at the expense of the environment. As a single-use device, a smart PFS tends to increase the amount of plastic and electronic waste. This mandates the implementation of design for environment (DfE) best practices to produce an environmentally friendly smart PFS. Starting from the material selection, one opportunity is to use environmentally sustainable resins generated from bio-based feedstock with a mass balance approach.[4] This is totally equivalent to traditional plastic in terms of grade, quality, and mechanical performance. Although, it has a lower environmental impact because it is generated from biomass and waste cooking oils, in contrast to traditional plastic which is made of carbon-based sources. The device (whether it is shaped as add-on or custom plunger) needs also to be designed to be easily disassembled at the product end-of-life. For example, no welding or hard fixing is used to seal the plastic elements together. The device can rely upon snap-fit features to ease the opening of the housing, and ultimately the separation of the plastic from the electronics during disposal. The product might still have some residual value after use:

- Both the add-on and the custom plunger could be separated from the PFS, potentially opening the opportunity to apply it as re-usable item on a new PFS.
- The plastic might be recycled (e.g., chemically, mechanically) and reused as raw material in a non-medical market.

## SUMMARY

PFS is becoming the norm for the administration of biological drug products via injections. Important factors driving the growth in the PFS market are the continuing prevalence of PFS-injectable drugs to treat chronic diseases, and the adoption in clinical trials. While these might be seen as different use cases, they share a common request: they demand accurate digital data to demonstrate medication compliance and adherence. A smart PFS provides HCPs with reliable data to monitor a patient's in-take behaviors, and clinical study teams with all data they need to assess the efficacy of new investigational drugs and therapies. Adding digital features to small-volume PFS is not trivial. Smart PFS design must be approached within a multidisciplinary framework. Well-proven design and development expertise in this field becomes paramount to surf this unique market opportunity. Recognizing that a one-size-fits-all approach does not always work, Flex is proposing a vari-

ety of technological solutions for a connected, smart PFS that can be turned into end-products. Preparing now for the transition from regular to smart PFS is essential. ◆

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## BIOGRAPHY


**Salvatore Forte** is Innovation Manager at Flex, leading an R&D engineering team in the company's Design Center in Milan, which works to bridge the gap between technology breakthroughs and successful design implementation for medical devices. He has a solid technical background in analogue and digital electronics, specializing in embedded system design for power-sensitive medical devices, such as wearable health monitors, disposable point-of-care, and automated drug delivery. He earned his MS in Electrical Engineering from University of Naples Federico II (Italy).



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# Special Feature

## Improving Bioavailability & Solubility: The Never-Ending Quest

By: Cindy H. Dubin, Contributor

Poor water solubility and bioavailability are long-standing challenges in the drug development process. Around 90% of the drug candidates in the current development pipeline and close to 40% of the marketed pharmacological products are associated with concerns related to solubility and/or permeability.[1] Therefore, industry continues on what seems like a never-ending quest to overcome these challenges.

Tamas Solymosi, Lead Scientist at Nanoform, points to several new technologies that have reached preclinical or clinical evaluation. These include nanoprecipitation, a bottom-up process to assemble amorphous or crystalline nanoparticles composed of the API and block-copolymers or other stabilizers. Among others, clofazimine and lumefantrin – two difficult-to-formulate APIs – were successfully processed using nanoprecipitation. "KinetiSol™ technology employs a high-energy kneading method to prepare solid

**Nanoform's Controlled Expansion of Supercritical Solutions (CESS®) technology enables the creation of API nanoparticles directly from solution.**

dispersions of APIs, which are otherwise difficult to incorporate, into ASDs using standard spray drying or melt extrusion technologies," says Mr. Solymosi.

Formulators turn to modifying physical and chemical API characteristics to achieve improved solubility and bioavailability. One strategy that has recently emerged are drug-drug co-amorphous (CAM) systems. They have gained interest in their ability to improve solubility as well as an alternative to traditional fixed-dose combinations. In CAM systems, low-molecular-weight coformers – sugars, organic acids, amino acids, and surfactants – are used instead of polymers to enhance physical stability. No energy of lattice rearrangement needs to be overcome during the dissolution process, enabling high drug solubility and rapid dissolution.[2]

Co-crystals, a class of crystalline materials composed of two or more components, have gained attention in sustainable and green research due to their potential for enhancing drug delivery systems.[3] By utilizing eco-friendly solvent systems and renewable starting materials, researchers are developing environmentally benign synthesis methods for co-crystal production. These sustainable approaches not only minimize the environmental impact but also promote the development of greener pharmaceuticals with improved solubility and bioavailability.[3]

There is also rising demand for cyclodextrins to enhance solubility. Their ability to form inclusion complexes with various drug molecules, improving bioavailability, has led to increased adoption in pharma and a possible market value of $579.6 million by 2032.[4]

Learn more about these, and other, technologies in this exclusive *Drug Development & Delivery* annual report.

## Adare Pharma Solutions: Value Creation & Differentiation for Customers

High Throughput Screening (HTPS) for lead identification has led to a significant surge in new compounds exhibiting poor aqueous solubility and/or bioavailability (BA). This can be attributed to HTPS's inherent tendency to identify lipophilic leads, which favor target binding for efficacy. Despite the strides made in pharmaceutical technology, the challenge of addressing solubility and BA issues in these compounds remains a formidable one for formulators.

"Efforts to tackle BA issues necessitate a comprehensive understanding of the underlying causes and mastery in the development of tailored formulation strategies," says Srinivasan Shanmugam, PhD, Executive Director of Pharmaceutical Sciences, Business Support & New Technologies, Adare Pharma Solutions. Poor bioavailability can stem from a variety of factors, including drug-related issues such as inadequate solubility, slow dissolution rate, limited permeability, and physiological barriers like first-pass metabolism and P-glycoprotein (Pgp)-mediated efflux.

While drug absorption is influenced by drug-related factors classified under the Biopharmaceutical Classification System (BCS), physiological barriers ultimately determine the overall BA of a drug. Therefore, devising effective formulation strategies means navigating both drug-related and physiological factors. A grasp of the physicochemical properties of drugs – such as logP, melting point, and ionization – is also crucial for selecting appropriate formulation techniques.

Adare Pharma Solutions utilizes both traditional and innovative technologies to enhance solubility. Dr. Shanmugam explains that on the traditional strategies front, Adare has extensive expertise in creating amorphous solid dispersions to enhance solubility of poorly water-soluble compounds with the use of spray dryer or hot melt extruder. Two examples include:

- Diffucaps®: "Our specialized Diffucaps technology creates acidic or basic micro-environments with organic acid or alkaline buffers to augment the solubility of pH-dependent and poorly water-soluble drugs," he says. Additionally, Diffucaps is a fluid bed-based process so it has the ability to create amorphous solid dispersions.
- Optimum®: This technology platform effectively enhances the solubility and permeability of various BCS class drugs by generating lipid microspheres and amorphous solid dispersions, Dr. Shanmugam says.

"Our technologies overcome the challenges of solubility issues with simplicity, speed, and efficacy," he says. "Our solubility/BA enhancement capabilities, combined with our expertise in developing easy-to-swallow and palatable patient-centric formulations, deliver value creation and differentiation for our customers."

## Ascendia Pharma: A 3-Pronged Formulation Approach

New Chemical Entities (NCEs) have created a bottleneck for improving the bioavailability of drugs for unmet medical needs. Thus, it has fueled the interest to adapt more innovative approaches such as lipid and polymeric nanoparticle technology for parenteral and oral delivery of drugs. Identifying the appropriate ingredients or excipients (lipids/polymers) for im-

**Ascendia's GMP-grade microfluidizer for nanoparticle manufacturing.**

proving solubility and bioavailability is challenging, even more so with the novel ingredients due to lack of safety and regulatory guidelines. In spite of these challenges, FDA-approved inactive ingredients compatible with lipid-based self-emulsifying delivery systems (SEDDS), amorphous solid dispersions (ASD), co-precipitation, and Kinetisol® among others, have been used to enhance the solubility and bioavailability of molecules.

Ascendia is taking a three-pronged, tailored formulation approach to address these challenges. First, is to find the right excipients, polymers, and solvent by screening molecules for maximum solubility. Second, is employing its platform technologies – LipidSol®, NanoSol®, EmulSol®, and AmorSol® – to identify the right technology for drug loading and stability. Three, is optimizing the formulation by using design of experiments (DOEs) and utilizing intrinsic properties of the compounds.

"These steps are relevant to find the most appropriate formulations with enhanced efficacy and stability of drugs," says Shaukat Ali, PhD, Senior Director Scientific Affairs and Technical Marketing at Ascendia. "Ascendia's core capabilities in formulation and analytical development, stability and microbial testing, coupled with cGMP manufacturing suites for sterile and non-sterile drugs, offer unparalleled benefits for expediting drug molecules faster to clinic."

LipidSol, NanoSol, and EmulSol are based on lipids and solubilizers, while AmorSol is ASD-based. "All are designed with FDA-approved ingredients, and have been applied extensively to improve the solubility and bioavailability of molecules across all modalities by oral and injectable route of administrations," Dr. Ali says.

As more new molecules are discovered, a good majority of them are lipophilic, BCS Class I and Class IV (with logP 2-8 and high melting) and require non-conventional approaches to improve their solubility and bioavailability. Conventional technologies such as pH modification or salt formation, complexation, and prodrugs due to their own limitation, may not be well suited for developing medium- to high-dose drugs, says Dr. Ali. Therefore, efforts continue to find the relevant technology to yield the desired solubility, bioavailability, and most importantly, stability, with the understanding of scalability in manufacturing of drug products under sterile or non-sterile conditions. "Ascendia Pharma's enabling platform technologies are poised to address the growing demands for new drug candidates with enhanced bioavailability, and therefore, primed to take the lead in formulation development and manufacturing of those innovative lipophilic medicines," he says.

## Croda Pharma: Specializing in Specific Enhancement Technologies

Croda Pharma specializes in four technologies aimed at improving bioavailability and solubility. First, nanoparticle-based delivery systems such as liposomes, nanoemulsions, and inorganic nanoparticles, offer large surface area to encapsulate drugs that further improves their solubility and bioavailability.[5] These systems can also protect active pharmaceutical ingredients from degradation and offer a targeted delivery. Additionally, there is reduction in toxicity, greater safety, and biocompatibility.[6] A tool that has recently emerged for the development of nano systems like solid lipid nanocarriers, nanoemulsions, liposomes, etc. is microfluidic technology, which provides enhanced precision, efficiency and functionality[7], says Kritika Bajaj, PhD, Research Scientist at Croda Pharma.

Second, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS) are formulations consisting of oils, surfactants, solvents, and drug substance. They spontaneously form oil-in-water emulsions when introduced to aqueous phase. These systems enable improvement in oral bioavailability of a poorly water-soluble drug.[8] These systems are easy to make, economical, and scalable, says Dr. Bajaj.

Third, amorphous solid dispersion (ASD) technology involves utilizing excipients to assist in dispersing a poorly soluble drug in a hydrophilic inert carrier matrix (usually a polymer) to enhance its solubility and dissolution rate upon administration. This technology works by increasing surface area, improving wettability, tailoring release profiles, and enhancing solubility by dispersing a drug in an amorphous state.

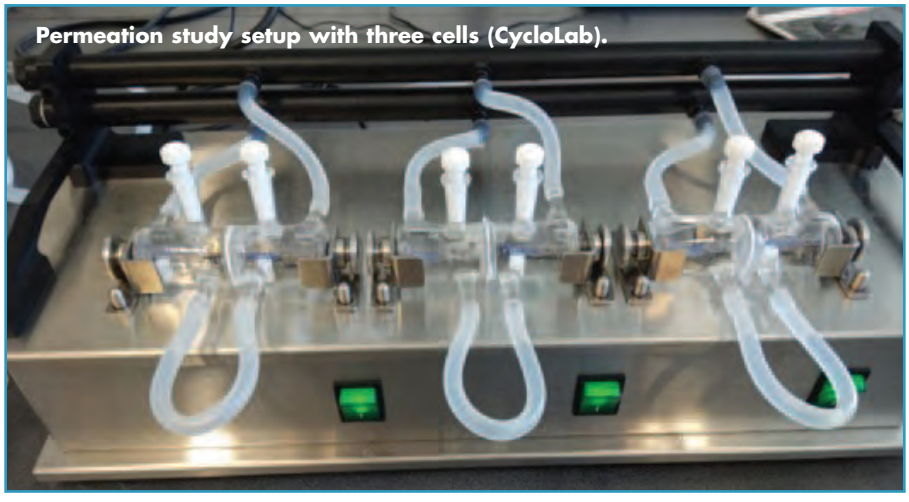
Finally, a permeation enhancer works by improving the transport of poorly absorbed actives across biological barriers such as skin, mucosa, or gastrointestinal tract. They can aid transdermal and oral drug delivery.[9,10] Compounds such as surfactants, dimethyl isosorbide (DMI), and lipid-based excipients can improve drug permeability across biological barriers, leading to increased bioavailability.[11]

Dr. Bajaj says: "Croda's portfolio of high purity excipients used as surfactants, permeation enhancers, polymers, lipids, and oils can play a significant role in the above-mentioned technologies to enable improved solubility and bioavailability of drugs."

## CycloLab: Cyclodextrins Are an Inventive Approach to Low Solubility

One of the key activities of CycloLab Cyclodextrin Research & Development Laboratory Ltd. is developing improved formulations with active pharmaceutical ingredients of low aqueous solubility (hence having poor bioavailability) by applying various cyclodextrins. As a result of cyclodextrin complexation, the pharmacokinetic parameters of these substances are favorably modified due to the inclusion phenomena. Several other technological properties (wettability, taste masking, physical disintegration) of the complex may become significantly more advantageous compared to traditional drug formulations, wherein a surfactant and/or a co-solvent is applied for enhancing bioavailability. Using cyclodextrins is still regarded as an inventive approach.

When an innovator applies to get approval for a new cyclodextrin-based composition of a known drug compound, the question of bioequivalence may be raised by the pharmaceutical authorities, even for injectables. The U.S. Food and Drug Administration Center for Drug Evaluation and Research (CDER) issued a draft guidance document entitled "Sameness Evaluations in an ANDA – Active Ingredients" in November 2022 stating that, in general, cyclodextrin complexes do not constitute new chemical forms that are considered in determining active ingredient sameness because they are not intended to furnish pharmacological activity (or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease) associated with the approved use of the drug product. Since this guideline is a nonbinding recommendation, the innovator may be interrogated to demonstrate bioequivalence of an already marketed composition compared to the novel complex, cyclodextrin-based formulation. Traditional



**Permeation study setup with three cells (CycloLab).**

animal studies to justify bioequivalence are demanding in terms of cost, duration of test, and documentation, including sensitive ethical issues. To bypass this difficulty, biowaiver data such as *in vitro* permeation studies are often found appropriate in the approval process of the cyclodextrin-based composition.

"CycloLab conducts relatively facile comparative *in vitro* tests for a relevant marketed product (as a reference) and a novel cyclodextrin-based formulation, regardless if the new product candidate is developed by CycloLab or previously elaborated by a third party," explains Dr. Istvan Puskas, Research Chemist at CycloLab. "By conducting *in vitro* permeation studies, the study sponsor may get insight into the characteristics of the dissolved drug in simulated biological fluids or in human plasma. The kinetics of the drug permeation rates in solution specific to the reference marketed product versus the cyclodextrin-based complex may establish adequate justification of bioequivalence. The test is performed using semi-permeable membranes of specific pore size distribution and retention capabilities according to molecular weight. This technique enables discrimination of unbound portion of drug substance from that included into cyclodextrin or associated to protein."

To further illustrate the strength of drug-cyclodextrin interactions, computer modeling on the noncovalent association, as well as aggregation studies in different dilutions, may be performed. If required, competitive cyclodextrin binding studies may be provided in human serum albumin (HAS) as well as in blood plasma. CycloLab's study report on the experimental data is issued ready for submission to relevant authorities to support the approval process.

**Application Laboratory Manager Masumi Dave in Gattefossé's Technical Center of Excellence.**

## Gattefossé: Lipid-Based Approach Improved Exposure by Two-fold for Poorly Soluble Active

Chemical modification approaches like salt formation, complexation, among others, are a few of the many approaches for improving a molecule's solubility, but modifications present a new set of challenges to overcome, and they may not outweigh the benefits, says Masumi Dave, Application Laboratory Manager at Gattefossé. "Any change to a drug's molecular properties can set the development clock back because the modified molecule's physico-chemical characteristics and pharmacokinetic parameters will then be different, which may impact solubility, stability, bioavailability, and safety."

Modification by formulation design using lipid-based excipients to prepare SEDDS/SMEDDS may enhance bioavailability and solubility by utilizing multiple BAE mechanisms, she says. Lipid-based excipients like Labrasol® ALF, Gelucire® 48/16, Gelucire 59/14, etc., can be used to deliver lipophilic molecules that are otherwise difficult to solubilize. Self-emulsifying, microemulsifying or nanoemulsifying drug delivery systems (SMEDDS/SNEDDS/SEDDS) can help improve solubilization and absorption of these compounds *in vivo*.

"We worked with a customer's API that was subject to first-pass metabolism and had solubility and bioavailability challenges," Ms. Dave explains. "Following a lipid-based approach, a formulation was developed with a combination of excipients having short chain fatty acid esters like Labrasol ALF and long chain fatty acid esters like Labrafil® M 1944 CS to aid tight junction opening, promote lymphatic uptake, and to generally improve systemic availability of this active. Excipient selection was based on solubility of the active in the excipient, excipient miscibility, and the target drug load. This formulation improved the exposure ($C_{max}$) in dogs by two-fold with lower variability compared to the control formulation."

## Hovione: Using ASDs to Turn Candidates into Drugs

Hovione has experienced some very marked trends that have driven the way the CDMO produces drug dosage forms. One of these is the increased prevalence of new chemical entities that are poorly

soluble and the establishment of amorphous solid dispersions (ASDs) as the predominant platform to overcome poor bioavailability – in great part leveraged by spray drying, which has become a reliable industrial-scale platform. Filipe Gaspar, Vice President Technology Intensification at Hovione, says: "This has led to significant advances on the formulation of amorphous materials, namely in material and analytical sciences, the development of novel (sometimes enabling, like Dispersome®) excipients, and the need for fit-for-purpose dosage forms."

He explains that Hovione's R&D portfolio is made up of more than 60 projects in different development phases, comprising active ingredients with solubility challenges. "Our approach starts by understanding the most appropriate bioavailability enhancement approach to increase solubility/bioavailability," he says. "In most cases, converting the active materials into an ASD provides the larger opportunity to turn these candidates into viable drugs. In these cases, we apply a thorough screening procedure with as little as 5g of active, Hovione's ASD-HIPROS, to identify the best composition, in terms of performance, physical stability, and manufacturability. Often, a ten-fold increase in performance – often more – is observed without compromising other drug attributes."

## Latitude: Drug Substance Nanoparticles Are Often the Only Option

Nanoparticles of drug substances, which are crystalline or non-crystalline insoluble API milled to about 100-200nm particles, have received a lot of attention recently. This form can provide enhanced dissolution and bioavailability for insoluble APIs while requiring only a very small proportion of excipients (about 10% of the total weight of the dosage form); thus a nanoparticle is probably the only feasible technology for an insoluble drug substance that has a relatively high oral dose, e.g., >250mg/dose (a High Dose Insoluble API).

In contrast, all other formulations that can enhance solubility/bioavailability require a large proportion of excipients – usually 50% to 90% of the drug dose to solubilize the drug, which inevitably leads to a bulky dosage form (e.g., a large tablet) that is inconvenient or impractical, says Dr. Andrew Chen, President and Founder of Latitude Pharmaceuticals. "A final oral dosage form can only realistically be provided with a limited total weight of about 1.5g for a tablet and 1g for a capsule," he explains. "Therefore, many other solubility/bioavailability enhancing formulations such as SEDDS, lipid-based nanoparticles, solid dispersion, liposomes, and solvent formulations are not suited for a High Dose Insoluble API. An oral formulation (e.g., tablet, capsule, granule, or oral suspension) containing nanoparticles of the drug substance is the best and often the only option."

Two different and innovative approaches were recently deployed to solve a client's bioavailability and solubility challenges. A client wished to develop an intravenous (IV) and an oral (PO) formulation for one API, but the API was very insoluble and had a high-dose (>350mg); the API was soluble only in dimethyl sulfoxide (DMSO). "We developed an IV formulation that was an oil/water nanoemulsion, with 150nm average droplet size, using a natural phospholipid and triglyceride oil," Dr. Chen describes. "The formulation was able to dissolve the dose completely, permitted IV infusion, and was very well tolerated. For the PO formulation, a variation of the nanoemulsion containing API nanoparticles was developed and achieved unprecedented oral bioavailability in test animals."

## Lonza: Weighing the Risks & Benefits of BAE Technologies

Improving a drug's solubility, dissolution rate, or permeability can be required to enhance bioavailability. When a drug is limiting oral absorption, a wide variety of bioavailability enhancement (BAE) technologies are available to address these risks. "At Lonza, we balance the risks and benefits of each approach and apply the best fit for a drug's specific BAE needs," says Dr. Martina Ribar Hestericová, Associate Director, Science Communications at Lonza. "The approach we take is dictated by the unique physiochemical properties of each new chemical entity (NCE) and the target product profile, including the intended dose and patient population."

Technologies used at Lonza for increasing the solubility of a drug can include forming salts, cocrystals, self-emulsifying drug delivery systems (SEDDS), and amorphous solid dispersion (ASDs). Using an integrated drug development approach bridging drug substance solid form selection (SFS) with BAE technologies, Lonza offers customers reduced time to clinic utilizing optimized formulations and processes, she says. Incorporating risk assessments for formulation and process development drives the identification of the key rate-limiting steps to absorption and rapid prototyping through clinical scale-up.

"Applying risk assessments, we rou-

tinely mitigate issues such as precipitation of a lower solubility form, chemical degradation, and physical state change when employing a BAE technology," says Dr. Hestericová. "Based on our experience, gaining a comprehensive understanding of a molecule's solid form landscape is essential to a successful drug development program, regardless of the final dosage form."

The selection of a polymorph that will minimize risk prior to a clinical formulation development effort will reduce risk in later stages. When a BAE technology is utilized, the in-going drug substance is often a crystalline form that will require acceptable shelf-life prior to downstream processing. As part of Lonza's integrated offering, the SFS team works side-by-side with the drug product development team to realize the benefits of an optimized solid-form lead selection.

In parallel with the lead selection of the solid form, Lonza's drug product development teams utilize a variety of *in silico* and *in vitro* techniques for technology selection. "Our bioperformance toolkit leverages proven methods to assess a drug's physiochemical properties," she explains. These properties are fed to predictive tools, such as physiologically based pharmacokinetic (PBPK) models, to perform an adsorption risk assessment. The output of these efforts ensures rapid formulation development and confidence that *in vivo* exposures have been optimized. Performing physical form manipulations, such as exploring various polymorphs, ASDs, micronization, or nanosuspensions, can be used to address the solubility or dissolution limitations of drugs. Chemical enhancement approaches, such as creating a salt form, can significantly alter the physiochemical properties to overcome absorption rate challenges after the compound lead selection has occurred.

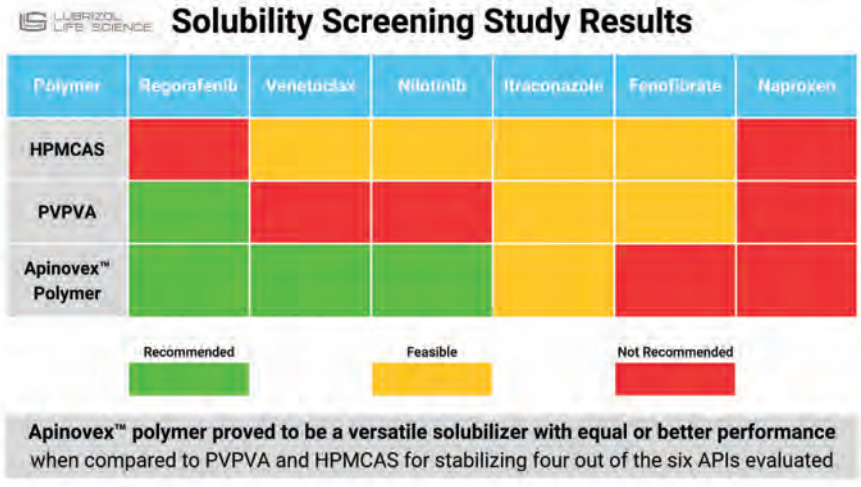
"At Lonza, we have extensive knowledge and experience employing BAE technologies on thousands of our customers' compounds that can be utilized to overcome absorption challenges and ensure sufficient exposure of the drug," says Dr. Hestericová. "We use a risk-based approach to successfully navigate the multitude of options available and align on a design that is needed for the drug, whether that is a simple crystalline form in a capsule or a more complex solubility-enhanced formulation utilizing one or more BAE technologies."

## Lubrizol: Two Novel Excipients May Rescue APIs From Solubility Issues

Solubility and bioavailability challenges are the most pressing needs in the field of small molecules. While there are traditional excipients and techniques available to address these issues, they often have low efficiency and lead to complex manufacturing processes or undesired side effects for patients.

"At Lubrizol, we believe that novel excipient technologies have the potential to overcome solubility and bioavailability-related challenges while producing more patient-friendly dosage forms and creating opportunities for new intellectual property," says Nick DiFranco, MEM Global Market Manager, Novel Pharmaceutical Technologies at Lubrizol.

Lubrizol Life Science's oral-grade Apinovex™ and injectable-grade Apisolex™ polymers were designed to overcome poor solubility using simple, scalable manufacturing techniques. Apinovex and Apisolex are excipient-grade polymers that offer IP-protection and life-cycle management for BCS Class II and IV APIs. Apinovex polymers are GMP-validated, high molecular-weight polyacrylic acid excipients designed to provide both processing and formulation benefits for spray-dried amorphous solid dispersions (ASDs), explains Mr. DiFranco. Apinovex polymers enable formulators to achieve stable, high drug loading (up to 80%) and up to 10x improvement in drug release for crystalline APIs. With Apinovex, formulators can develop efficient, IP-protected oral




**Recent data on Lubrizol's Apinovex polymer showcases its ability to solubilize a range of small molecules with different properties and glass-forming abilities.**

solid dosage forms for a range of poorly soluble APIs.

Recently, Lubrizol and Research Center for Pharmaceutical Engineering (RCPE) published new data on the Apinovex polymer, showcasing its ability to solubilize a broad range of small molecules with different properties and glass-forming abilities. Mr. DiFranco explains that the data demonstrated that Apinovex polymer could match or improve upon the performance of traditional excipients such as HPMC-AS and PVP-VA for several drugs, including challenging beyond-rule-of-5 compounds such as venetoclax, nilotinib, and itraconazole. Apinovex polymer demonstrated good solubilizing properties and supersaturation maintenance in the initial study, and new spray drying trials will be run later this year.

"Given these results, we view the Apinovex polymer as a promising new tool for formulators working on oral amorphous solid dispersions (both NCEs and 505(b)(2) products)," he says.

The Apisolex polymer is an injectable-grade poly (amino acid)-based co-polymer that has been shown to increase the solubility of hydrophobic APIs by up to 50,000-fold where other commonly-used excipients fail (such as surfactants, PEG-based polymers, and cyclodextrins). "Robustly patented, safe, efficient, and scalable, Apisolex formulations can achieve drug loading of up to 40% and dramatically increase the achievable concentration of API in water," says Mr. DiFranco.

The Apisolex polymer utilizes straightforward mixing/homogenization techniques coupled with filtration or lyophilization to produce the desired end product – either a powder for reconstitution or a ready-to-inject sterile liquid. He says: "The technology has proven effective with a broad range of small molecule APIs, demonstrating both improved compatibility and efficiency when compared to traditional surfactants and polymeric solubilizers. The effectiveness of this tool, coupled with its ease of use, led to two formulation awards last year, including first place in the Finished Formulation category at the 2023 CPhI Pharma Awards. The Apisolex polymer was recognized as the latest solubility-enhancing excipient to be launched specifically for parenteral use in decades, and we view it as an enabling technology for rescuing new APIs from solubility and bioavailability challenges."

## Nanoform: Proprietary Green Nano Tech Obtains Desired Particle Size

As the complexity and lipophilicity of drug candidates are expected to further increase, there is a constant need to evolve druggability-enabling technologies – and to develop new ones. Several new technologies have reached preclinical or clinical evaluation. One of the most recent strategies to reduce particle size, and in certain cases alter the crystallinity of compounds, is Nanoform's Controlled Expansion of Supercritical Solutions (CESS®) technology, which works by dissolving an API in supercritical carbon dioxide, then controlling precipitation to obtain the desired particle size distribution and polymorphic form. Because it does not require the use of excipients and organic solvents, CESS is a green technology.

"It is powerful in helping formulators decrease the particle size of a pure drug substance to the very lower edge of the nano range, which might offer dose reduction, elimination of food effect, and lower variability in pharmacokinetic parameters," says Tamas Solymosi, Lead Scientist, Nanoform.

The majority of Nanoform's partners present drug candidates that are notoriously difficult to formulate, with dissolution and/or solubility limitations. Mr. Solymosi says that at this point of development, usually after salt and polymorph screening and initial PK and toxicology studies, chemical modification is a last resort and partners prefer size reduction, solubilization, and solid dispersion strategies. He explains: "In certain projects, nanoforming by CESS technology has enabled an order of magnitude increases in bioavailability, or the complete elimination of food interaction. Moreover, in general we do observe lower variability in PK parameters compared to reference formulations."

One partner presented Nanoform with a prototypical poorly soluble drug candidate that was intended to be formulated as a hydrogel. Previous solubilization techniques failed, and nanomilling turned out to be suboptimal. Nanoforming proved to be successful and the nanoformed API could be incorporated into high drug load formulations, eliminating a major roadblock to the partner's investigational new drug (IND) application.

The decrease in inter-individual variability of PK parameters renders nanoformed drugs attractive as erratic dissolution from conventional drug formulations, coupled with inherent differences in gastrointestinal pH, motility, bile salt secretion, and other physiological factors that can lead to extreme variabilities. Mr. Solymosi says: "For certain drug products, this can result in subgroups of patients necessitating dose reduction due to toxicity issues or, on the other hand, yields underdosed patients with subtherapeutic drug levels leading to worse outcomes." ◆

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# CLINICAL TRIALS

# Digital Endpoint Integration in Clinical Trials: Key Considerations & Nuances Per Therapeutic Focus

By: Tapan Raval, PhD, and Ganesh Gundi, MD

## INTRODUCTION

In today's clinical trials, industry stakeholders of all kinds are embracing the use of digital health technologies (DHTs), such as medical-grade sensors and wearables, to collect respiratory rate, oxygen saturation, blood pressure, and other physiological parameters directly from trial participants in the comfort of their homes or wherever they live or work.

The popularity of this data collection modality is unprecedented. Patients have more flexibility to participate in trials and improved experiences while providing trial sponsors with richer insights captured in near real-time to guide smarter decision-making. The number of trials integrating DHTs across therapeutic areas is growing by the day. With regulatory support and guidance, these tools are allowing sponsors to include patients who potentially could not be in trials due to geographical and logistical barriers. They also evaluate diseases and related patient experiences in near real-time with continuous data collection and review opportunities.

However, when considering incorporating digital endpoints into trial design, it is essential sponsors, their CRO partners, and site teams account for the key clinical and operational aspects discussed further, which can impact how well clinically meaningful outcomes are captured and analyzed. This includes planning to ensure patient safety and data quality are not compromised. Additionally, therapeutic needs can vary greatly among patient populations and conditions being studied. Taking those nuances into consideration is critical to optimizing trials with digital endpoints.

## KEY COMPONENTS FOR DHT INTEGRATION

Before all else, sponsors and CROs need to ensure the right mix of experts is engaged in the trial design process and give the following careful thought:

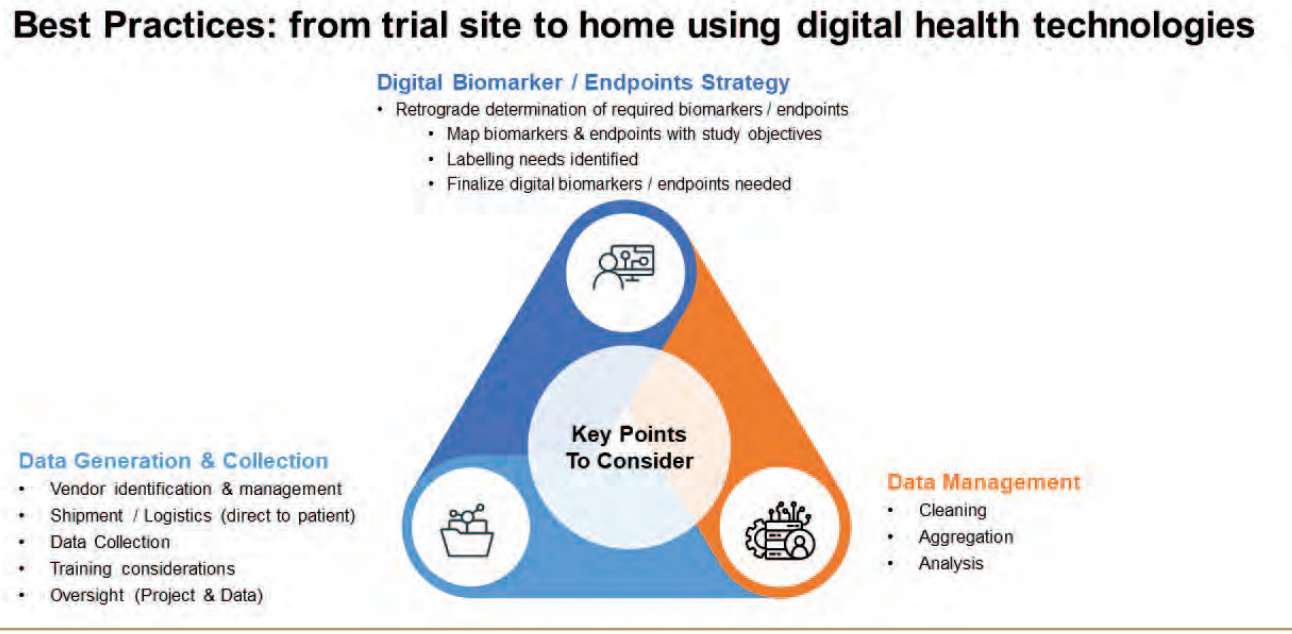
- Protocol/program-specific digital endpoint strategy
- Data generation and collection
- Data management

## PROTOCOL/PROGRAM-SPECIFIC DIGITAL ENDPOINT STRATEGY

One of the first critical steps toward trial execution effectively using DHTs is the selection of the device(s) and making sure they are fit-for-purpose to program objectives and endpoints. In the ever-evolving drug development landscape, there are numerous devices capable of collecting and providing similar physiological parameters. Therefore, best practice requires matching the study objectives with digital endpoints that can prove the hypothesis as per study design and include the specific functionalities that can generate those specific digital endpoints.

Additionally, leveraging therapeutic experts' insights during trial planning can help determine what device(s) may work best for specific patient populations. For example, using impulse oscillometry technique or cough monitors for respiratory diseases may seem attractive. However, we must be aware of the regulatory acceptance of the data, given most data is under research, and currently, no acceptable digital endpoint exists. In this case, sponsors of respiratory disease trials are advised to rely on established spirometry data for pulmonary function testing but to keep up with newer technologies coming to market so they may be used, as appropriate, when acceptable digital endpoints are

FIGURE 1




available.

Another example is choosing devices that have blood pressure cuffs appropriate for a wide range of arm circumferences possible in various patient populations. Blood pressure readings can be grossly erroneous if inappropriate cuff sizes are used.

Further, deriving the target quantity of data needed along with the timeframe and frequency of data collection is imperative for both the digital endpoint strategy and labelling needs for marketing.

## MANUFACTURER CHECKLIST

When the endpoint strategy is finalized, the risk-based qualification of the device manufacturer is the next step in the journey. Sponsors should consider the checklist below to ensure their partner manufacturer can fulfill end-to-end needs for the trial's duration:

- Review the manufacturer's data security, privacy, scalability capabilities, and financial standing. This includes knowing whether local to global reach and services are available, including device shipping to and from sites and patients.
- Ability of its team to participate in training in appropriate disposal of devices and their parts (eg, batteries) or medical waste. It is also key for patients and sites to be trained in this for process compliance.
- Provision of mobile phones and laptops along with internet connectivity and managing devices remotely, including remote technical troubleshooting and coordinating software updates.

Specifically, regarding device shipping and logistics, while connectivity for individuals worldwide is much improved in recent years, making sure medical devices reach end users as planned and within timelines in multiple countries has its own challenges. Among other issues, this means accounting for variations in customs, import requirements, device approvals, and appropriate labelling of devices.

The sponsor's shipment oversight partner will need to have strong and detailed global kitting and supplying/re-supplying capabilities to meet start-up timelines and needs throughout the trial. Consolidating all necessary materials, such as investigational product(s), device(s), and training instructions and content, within one or few shipments can improve the patient experience and burden.

## EFFECTIVE DATA GENERATION & COLLECTION

Given the continuous data monitoring and collection coming in from DHTs, sponsors can gather numerous insights from one device. When considering hundreds to thousands of patients sharing data via a DHT in one study, sponsors have potential to gather millions of data points. This can be an exciting opportunity for diving deep into patient experiences. However, there is an acute need to balance actions between digital data collection and ensuring patient safety remotely, and again, obtaining adequate data quantity to meet the study objectives.

The main factors affecting successful and quality data collection without compromising patient safety include the

following:

**Purposeful DHT Integration:** While there are many devices in use that have endpoints cleared in one or two countries, as the industry strengthens its commitment to improving access to trials to traditional underserved patient populations globally, closely evaluating the device from a patient's perspective will be critical. This includes gauging ease of use, local language capabilities, accommodating internet connectivity challenges and more.

**Ease of Data Transfer:** Along with ease of use, ensuring the device design allows patients to transfer data to central software or database with minimum effort will be beneficial to collecting insights as needed. As device functions are interactive and straightforward, the likelihood of securing adequate quality data will increase.

**Enhanced Passive Data Collection:** To further reduce patient burden, a shift to passive data collection can be critical. It is vital to think about trial participants' physical and mental condition, ages and ability to comprehend the requirements of data collection.

For example, if participating in an oncology study in which enrolled patients' life expectancy is less than 1 year, sponsors and CRO partners must consider the patients' perspective and emotional mindset before adopting a DHT that requires the patient to manually collect multiple spirometry records on a regular basis. Or, if considering pediatric patients, explaining the appropriate spirometry device usage and technique(s) and ensuring children follow instructions can be difficult, so reduced compliance is a distinct potential. For children, alternative technologies, such as forced-oscillation technique, which requires the young participant to breathe normally, should be considered. While this is attractive, the outputs are yet to be cleared from a regulatory perspective. The technology is being widely used in clinical practice to facilitate better insights for physicians; however, use in clinical trials for primary or secondary endpoints is approximately a year or more away.

Looking ahead, sponsors and CRO partners are moving toward creating device-agnostic software with integration capabilities that enable electronic source (eSource). This capability can help eliminate manual data entry and transcription and traditional methods of source data verification. It can also help reduce administrative burdens on site teams and patients, who will not have to remember multiple usernames and passwords to access DHT software.

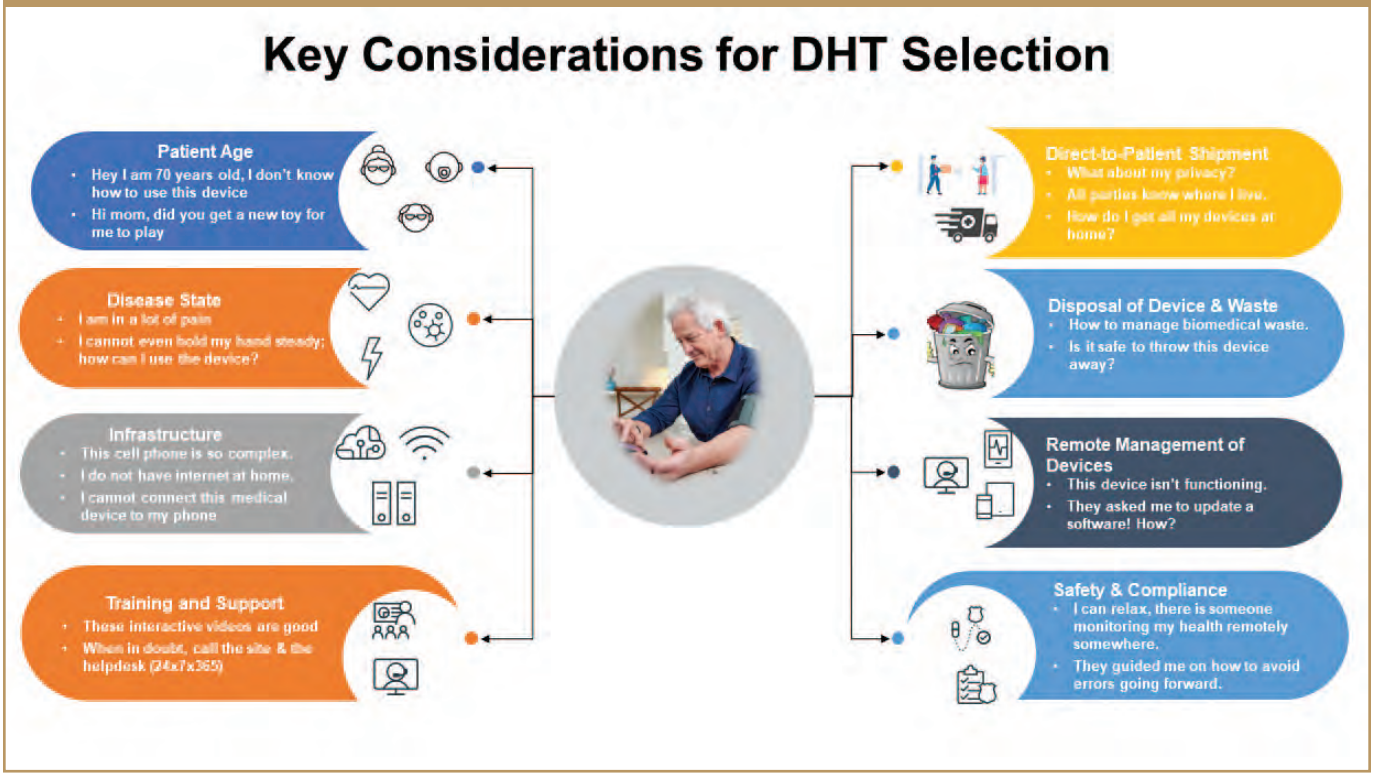
## TRAINING & SUPPORT

Another integral component to an effective digital endpoint strategy is adequate training and support services to ensure proper DHT use and data collection. Some key aspects to consider include the following:

- Training will need to be timely and as early in the process as possible but not so early that the end user forgets it. Challenges during use and execution are to be expected but timing of problems cannot be anticipated.
- Tailored and adequate training materials should be made available to users anytime via the applications and other systems.
- Performing psychometric analysis of the material provided can help gauge how well the end users understand what is needed of them and the importance of being compliant. This is still a new and evolving methodology but has demonstrated benefits to sponsors.
- Efficient 24/7/365 local language support to sites and patients. Having a support team for patients must be the collective goal of the sponsor, CRO partner and site team. However, this is not without challenges.

For example, the US FDA's guidance for assessing pressor effect, which helps gauge how much blood pressure is increased due to treatment, notes that cardiovascular risk evaluation of any drug administered for more than 12 weeks is imperative. During execution, recording ambulatory blood pressure data for a finite period or during visits is important, and the devices are blinded to the subjects. The variance window to repeat these measurements is also limited. This means efficient collection of requisite data must be conducted correctly the first time, and as such, training and ongoing support for patients and site teams is necessary. Appropriate education may include hands-on training and simulations in front of expert technicians, easy-to-digest instructional videos, and other assets. This can help participants feel comfortable using the device on their own and ensure any potential stress is curbed to not alter blood pressure measurements. On the other side, making sure clinicians are equipped with near real-time feedback through compliance reports upon data upload helps ensure details about patient errors are promptly addressed with re-training, etc.

FIGURE 2




## MANAGING PATIENT SAFETY

As we transition from sites to patients' homes, monitoring patient safety and medical management is a challenge and a new opportunity. While data can be made available in near real-time, sponsors and study teams have an opportunity to better manage the patient's condition that was not available prior to DHTs. However, it is vital sponsors and CRO partners thoroughly walk-through concerns regarding data privacy, making sure patients' identifiable information is secure per regulatory requirements and Good Clinical Practice guidelines.

Developing key metrics early in trial planning and design can help ensure adequate support for patients, including asking such questions as:

- What is the threshold of acceptable data quantity?
- What are specific parameter ranges that should trigger a patient health review either remotely or in person?
- What are the appropriate compliance metrics to guide study teams and help identify re-training needs for patients based on trends of data collected during the trial?

## HOLISTIC DATA MANAGEMENT

It is not physically feasible to manually perform such tasks as data cleaning, aggregation, and analysis effectively in trials with digital endpoints, given the vast amount of data being collected. As such, sponsors may consider the tips below for a holistic approach to data management and oversight:

**Data Cleaning:** Build in automatic edit checks at multiple levels to receive clean data right from the beginning and include:

- Data entry by site (for first time), and thereafter, patient from their home.
- Demographic discrepancy edit checks (per device used). For example, edit checks for absurd values from devices being transferred, subject's age, gender, race, etc., that need to be collected as per the device needs:
  - For electrocardiogram data, age and gender are key.
  - For blood pressure data, age, gender and dominant hand are key.
  - For spirometry data, age, gender and environmental factors are key.
  - Provision to share immediate feedback to patients and site teams on compliance (data quantity and quality) upon data upload.

**Data Aggregation:** Time-synchronized data aggregation for patients using multiple devices and across different geographies can be leveraged to derive succinct data covering evaluation and event management according to time zones and daylight savings time. Data conversion into a standardized format compatible with downstream needs related to derive meaningful outcomes is an important and complex aspect to consider. For example,

this is key when there is a need to combine the dose administration time with before and after spirometry data. Accurately collecting and combining data from multiple sources is extremely important.

**Data Analysis:** Lastly, statistical analysis that is capable of proving the study hypothesis and outcome is essential. This can run smoothly if the data pertaining to the objectives is accurate and complete. For use cases related to algorithm development or using an existing algorithm for the first time on a compound, interim evaluation of effectiveness of developed algorithms is necessary to identify issues early and either course correct or shelve the project with limited loss.

## SUMMARY

As previously noted, enhancing the patient experience and reducing burden is key in today's drug development landscape, and DHTs can certainly play a positive role in that. However, there is quite a lot for sponsors and related stakeholders to consider when determining whether it may be beneficial to integrate DHTs into trial design to better meet the needs of the specific patient population while maintaining their safety and upholding data integrity. When thoroughly considering varying nuances for optimal trial design with digital endpoints, it is possible to successfully plan for and execute clinical trials using DHTs, allowing sponsors to make more informed decisions for the patients they aim to serve. ◆

## BIOGRAPHIES

**Dr. Tapan Raval** is the Director, Medical Devices Science and Technology, Strategic Solutions, Connected Devices at IQVIA. With a medical and clinical research education background, he has nearly 2 decades of experience working in the CRO industry. He has helped oversee centralized cardiac safety operations and data management as well as create end-to-end solutions for using data generated by medical devices as part of clinical trial safety and efficacy end points.

**Dr. Ganesh Gundi** is the Director, Medical and Lab Operations Connected Devices at IQVIA. He offers more than 15 years of experience with IQVIA, managing clinical trials that have supported more than 150 customers primarily with ECG, Holter, patches, telemetry services, and medical monitoring.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# DRUG DISCOVERY

## Benefits of Acoustic Liquid Handling in Drug Discovery

By: John Fuller, PhD

### INTRODUCTION

The pressure isn't easing to get more done in the laboratory, nor is the push to get results faster. Innovation remains steadfast with 53 FDA-approved novel drugs in 2023.[1] In 2022, 37 novel drugs were FDA approved, with 24 (65%) using an expedited program - underscoring the priority to bring life-saving treatments to market.[2]

Today's drug discovery favors precision drug therapy, pushing complex modalities like combination therapies and biologics, along with cell- and gene-based therapeutics to the forefront. Major advances in areas, such as synthetic biology, advanced disease model creation, and integrated multi-omics-based workflows, continue to evolve our understanding of human pathophysiologies and bring the hope of new treatments to the market with an ever-increasing speed. Aside from being intricate, they are also lengthy processes with greater risks for human error.

There are clear needs in the market for simple but robust solutions that streamline workflows, provide precision and accuracy, and provide a pathway to future workflow expansion as life sciences technologies evolve.

On the heels of the pandemic is an inevitable drop in drug discovery ROI that stands in contrast to the pandemic's height.[3] Though investments remain robust, so is the call to reduce costs.

Supply chain issues and inflation are further straining laboratories. The three-fold pressure of heightened complexity, accelerating pace, and financial strain calls for a revolutionary, economical solution that enables labs to accelerate research efforts and minimize errors.

Automated acoustic liquid dispensing technology is a game changer - whether deployed as a stand-alone instrument, in a scaled-up workstation, or as part of fully integrated systems - when it comes to handling complexity in efficient and cost-effective ways.

In a single primary screening campaign, labs can be required to screen up to 1-3 million molecules to locate potential new medicines. Imagine manually transferring an astounding 500,000 samples each day! That's how many samples can be run on an acoustic liquid handler.[4]

However, with burgeoning advances in protein-target relationships, AI-enabled target-toxicity profiles, polypharmacology, and biomarker-based stratifications, early stage discovery efforts may need to instead profile fewer primary molecules, but test those candidates over a higher number of complex assays and target-agnostic workflows, such as high-throughput transcriptional profiling.


**The Echo 525 Acoustic Liquid Handler from Beckman Coulter Life Sciences for aqueous-based acoustic transfers is designed for biochemical and genomic sample and reagent transfer.**

Acoustic technology is well-poised to accelerate research decisions for new and existing molecules.

## TODAY'S CHALLENGES IN THE DRUG DISCOVERY PROCESS

With drug discovery science increasing in complexity, labs researching new therapies face several common challenges. The first is fixed annual budgets, despite continuously changing research priorities. Additionally, inflationary pressure impacts labor, cost of raw materials, and more - not to mention the cost of laboratory space in tech hubs. This makes it even more critical to ensure no errors are made along the way - something to which tedious manual workflows are prone.

Labs also face time and resource limitations for staff, independent verification, and validation programs that require training and re-training staff. The skilled labor shortage added to the high process standardization required for staff accreditations and certifications creates bottlenecks - particularly for highly manual laboratories.

Variability among human operators overseeing manual processes can complicate standardization, reproducibility, and accreditation. Daunting sample and data management can overwhelm lab staff who rely on manual processes. Laboratories also face the evolving complexity of balancing today's liquid handling needs with tomorrow's unknown demands.

All of these constraints detract from critical work but can be addressed through a trustworthy instrument scaled to laboratory needs while facilitating better data and sample management.

## WHY AUTOMATE LIQUID HANDLING ACOUSTICALLY?

Automated acoustic liquid handling is a synergy of reliable, contact-free instrumentation paired with intuitive software that creates rapid reproducible success, especially beneficial in a race against time. In particular, miniaturized sample management and assay-ready plate creation benefit from the unparalleled accuracy and precision provided by acoustic dispensing.

Lengthy cycle times, physical space constraints, and lack of skilled labor hinder laboratories that rely on manual liquid dispensing, making reproducibility challenging and high-throughput


**The Echo 650 Series Acoustic Liquid Handler series from Beckman Coulter Life Sciences enables assay miniaturization in a broad range of applications.**

screening (HTS) nearly impossible. Some liquid handlers demand repeated cumbersome calibrations be verified on every instrument transferring the reagent. These are limited in volume ranges and workable reagent types and, at best, approximate against inconsistent reagents.

Acoustic liquid handling, on the other hand, deploys sound energy to eject precisely sized droplets from a source onto a microplate, slide, or other surface. Our instrument applies Acoustic Droplet Ejection (ADE) to accurately and rapidly transfer up to 700 drops of fluid per second.[5] The acoustic liquid handler is inherently capable of combinations of various sample types: capable of providing 1-to-1, many-to-1, and 1-to-many, and many-to-many complex combinatorial transfers to either dry or prefilled wells.

The technology has become a mainstay in sample management, HTS, and assay development workflows. Acoustic dispensing is enabling previously unimaginable throughputs in advanced synthetic biology and genomic applications.

Acoustic liquid handlers apply Dynamic Fluid Analysis (DFA) for fluid transfers that simplify experimental setup and enable a higher degree of experimental and workflow flexibility. The instruments determine transfer parameters automatically at runtime for


**Echo acoustic liquid handlers from Beckman Coulter Life Sciences are frequently integrated with robotic platforms such as the pictured Access Laboratory Workstation to readily create assay-ready plates for various workflows in drug discovery.**

a specified fluid set, eliminating the need to adjust transfer parameters. They are versatile enough to work with complex reagents that can vary in fluid properties and are exceedingly accurate due to DFA's ability to adjust the acoustic energy to changing fluid properties.

## HOW ACOUSTIC LIQUID HANDLERS STREAMLINE LAB OPERATIONS

Acoustic liquid handling automation uses substantially fewer materials, reducing laboratory costs through miniaturization and maximized HTS campaigns. It also minimizes human error and risk of handling toxic or hazardous materials.

Increased assay precision reduces replicate requirements, extending samples across a greater number of possible experiments. For example, direct dispensing of cherry-picked samples extends sample life, while acoustically diluting small molecules and biologics generates vitally accurate and reliable concentration information.

With cell-based assays in demand, acoustic liquid handlers offer the ability to readily generate assay-ready plates for testing various samples. Assay-ready plates provide a convenient pause point to synchronize with cell cultures that are ready for plate seeding. Automated cell culture enables large amounts of multiple robust and reliable cell lines for assays, facilitating timelier responses to simultaneous requests from assay-development labs for screening or supply batches.

Acoustic liquid handling automation solutions also facilitate improved data and sample management as workflows become increasingly complex. With better sample traceability, the technology makes feasible identification of many targets through the biotech revolution and numerous combinatorial technologies for compound collection.

## ACOUSTIC LIQUID HANDLING IN ACTION

Evotec, a drug discovery company in France, implemented acoustic liquid handling as part of an integrated system. "In 2020, we prepared around 27 million compounds with our Echo platforms to support the primary assay up to the profiling step," said Marion Stodel, Research Scientist, Sample Management at Evotec.[6]

They achieved the sample management goals of maximizing inventory usage, introduced flexibility of collection assemblies, increased delivery efficiency of assay-ready plates in nano-volumes, and improved data management efforts.

"Echo is the heart of the platform and works with acoustic transfer technology," Stodel continued. "It transfers compounds from compatible source plates to destination plates. The smallest volume that can be transferred is 2.5 nanoliters. Dispensing such a small volume saves expensive compounds and time, as it allows minia-

turization into 1536-well plates."

Acoustic liquid handling saved them costly resources and allowed for miniaturization. The integrated process empowered complete oversight of assay-ready plates from order to delivery, both internally and externally. An integrated Access DRS robotic platform, also available from Beckman Coulter Life Sciences, utilizing our acoustic liquid handlers prepared assay-ready plates and acoustic tube plates for HTS support.

Evotec significantly reduced cycle time, performing intermediary steps. They reaped transformative benefits, such as precise and accurate acoustic dispensing throughout. This saved time from store into plates with cherry pick or dose-response protocols running directly from tubes to destination plate. This also created compounding savings (nanoliter dosage allowed for minimal resource usage) and the flexibility to work with selected collections. It should be noted that while acoustic liquid handling excels at contact-free nanoliter-scale transfers, at Beckman Coulter Life Sciences, we are strong proponents of "right tool for the right job." In that effort, the instrument is complemented well with larger-scale transfers. At Evotec, our Biomek i7 Workstation facilitated transfers from other storage media into compatible labware.[6]

Outside of traditional DMSO-based sample management, high-throughput DNA assembly is another application area that has been transformed by acoustic liquid handling. Codex DNA shares impressive statistics on workflow enhancements made by transforming their lab using acoustic liquid handling.

"Processes that would take 8 hours with traditional liquid handlers now take less than 30 minutes with the Echo Liquid Handler and tipless liquid handling, enabling the construction of DNA variant libraries at unprecedented scale," said John E. Gill, Sr., Director of R&D at Codex DNA.[5] "Moreover, the high accuracy and precision of non-contact acoustic technology transfers ensure that we're delivering every oligonucleotide with utmost confidence."

## LOOKING AHEAD

Automated acoustic liquid handling allows labs focused on drug discovery to be active, agile, and responsive to evolving demands and keep up with the increasingly complex pace of today's laboratory. It also allows researchers to shift their focus from the tedium of the workflow to the research work at hand. As therapeutic technologies and discovery pipelines evolve, scalable platforms like acoustic liquid handling can help labs stay ahead of the curve.

In 2023, the global drug discovery market is estimated to be worth $93.91 billion with a 6.59%(CAGR) from 2023-2028.[7] The tremendous investments and laboratory efforts being made to treat diseases and prolong life, paired with innovative solutions, can rapidly accelerate vital cures for complex, multi-faceted diseases, such as Alzheimer's and cancer.

The drug discovery journey has focused its innovation on speed and accuracy. Critical breakthroughs in liquid handling automation offer a trajectory out of conflicting pressures, making it a practical solution for labs. Though we may not be able to control inflation, we can strategically shorten the time it takes for life-saving drugs to reach patient bedsides.

For those of us with loved ones who have suffered through illnesses, we uniquely know that reducing the time required to discover a cure is priceless. ◆

*NOTE: Echo Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.*

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## BIOGRAPHY


**John Fuller** is the Commercial Product Manager for Echo Instruments at Beckman Coulter Life Sciences. He was previously a Field Applications Scientist for Labcyte. He holds a PhD. from the U.N.T Health Science Center and completed a postdoctoral fellowship at Johns Hopkins University School of Medicine.

# DATA SHARING PLATFORM

## How to Overcome the Challenge of Differing Digital Maturity Levels in Biopharmaceutical Supply Chains

By: David Brick, MS, and Harlan Knapp, MS

### INTRODUCTION

The past decade has brought new challenges to the single-source manufacturing model with the adoption of more flexible manufacturing networks, utilizing in-region capacity through a combination of outsourcing to contract organizations and the deployment of more flexible facilities utilizing single-use technologies.

This evolution toward a network of partnerships between drug sponsors and contract manufacturers has highlighted the challenges of technology transfer, capturing, monitoring, analyzing, and reporting manufacturing process and quality data as it relates to data integration between organizations.

Predictably, data sharing becomes more cumbersome as more partners and technologies are involved – with impacts not just on incidental process data, but also on critical quality and approval data that can impact product release and regulatory filings. As a recent BioPhorum report (Vision for Digital Maturity in the Integration Between Biomanufacturers and Partner Organizations) indicates, companies share data at different levels of digital maturity depending on their specific business priorities and internal digital maturity.[1] Therefore, the efficient and timely delivery of therapeutics with complex manufacturing needs cannot be achieved by simply unifying the entire pharmaceutical landscape within the most advanced digital maturity level. A better approach is to implement a flexible data-sharing platform that can accommodate partners with different data management capabilities.

### FROM SINGLE-SOURCE TO AN AGILE MANUFACTURING NETWORK

The pharmaceutical industry is transitioning to an agile manufacturing network composed of acquisitions, collaborations, and partnerships to support an increasingly complex pipeline from different therapeutic modalities and different geographies. This allows for an agile supply chain with increased capacity for producing pharmaceutical products using partners' existing infrastructure, including CMOs (Contract Manufacturing Organizations), that are more rapidly deployable and cost effective than building new infrastructure optimized for a given modality.

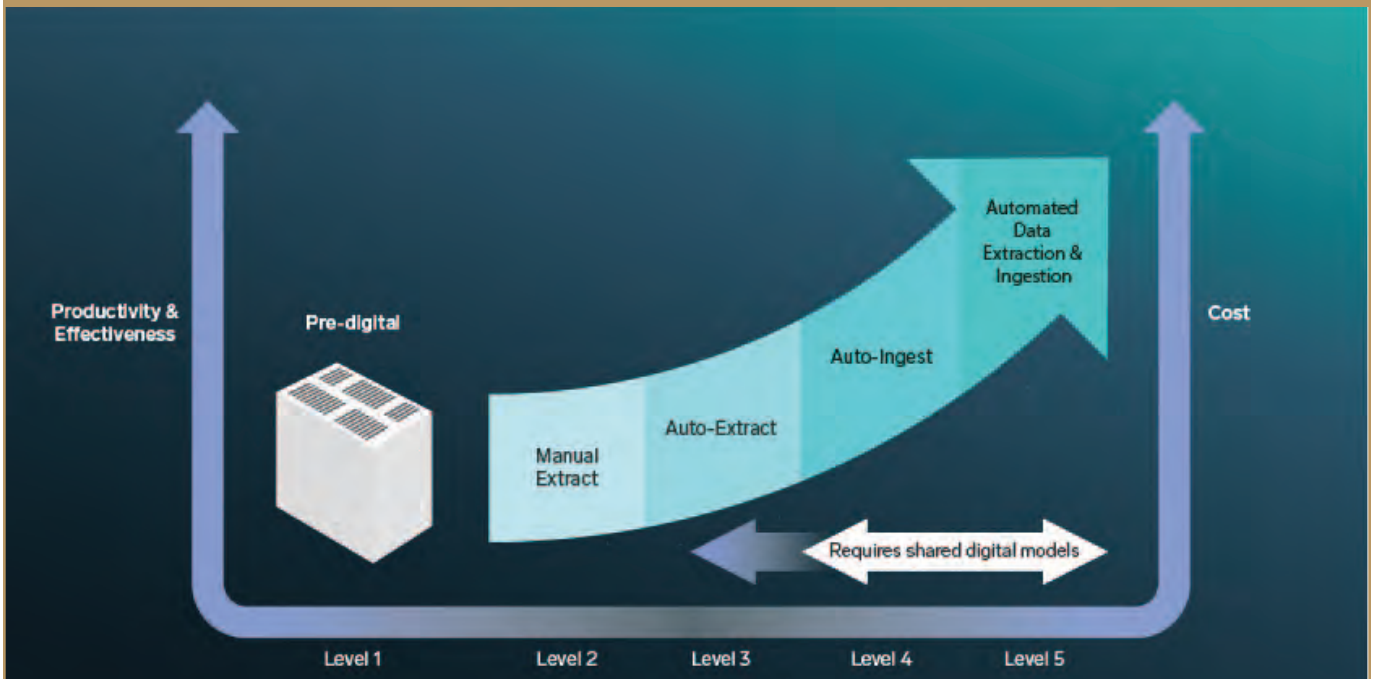
Furthermore, sponsors can improve their global presence and rapidly deliver therapeutics to new markets by leveraging these agile networks, especially in new modalities that require a complex manufacturing supply chain, such as cell and gene therapies.

As therapeutic pipeline complexity continues to increase and multi-regional clinical trials are conducted, sponsors are becoming increasingly reliant on partnerships with multiple CMOs for manufacturing, inventory management, and quality control.

### BIOPHORUM: DIGITAL MATURITY PATTERNS

The new paradigm for pharma collaborations calls for an assessment of data sharing and transfer. To that end, BioPhorum's IT member companies established a Digital Plant Maturity Model (DPMM) to determine the digital maturity with regard to the business capabilities and enabling dimensions of any given facility.

FIGURE 1




**Digital maturity for data sharing between sponsors and contract organizations. Representation of BioPhorum IT's Digital Maturity Patterns (Courtesy of IDBS).**

This was later extrapolated to evaluate the digital maturity for data sharing between sponsors and contract organizations.

The scale consists of five patterns of maturity. Pre-digital communication does not involve integration but rather represents the exchange of physical documents and phone calls or the direct use of a partner's applications. This approach is often preferred by small organizations that lack digital operational systems or for small projects in which digital integration is not cost effective.

The next level is the manual extract, in which data exchange is manually carried out via email with manual export/import of data using Microsoft Excel, CSV/text files, or Portable Document Format (PDF) files.

Automation starts at level 3, also known as auto-extract, in which the contract manufacturer automatically transmits standard data sets periodically from its source systems to their partner while there is still a manual process to consume the data on the sponsor side.

Level 4 "auto-ingest" adds another layer to the system by automating the ingestion of data into the operational systems on the receiving end. Here, data is typically transmitted using machine-friendly data structures, such as XML, and delivered via RESTful API or SQL query access.

Finally, level 5 involves automated data extraction and ingestion in near real-time instead of being timed with batch release or process step progression. Near real-time communication can be beneficial when partners wish to receive instant updates about manufacturing milestones or issues to work in tight partnerships. Additionally, level 5 can include interactive services, such as slot scheduling in personalized therapies outside of sharing process data.

## CURRENT TRENDS & BOTTLENECKS IN DATA SHARING

BioPhorum's Digital Integration of Sponsor and Contract Organizations team surveyed 31 companies consisting of large pharmaceutical sponsors, contractors, and IT support teams. A synopsis of the findings and subsequent survey insight confirms these trends and expectations in terms of digital maturity.

As with the DPMM, there is typically an aspirational level (5), which is not what every organization may strive for. Level 5, real-time data sharing of manufacturing data, is not needed and is not useful in many situations. Instead, auto-ingest (level 4) is currently the main aspiration in terms of quality, supply chain, and manufacturing. Additionally, automation of data exchange is a lower priority for less-frequent activities like Tech Transfer.

Another significant takeaway from BioPhorum's report is mismatched expectations between sponsors and contract organizations due to differences in maturity levels. The biggest mismatch occurs when large BioPharma sponsors desire real-time data from the contractor, whereas the contractor's standard processes only provide batch records in PDF or Excel files for

weekly or monthly submissions. Conversely, a biotechnology start-up with limited IT capability might be collaborating with an experienced and digitalized contract organization.

In both cases, there will be a significant difference between the visibility of and sophistication of process-related data and the ability to reap benefits from data sharing. This disconnect can be exacerbated by the lack of clarity in the contractual framework to convey the sponsor's expectations.

When data sharing is left as a "detail to be worked out later," subsequent unapproved requests from the sponsor, which may seem trivial to a more digitally mature organization, may require partners to revamp their entire process.

Frequently, manual extraction still plays an important part in data exchange. Organizations working with multiple sites, partners, and/or sponsors often use data lakes to aggregate process data. Instead of allowing external parties to directly access this data or the original data sources, which would compromise the confidentiality of other partners or sponsors, they may have to manually extract a modified version of sponsor-specific data.

However, through this process, information exchange is predisposed to cybersecurity risks, human errors, and potential delays. Furthermore, data misinterpretation can happen due to the variances in terminologies used by multiple parties. Ultimately, this could jeopardize data integrity and compliance, affecting the product release timelines and delivery to patients.

Sponsors and partner manufacturers have attempted to remediate this challenge by implementing point solutions, but eventually, these solutions failed due to the complexity of the data shared, challenges in managing, and syncing changes on both sides and lack of scalability. Such point solutions struggle to support a range of digital platforms and siloed manufacturing and lab applications across partner networks.

## SHOULD ORGANIZATIONS STRIVE FOR HIGHER DIGITAL MATURITY LEVELS?

Ideally, all organizations could upgrade their manufacturing systems or deploy novel digital integration tools for a nearly homogenous digital maturity level. This solution is not realistic as it would force organizations to delay deploying capacity while upgrading a facility and incur capital expenditures beyond the value of many projects.

In particular, achieving a digital maturity of level 4 (auto-ingest) and beyond can be costly both in terms of time and capital, especially when both the sponsor and partner must be at the same level of maturity.

Costs aside, an industry-wide upgrade is not necessarily constructive in every situation. Paper batch records and manual review may suffice for infrequent or small batch manufacturing to support early clinical trials. On the sponsor side, new companies with new molecules may receive little benefit from higher levels of automation until their products are produced at large scales. Therefore, aspiring for the most advanced digital maturity level across the entire biopharmaceutical industry could be counterproductive.

The better alternative is to standardize data sharing in a way that accounts for sponsors and partners with varying digital maturity levels.

The first step to standardizing digitized data exchange between partnering organizations is for both parties to align expectations and requirements. The contract agreement must clearly define the scope of data to be included and excluded as well as the expected timeline for data exchange. This may also need to take into account regional differences in data governance as determined by the geographic locations of the parties involved and the intended markets. The GxP compliance requirements must also be established as well as the plan of action in case of revisions to previously shared data.

Addressing the fundamental questions of data management will help ensure the partner organization – whether it is a CDMO for the development of the therapeutic or a CMO for its mass production – will provide the optimum solution for the project proposed by the sponsor while attenuating the process interruptions caused by miscommunication.

## DIGITAL INTEGRATION: AN EFFECTIVE STRATEGY TO BRIDGE THE GAPS BETWEEN VARIED DIGITAL MATURITY LEVELS

For partnerships between organizations with varying maturity levels, shared digital solutions can provide tremendous value.

Cloud-native software that can offer seamless data integration and sharing across the manufacturing network is emerging as a promising solution. Such platforms can enable the partners to log in and enter, upload, or query data within a shared process context, while allowing the receiving end to pull the data directly when needed, maximizing efficiency and transparency.

Cloud-based software can satisfy several principles of digital integration. Data contextualization is a key strength that enables coherent capture of all critical processes and quality data with higher data integrity. Thus, the data is immediately rendered meaningful, scientifically relevant, and interpretable for both the sponsors and manufacturing partners.

Furthermore, contextualized data can easily be converted into analytical output compliant with FDA regulatory requirements, such as Continued Process Verification (CPV) and Annual Product Quality Review (APQR). The ability to track data entry and approvals and add electronic signatures supports data GMP principles and regulatory compliance with 21 CFR Part 11 and Annex 11.

Accommodating varied levels of digital maturity, cloud-based digital integration and data management software can establish streamlined communication between sponsors and their partners, which helps build trust between the organizations and is one of the key ingredients of an agile manufacturing network.

Designed to give visibility to both parties, these systems enable them to jointly optimize manufacturing processes. Improved data digitalization capability also reduces the need for manual activities and removes human errors. In addition, sponsors and manufacturing partners can view process data cooperatively for a systemic evaluation of performance against the quality assurance targets. This allows early prediction of manufacturing pain points and timely intervention to mitigate them, ultimately reducing batch failure and releasing product for patients faster.

Perhaps one of the most noteworthy premises of cloud-based digital integration is its flexibility to accommodate partners regardless of their digital maturity level and data management strategies. CMOs can continue to use their streamlined processes and systems to remain efficient in their manufacturing while replacing manual data file generation, review, and delivery with a focused, purpose-built tool to simplify and speed sharing with their customers.

For the sponsor, applications focused on process context can automatically map the data structures of multiple partners along with internal data to a standard, user-friendly context that can be interpreted by all authorized parties and implemented with minimal training.

Cloud-based data contextualization and management tools will create new possibilities in the biopharmaceutical industry by minimizing hindrances caused by complicated data exchange requirements and facilitating collaboration. Rather than imposing the most advanced digital data-sharing systems on their partners, sponsors must encourage active participation in standardized digital integration. Meanwhile, contract organizations can maintain efficient operations and meet their customers' data needs by supporting standardized digital integration.

The collective efforts from drug sponsors and their various partners will broaden the limits of the pharmaceutical industry and make it adaptable to the emergence of new therapies and health crises requiring the rapid deployment of agile manufacturing networks to efficiently manufacture novel therapeutics and vaccines. ◆

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## BIOGRAPHIES

**David Brick** is Senior Director of Data Services at IDBS and has more than 30 years of experience in consulting, project management, data management, and data warehousing for reporting and analytics applications. He has spent more than 20 of these years focused on pharmaceutical and biotech manufacturing and process development. As part of Skyland Analytics and IDBS, he has been responsible for data management, connectivity, and sharing aspects of PIMS as well as technical implementation project delivery. Prior to joining Skyland Analytics, he served as Director, Professional Services for Dassault Systèmes BIOVIA (and its predecessors Accelrys and Aegis Analytical) where he had responsibility for all implementation activities for Discoverant, the world's leading informatics software for Life Science manufacturers, and for the Nexus data access and aggregation components of the product. His clients included more than 50 process development and manufacturing facilities worldwide. He earned a BS in Applied Mathematics with University Honors and a MS in Statistics from Carnegie Mellon.

**Harlan Knapp** is Head of Digital Manufacturing Solutions at IDBS and drives digital solutions for tech transfer and manufacturing control strategies to help accelerate approvals of therapeutics. He has spent the past 20 years in the biopharmaceutical industry in a variety of roles, including R&D, manufacturing, product development, management, sales, and C-Suite business development. He earned his Master of Science in Biology from Eastern Washington University.

# VACCINE DEVELOPMENT

## A Healthier Global Population Through a Universal Influenza & Multi-Virus Vaccine

By: Jeff Fischer, MBA

### INTRODUCTION

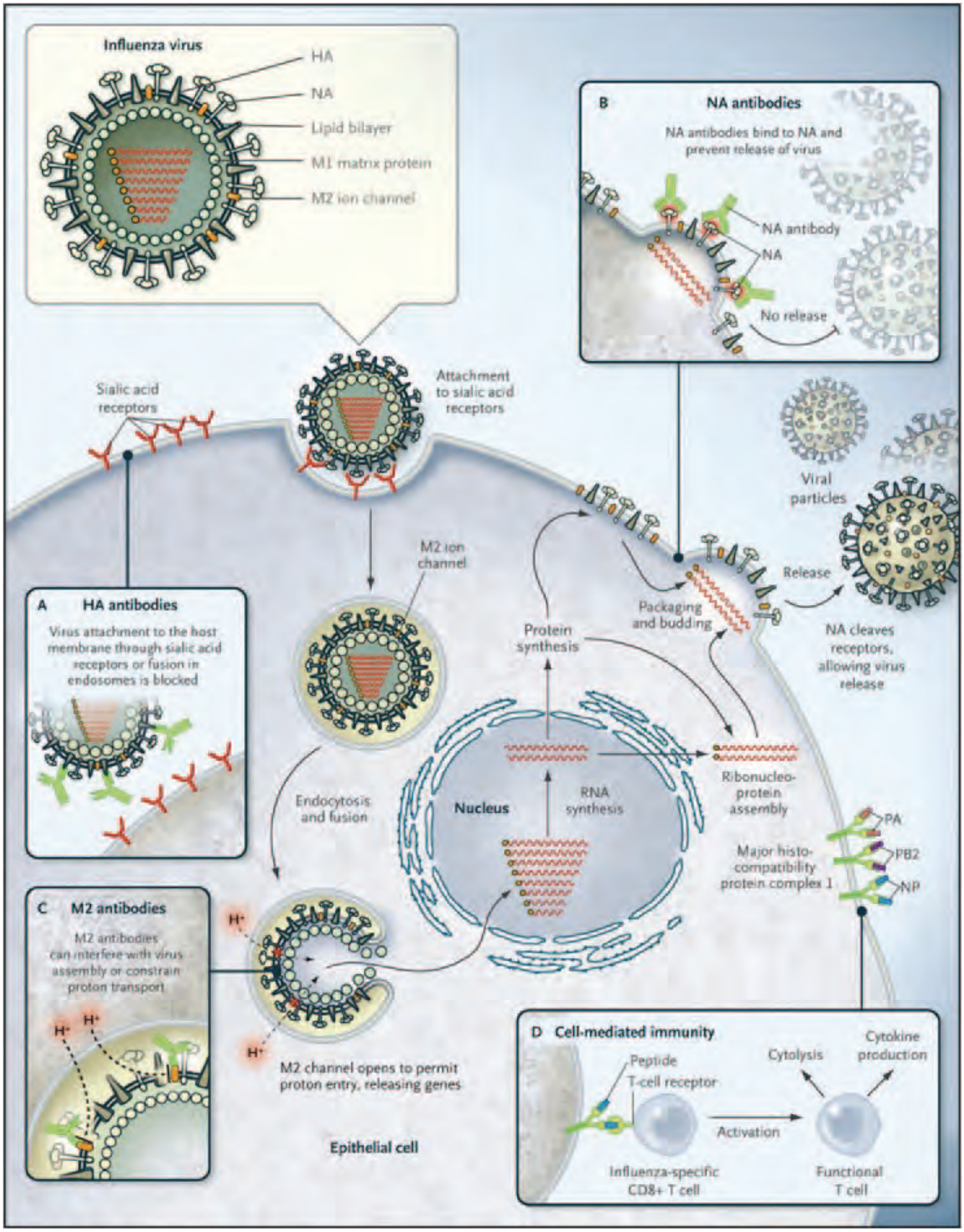
The global vaccine market was put into a tailspin and tested to its limits with the advent of the Covid-19 pandemic. While the world spent almost 3 years grappling with a severe health calamity (and, to an extent, continues to do so), Covid-19 vaccines were quickly developed to combat the high rates of sickness and death. But, we can't forget the infectious diseases that we were facing before the pandemic and continue to contend with today: influenza, RSV, and tuberculosis, among others. All continue to be public health crises. Seasonal influenza alone can cause anywhere between 290,000 to 650,000 global deaths each year.[1] With this reality as backdrop, the US needs to address how the vaccine technology of the future is going to overcome the current challenges of efficacy and durability to ensure we re-establish faith in vaccines and make certain that all members of society have access to protection. More specifically, we need to have an eye toward universal influenza or multi-virus vaccines that have the ability to provide broad coverage as viral strains continue to evolve.

### RECENT GOVERNMENT RESPONSES TO INFECTIOUS DISEASES

In August 2023, the US Government announced a new agency with focus on global health and pandemic prevention: The Bureau of Global Health, Security and Diplomacy.[2] The purpose of this agency is to organize diplomacy, foreign assistance, and national policies to be better prepared for infectious diseases and future pandemics. While the mandate for the agency is quite broad, it's a step forward by the US Government in the aftermath of Covid-19, especially with recent global outbreaks of diseases such as mpox.

When Covid-19 first spread within the US, the government enacted Operation Warp Speed (another ambitious initiative), which was a partnership between the Department of Health and Human Services and the Department of Defense to accelerate the development of a Covid-19 vaccine.[3] To ensure the highest rate of success under Operation Warp Speed, drug companies took steps to set-up large-scale manufacturing while running clinical trials to get quicker vaccine approval from the US FDA. As a result, we saw vaccine approvals for Pfizer, Moderna, and Johnson & Johnson. Today, more than 64% of the global population has been fully vaccinated, with the US donating more COVID-19 vaccines than any other country.[4] With the efficacy data that spans the past 3 years, we have greater insight into what works and what doesn't.

Developing and delivering a vaccine in the middle of pandemic remains challenging. The delay in immunizing the public causes social and economic damage. For viruses such as Covid-19 and influenza, time from development to delivery allows the virus to mutate. Continued morbidity and mortality from reduced efficacy and lack of durability can cause vaccine fatigue to the point where people choose not to get booster shots and additional vaccines — even if they need them.




**Vaccines composed of peptides targeting multiple epitopes produce an immune response that interferes with several stages of the influenza virus life cycle simultaneously such that broad protection can be achieved. The key conserved epitopes produce neutralizing antibodies specifically targeting the ability of influenza virus to attach to host membranes (HA antibodies – Panel A), replicate within cells (M2e antibodies – Panel C), and be released from the host cell (NA antibodies – Panel B). Additionally, T cell activation induces cell-mediated immunity for direct killing of infected cells and further coordination of virus clearance (Panel D).**

## SHINGRIX: A CASE STUDY IN SUCCESSFUL VACCINE DEVELOPMENT

Shingrix, an adjuvated recombinant protein vaccine for shingles developed and sold by GlaxoSmithKline (GSK), replaced Zostavax, a predecessor to Shingrix that was less efficacious and had shorter durability. Zostavax was discontinued in the US 3 years after the launch of Shingrix, which opened the opportunity to target more of the shingles market for GSK.

Unlike Zostavax, which delivered the entire varicella zoster virus, Shingrix delivered a key protein along with a potent liposomal adjuvant. The role of adjuvants is to serve as immune activators, heightening the body's recognition of the vaccine target and facilitating a more robust and longer-lasting protective response.

With such technology behind it, Shingrix revealed itself as a good example of an adjuvated recombinant protein vaccine with characteristics that developers should strive to achieve across other vaccines.

## THE SEARCH FOR BROAD INFLUENZA VACCINES

Scientists in the anti-viral medicine space have worked for decades to find a target within the influenza virus that is conserved and immunologically important across multiple strains to overcome the virus' rapid mutation. The most common focus is on conserved regions of hemagglutinin to prevent binding to the cell surface. The Hemagglutinin Inhibition Assay has been the standard release criteria for the annual influenza vaccine. Another key target has been the matrix protein, a key component in viral replication of the virus. A t-cell epitope is attached to the matrix epitopes to enhance cytokine activity and improve clearance. New interest has developed in the neuraminidase (NA). The NA is important to release the influenza virus from an infected cell. A t-cell epitope is added to this target as well to enhance cytokine activity to improve clearance.

Seasonal vaccines currently offer the best protection against influenza as it is an endemic disease that follows a predictable seasonal pattern: it causes outbreaks during colder months and recedes in warmer seasons. While access to seasonal vaccines is generally good, the issue is that these vaccines tend to lag against the most current influenza strain. The yearly influenza vaccine is designed based on circulating strains from the prior season and circulating strains in the Southern Hemisphere 3-to-6 months prior to influenza season in the US. These predictions aren't always perfect, and the virus can change and mutate quickly, rendering the vaccines less effective. The significant annual variation in efficacy builds a strong case for a universal influenza that would generate consistent efficacy from season to season.

## DEVELOPING A UNIVERSAL INFLUENZA VACCINE

Unlike seasonal influenza vaccines, a universal vaccine doesn't target influenza broadly. The vaccine is targeted to one or more key parts of the virus that are common across strains. By focusing on these more stable regions of important proteins, the vaccine teaches our immune system to recognize and fight against the virus regardless of its unique mutations. Imagine the influenza virus as a puzzle, and the universal vaccine figures out which pieces of the puzzle stay the same. Even if the rest of the puzzle changes, the core pieces stay constant, making it easier for our immune system to identify and fight the virus. The idea is that with a universal influenza vaccine, we wouldn't need a new vaccine every year. It could provide protection against a wide range of influenza strains, including ones that haven't appeared yet.

To move forward, companies like Longhorn are taking different approaches to attack the virus at different stage of its lifecycle: binding and entering the cell, replicating inside the cell, and as it attempts to exit the cell. It is important the vaccine generates both cellular and humoral immunity to ensure longer efficacy. A broad immunological response as provided by t-cell epitopes and adjuvants is important to combat a virus that has demonstrated the ability to continuously evolve and overcome the immune system.

The development process for a universal influenza vaccine doesn't come without challenges. It's important to target more than one area of the virus, so vaccines need to incorporate multiple targets while having anti-viral activity occur both with individual targets and as a complete set. That way, the vaccine can protect against multiple strains of influenza.

To get around this challenge, Longhorn developed a patented composite peptide approach that allows epitopes to be strung together into a unique peptide. The process involves finding conserved epitopes for each target, putting them in a specific order, and including a t-cell epitope to further stimulate the immune system. Longhorn teamed up with the Walter Reed Army Institute of Research (WRAIR), one of the world's foremost developers of adjuvants, to include ALFQ, their novel liposomal adjuvant. ALFQ, a similar but im-

proved formulation of the liposomal adjuvant in Shingrix, has repeatedly demonstrated the ability to generate a robust balanced immune response while significantly reducing reactogenicity. This combined approach also has the potential to evolve into a multi-pathogen vaccine that combines immunity against viruses such as influenza and Covid-19, or other pathogens.

If Longhorn's universal flu vaccine is successful, it could also be used to stop the spread of animal-borne influenza strains. The avian influenza strain H5N1 has been spreading through the US poultry industry throughout the past year, which has caused food prices to rise and increased the risk that a new pandemic virus could emerge. While currently the virus does not spread from human-to-human efficiently, an intermediate host such as pigs could allow the virus to mutate and create a novel virus. Pigs are the most likely source of new pandemic influenza strains because they are naturally infected with human and avian influenza strains.

Adjuvanted recombinant protein vaccines have decades of safety data, are easily manufactured, and are well accepted by the general population, making them ideal for large populations. Demonstrating high efficacy and long-term durability is far from certain; however, the potential benefits of enhanced immunogenicity, cross-protection potential, and improved safety profiles are compelling. As research and development efforts continue, these vaccines could play a pivotal role in reshaping the way we combat influenza through a universal vaccine, and other infectious diseases through a multi-pathogen vaccine, ultimately contributing to a healthier global population. ◆

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**Jeff Fischer** is the President of Longhorn and is responsible for oversight of all non-scientific aspects of the organization to include regulatory affairs. He co-founded the company and has served as its Chief Financial Officer from 2007-2017. From 1998-2005, he served as an Executive Vice President and CFO in the biotechnology industry. He is a former infantry officer in the United States Marine Corps and earned his MBA from the University of Texas at Austin.

# THERAPEUTIC FOCUS

# TTHX1114: Improving Outcomes for High-Risk Patients After Cataract Surgery

By: David Eveleth, PhD

## INTRODUCTION

Cataracts are one of the most common ailments of old age.[1] The condition, which is caused by the degradation of proteins in the lens of our eyes, creates a progressively opaque mass in the center of a person's vision, blurring and obscuring vision. Without proper treatment, it can lead to major problems with sight: In 2020, an estimated 15 million cases of blindness worldwide were attributed to the disorder. The prevalence of cataracts is similar around the world, but the majority of blindness occurs in low- and middle-income countries, primarily due to limited access to quality eye care services and a shortage of trained professionals.[2]

Fortunately, in areas with access to modern ophthalmic care, treatment for cataracts is relatively routine. A proven and straightforward surgical procedure can remove the damaged lens and replace it with a new one, restoring sight in most individuals. In the US, more than 4 million people undergo this operation annually with remarkable success. While the surgery is generally safe and effective, there are still certain risks associated with the procedure, particularly in patients with pre-existing ocular conditions, advanced age, and systemic diseases like diabetes.[3]

## POST-SURGICAL COMPLICATIONS IN HIGH-RISK CATARACT PATIENTS

In high-risk cases, surgery may replace one problem with another as oxidative stress from the surgical procedure damages sensitive endothelial cells that line the back surface of the cornea. Even under ideal conditions, these cells can be damaged and slowly recover, but in people with pre-existing corneal disease or advanced cataracts, the insult to the cornea's endothelial layer from surgery is larger than usual, leading to corneal edema that can defocus light coming into the eye. This creates visual impairment that may lead to long-term follow-up treatment and prolonged compromise of daily activities.

High-risk patients face the risk of corneal endothelial damage via ferroptosis, a process that triggers cell death.[4] When sensitive eye tissues are disturbed during cataract surgery, reactive oxygen species (ROS) accumulate nearby, oxidizing the lipid membranes of corneal cells and triggering the release of iron inside the cell. This phenomenon hinders the cells' ability to neutralize oxidative molecules, while allowing excess iron to accumulate inside of them. The resulting biochemical reactions can damage or kill the cell.

If edema associated with ferroptosis occurs after cataract surgery, the consequences can include increased inflammation, delayed wound healing, and compromised visual outcomes. These side-effects can prolong recovery time and potentially affect the overall success of the surgery. Fixing the problem permanently often requires long-term follow-up treatments, or in a worst-case scenario, a corneal transplant, requiring patients take immunosuppressant drugs for the rest of their lives.

## REASONS FOR DELAYED HEALING AFTER HIGH-RISK CATARACT SURGERY

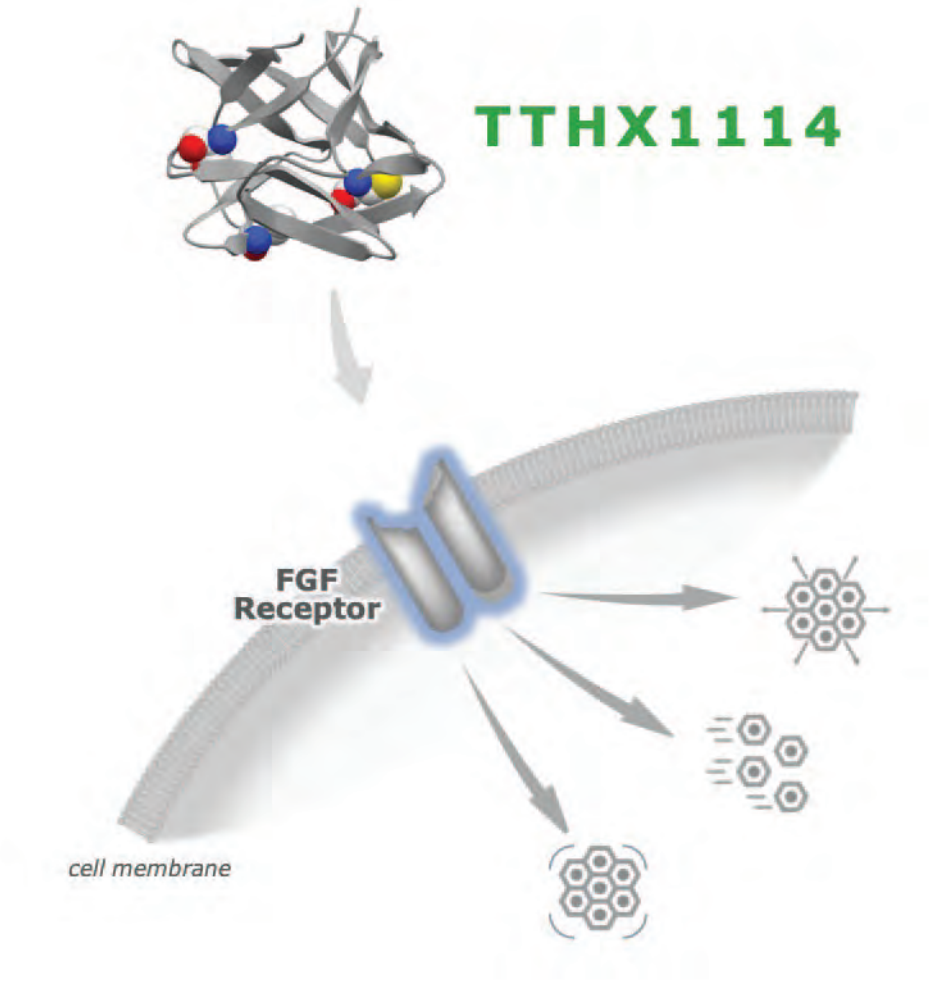
Under ideal conditions, the body initiates a healing response immediately after tissue damage, releasing a molecule called Fibroblast Growth Factor 1 (FGF1). This molecule, also called acidic fibroblast growth factor (aFGF), stimulates the proliferation and migration of various cell types involved in wound healing, such as endothelial cells, keratocytes, and epithelial cells.

FGF1 can also modulate the inflammatory response in the

cornea, influencing immune cells like macrophages and modulating their production of cytokines and growth factors. This modulation ensures a controlled and balanced healing response. Through these mechanisms, FGF1 orchestrates the regeneration and restoration of corneal tissue following injury or surgery, promoting wound closure, re-endothelialization, and the formation of a functional corneal structure.

Unfortunately, several factors complicate corneal healing. Production of FGF1 in the eye goes relatively dormant after infancy, leaving low levels of the protein available in adult human aqueous humor, the fluid that bathes and supports the cornea. Native molecules of FGF1 are also highly unstable and have a short duration of biological activity. When oxidized, the proteins quickly unfold, losing their shape and their ability to bind with receptors on the surface of corneal cells. In cases where endothelial cells are already weakened by pre-existing conditions like Fuchs' dystrophy or diabetes (or by excessive ultrasound required to remove advanced cataracts), the cornea is not always able to fully repair tissue damage inflicted by cataract surgery.

Although increasing the amount of FGF1 present in the eye could theoretically improve healing — and indeed, several other groups of researchers have explored therapeutic versions of the protein for use in dermal wounds — these efforts have been stymied by issues with the molecule's stability. Given its propensity to break down, using the molecule to promote growth in the cornea would require near-daily injections into the eye, a procedure few patients would be willing to tolerate.




## MODIFIED GROWTH FACTOR TTXH1114 CAN ACCELERATE CORNEAL HEALING

Trefoil Therapeutics is actively working toward improving outcomes for patients who experience poor vision because of corneal edema after cataract surgery. Its investigational therapy, TTHX1114, has been developed to stimulate natural cellular processes associated with native FGF1 that accelerate tissue healing and regeneration.

TTHX1114 is a modified version of FGF1 that binds to the same receptors on corneal cells, but has been engineered to be more durable, with enhanced persistence in the eye and improved binding capabilities to cell receptors. By mapping the three-dimensional structure of FGF1, Trefoil's researchers were able to address identified weak points in the molecule, such as amino acids susceptible to oxidation. These amino acids have been replaced with similar structures that are less prone to oxidation. Trefoil has also improved the way the molecule folds, reducing the amount of free space available within its structure. By pushing its amino acids closer together, the protein effectively becomes tighter and more robust.

In addition to these improvements, Trefoil also inserted a new internal bond between two distant parts of the protein chain, locking the structure of TTHX1114 into place so that it cannot unfold. These changes greatly increase the overall stability of the protein, allowing it to remain in the eye long enough for effective receptor binding to create a therapeutic effect.

TTHX1114 retains all the natural functions of FGF1, including binding to tis-

sue, regenerating cells after oxidative stress and other insults, and accelerating healing. Trefoil has demonstrated the potent protective and proliferative effects of TTHX1114 on corneal tissue in preclinical models and in human clinical trials.[5]

## A BRIEF HISTORY OF TTHX1114

The scientific story behind the development of TTHX1114 stems from the exploration of factors that control the division and growth of corneal cells. Trefoil Therapeutics' researchers built upon a large body of work that had established FGF1 as a critical factor in early development of the eye and cornea, setting up the pattern and promoting growth in the cornea and the eye overall.

Trefoil researchers sought to replicate this developmental environment in adult corneal tissue to promote growth, division, and tissue healing. FGF appeared to be a logical target in this context. The journey toward TTHX1114 involved building on previous work conducted in the lab of Ralph Bradshaw, a biochemist at the University of California, Irvine, who conducted pioneering work on nerve growth factors. Building from that work, Trefoil co-founder Mike Blaber — who is a former member of Bradshaw's lab and Professor at Florida State University — made significant contributions to understanding the three-dimensional structure of FGF and its receptor binding properties.

In order to produce TTHX1114, Trefoil expresses the protein synthetically in E. coli via modifications in its amino acid sequence. The selection of specific amino acids to change required a comprehensive understanding of their impact on receptor binding and the physical stability of the molecule. Through years of research and experimentation, the team gained insights into altering the stability and affinity of FGF for the receptor. The design process involved careful selection of molecules from a library of variants, with further modifications guided by three-dimensional and free-energy modeling. A key focus was on preventing the unfolding of the protein, as its native structure was crucial for receptor interaction and preventing degradation. By fine-tuning the amino acid sequence, the team at Trefoil aims to ensure that TTHX1114 remains active in the biological environment for a long period of time and effectively interacts with corneal cells to facilitate tissue healing.

## DELIVERY MECHANISMS FOR TTHX1114

Trefoil is currently developing two distinct delivery systems for this promising molecule. The first, an intracameral injection directly into the eye, targets endothelial cells on the back of the cornea, helping to improve recovery after surgeries that disturb or otherwise damage those tissues.

Phase 2 clinical trials of Trefoil's intracameral injection have already shown promising results for patients undergoing a Descemet Stripping Only (DSO) procedure. This procedure is an alternative to corneal transplantation that does not require donor tissue and thus avoids the potential for graft rejection and the need for postoperative immunosuppression. In these cases, a single intracameral application of TTHX1114 led to recovery of vision to 20/40 in a mean time of 4.2 weeks following DSO.[5] Trefoil researchers observed a significant dose-dependent effect of TTHX1114 on both the recovery of good vision and the deturgescence of central corneal thickness (CCT), with median CCT recovered to better than baseline by day 84.[6] Restoration of corneal function and visual recovery indicated treatment with the drug accelerated healing through endothelial cell regeneration and migration.

In addition to its injectable form, Trefoil is also developing a topical solution of TTHX1114 that can treat epithelial cells on the cornea's outer surface. Corneal epithelial defects are a hallmark of several conditions, including Sjogren's syndrome, neurotrophic keratitis, and herpes virus infections.

Activation of herpes viruses, which cause oral and genital sores as well as chickenpox and shingles, for example, can lead to damage in the outer layer of the cornea. Ocular herpes (herpetic keratopathy) can produce corneal ulcers that blur vision and induce intense pain and inflammation — yet there are currently no treatments on the market used specifically for these ulcers.[7] Trefoil's topical formulation of TTHX1114 has been shown to reduce herpetic keratopathy and accelerate corneal ulcer wound healing by stimulating epithelial cell growth in preclinical models.

## SUMMARY

In many cases, high-risk patients have few options for complication-free treatment of cataracts or other degenerative eye disorders. By filling this unmet need, TTHX1114 has the potential to significantly improve vision health, enhance the well-being of high-risk individuals, and alleviate economic burden on healthcare systems and economies. If successful, the drug could make it possible to effectively

manage cataracts and corneal disease even in areas without easy access to surgical intervention — thus reducing visual impairment and restoring the quality of life for millions of individuals worldwide. ◆

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## BIOGRAPHY

**Dr. David Eveleth** is CEO of Trefoil Therapeutics. He has spent nearly 30 years in leadership positions at pharmaceutical and biotechnology companies, with a focus on ophthalmology and neuroscience. He has been involved in or led seven pharmaceutical INDs, the approval of several new drugs, and the approval of two medical devices. Prior to joining Trefoil, Eveleth led the Ophthalmology Medicines Development Group at Pfizer as vice president, a franchise that achieved peak revenue of $1.8 billion, and advised numerous biotechnology companies and venture capital groups. He earned his PhD in Biology from the University of California, Irvine.


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# ENGINEERING BIOLOGY

## Scaling Engineering Biology to Accelerate Advances in Healthcare

By: Raquel Sanches-Kuiper and Matthew Hayes, PhD

### INTRODUCTION

In recent years, significant progress in engineering biology has transformed the world of healthcare, providing researchers with revolutionary biological tools to develop novel therapeutics. From monoclonal antibody therapies — the fastest growing area of biotherapeutics — to mRNA vaccines, CAR-T cell therapies, and drug biosynthesis, engineering biology is providing solutions to some of the biggest challenges in healthcare.

The potential of this transformative technology to support global healthcare challenges was demonstrated during the response to the COVID-19 pandemic, facilitating the rapid production of diagnostic tests, preventative vaccines, and therapies. The pre-existence of biofoundries — facilities containing high-throughput bioengineering and robotic capabilities — enabled engineering biology to be effective during the response to the pandemic, scaling global testing and vaccine manufacture.[1]

In part, due to the sheer scale of resources recruited to tackle the pandemic, vaccine development was incredibly quick compared with the development of traditional vaccine technologies, which on average spans 10 years, accounting for preclinical, Phase 1–3 testing, filing, and registration. In contrast, once the SARS-CoV-2 sequence was released, it only took 11 months for the first COVID-19 vaccine to be approved by regulatory bodies in the UK, US, and Europe.[2]

However, numerous healthcare challenges exist beyond COVID-19. The extensive list of conditions engineering biology is poised to address includes cancer, neurodegenerative diseases, infectious diseases, and many others. But how can we tackle all these challenges at pace and scale when it took the collective efforts of the world's biotechnology and pharmaceutical industries to conquer just one?

While engineering biology has already proven to be transformational to some of healthcare's greatest challenges, we simply don't yet have access to all the tools to benefit from it at the scale required.

### TIME & SCALE IN HEALTHCARE

While the need for speed and scale is obvious in the context of the recent pandemic response, the everyday relevance of these factors is understood acutely within the pharmaceutical industry. Cost, speed, and quality are essential elements for enabling leading pharmaceutical companies to stay ahead of the curve. Each has been improved by applying principles from engineering biology, but there is great potential to further optimize workflows that could, in turn, deliver life-saving treatments to patients faster and at lower prices.[3]

Advances in the treatment of hematologic malignancies represent an example of how improved workflows can impact patient outcomes. Engineering biology played a significant role in the development of CAR-T cell immunotherapy, which uses novel cell therapy and genetic reprogramming methods to genetically alter patients' T cells, reprogramming them to target the patients' tumor cells.[4] The first CAR-T cell therapy was approved for the treatment of previously incurable hematologic cancers in 2017. Six CAR-T cell therapies have since been approved by the US Food and Drug Administration (FDA), costing over $350,000 per infusion — a cost largely associated with laborious and complex

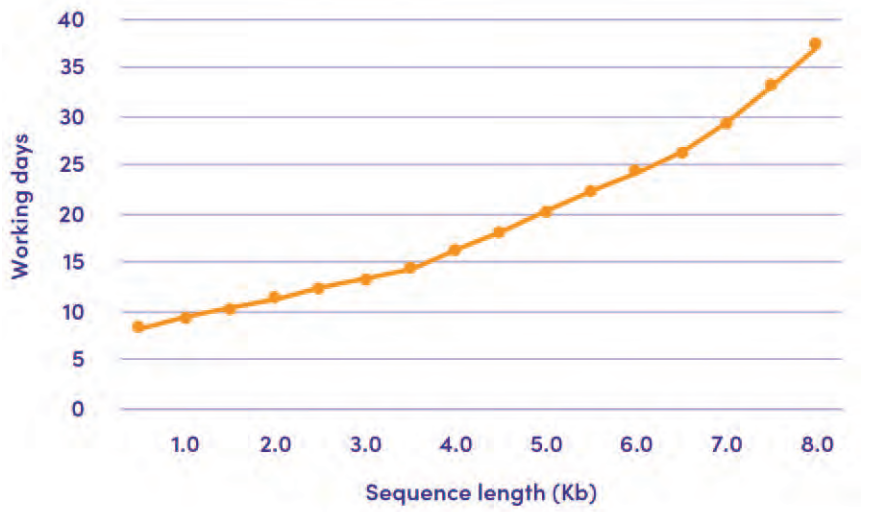
manufacturing processes. Implementing innovative technologies to scale and optimize CAR-T cell production may help improve access and meet the increasing demand for patients with cancer.[5]

## THE NEED FOR IMPROVED GENE SYNTHESIS

Engineering biology workflows follow an iterative Design-Built-Test-Learn (DBTL) methodology. To assess the capabilities of the DBTL cycle, the US Department of Advanced Research Projects Agency administered a "pressure test" to evaluate a biofoundry, where the team was given 90 days to generate organisms that would produce 10 molecules.[6] Despite the identities of the molecules being unknown in advance, 6 out of 10 targets were successfully generated during the performance period. However, sourcing DNA was the major bottleneck, accounting for around half the allotted time.

This bottleneck is poised to become much more acute with the advancement of artificial intelligence (AI) in protein design. Scientists now have access to powerful tools to facilitate the discovery of de novo structures with useful functions, such as improved stability or new catalytic activities.[7] To engineer these functions, specific mutations may be introduced and tested in iterative cycles. Machine learning (ML)-based methods using combinatorial libraries of mutations create opportunities to investigate sequence space more efficiently and screen protein variants rapidly *in silico*.[8] As these programs continue to expand, the need for DNA to iteratively test these sequences and reap their benefits will expand enormously.

**FIGURE 1**




**Time to validate a DNA sequence increases as the length of the DNA product increases.**

## TECHNOLOGICAL CHALLENGES FOR DNA AVAILABILITY

Despite the evident need, increasing access to gene-length DNA isn't straightforward. Numerous innovations in DNA sequencing technology have been made, but the barriers to the synthesis of long and complex DNA sequences at scale need to be overcome to drive engineering biology forward.[9]

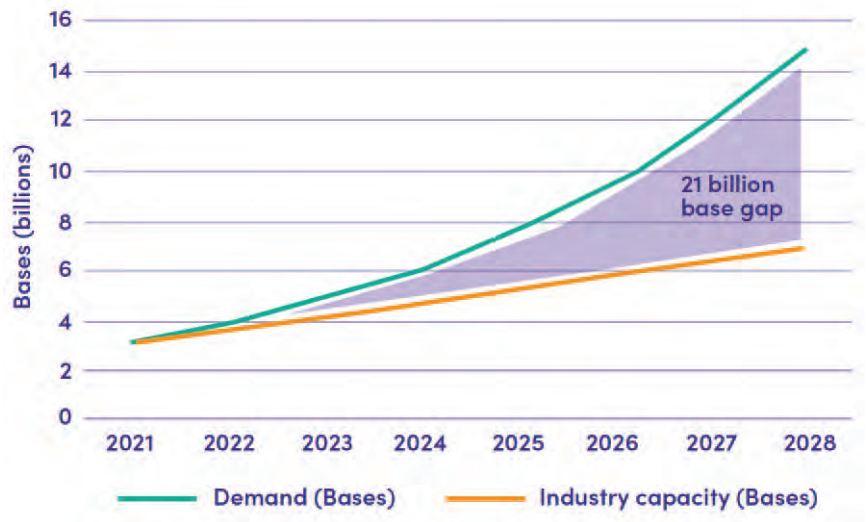
Efficiency of the elongation cycle is one of several factors contributing to the challenge of producing gene-length DNA.[10] With increasing DNA length, the yield of error-free DNA decreases significantly. For example, with an elongation cycle efficiency of 99%, the theoretical yield calculation for an oligo composed of 120 bases is as follows: $(0.99^{120} \times 100\%) = 30\%$.[9] The yield for an oligo of 200 bases, however, decreases to 13%, and increasing the DNA length to 1 kilobase causes the yield to plummet to less than 0.01%.

For sequences longer than 1 kilobase, DNA is assembled from a pool of synthetic oligonucleotides, leading to increased challenges associated with the time and cost of oligo assembly and error correction. This process is error-prone, and validating a sequence becomes more time-consuming as the length of the DNA product increases (Figure 1).

Screening for the correct product generally involves cloning into a vector, transformation into bacteria, isolation of individual clones, and validation of the sequence by Sanger sequencing. This method is simple and efficient for sequences of up to 300 base pairs; however, the probability of error increases significantly with increasing DNA length. Many clones must be generated and sequenced to obtain the correct product, adding to the time and costs required for DNA synthesis.

Current DNA synthesis methods are effective for the synthesis of short DNA sequences, but new technologies are critical to enabling parallel synthesis of many sequences, assembly of gene-length DNA, and error correction via built-in programs.

## FIGURE 2




**As DNA demand accelerates, supply constraints created by centralized services lead to a 21 billion base gap.**

## BENCHTOP GENE SYNTHESIS

The development of benchtop DNA printers represents a new breakthrough in DNA synthesis technologies and a potential solution to the growing demand for this process to become more affordable, flexible, and scalable than is currently available through service labs (Figure 2).[11]

Having an instrument capable of synthesizing and assembling gene-length DNA, correcting errors, and producing large quantities of sequences in parallel will simplify and accelerate each iteration of a complex experiment, improving access to long, accurate DNA sequences and promoting unprecedented speed and control of synthesis. Current benchtop DNA printing is limited to shorter DNA strands. As errors increase exponentially with the length of DNA, developing a benchtop machine to synthesize error-free long DNA requires the re-imagination of DNA synthesis technology.

## THERMAL SEMICONDUCTOR CHIP TECHNOLOGY BRINGING GENE SYNTHESIS TO EVERY LAB

Innovative technologies to address the limitations of current benchtop DNA printing have recently emerged, bringing researchers closer to accessing gene-length, error-free DNA synthesis.

For example, recent microarray formats enable parallel synthesis of sequences at distinct reaction sites, with semiconductor chips greatly increasing multiplexing capabilities for DNA synthesis. Combining highly parallel synthesis on a silicon chip with precise, thermal control of DNA synthesis increases the control and accuracy of DNA synthesis (Figure 3).

Re-engineering DNA synthesis chemistry can allow for selective elongation at specific synthesis sites via temperature-sensitive protecting groups at the sequence termini.[12] After the initiation of an elongation cycle, nucleotides will only be added to chains present at heated reaction sites, enabling precise control over the synthesis of thousands of heterogeneous sequences in parallel.[13]

Methods are also being developed to integrate DNA synthesis with a staged assembly and error-removal process. For example, the Binary Assembly® process joins complementary DNA strands by selective transfer of DNA from synthesis sites to assembly sites on silicon chips.[14] After complementary strands are annealed, the assembly sites are heated to sequence-dependent temperatures that promote rapid dissociation of imperfect matches from the chip, thereby separating and removing error-containing sequences whilst maintaining those with correct homology during the assembly process.[14] This offers significant advantages over conventional approaches by lowering error rates and eliminating time-consuming post-synthesis steps.[12] Duplex DNA fragments can be joined, and the process is repeated to assemble gene-length sequences.

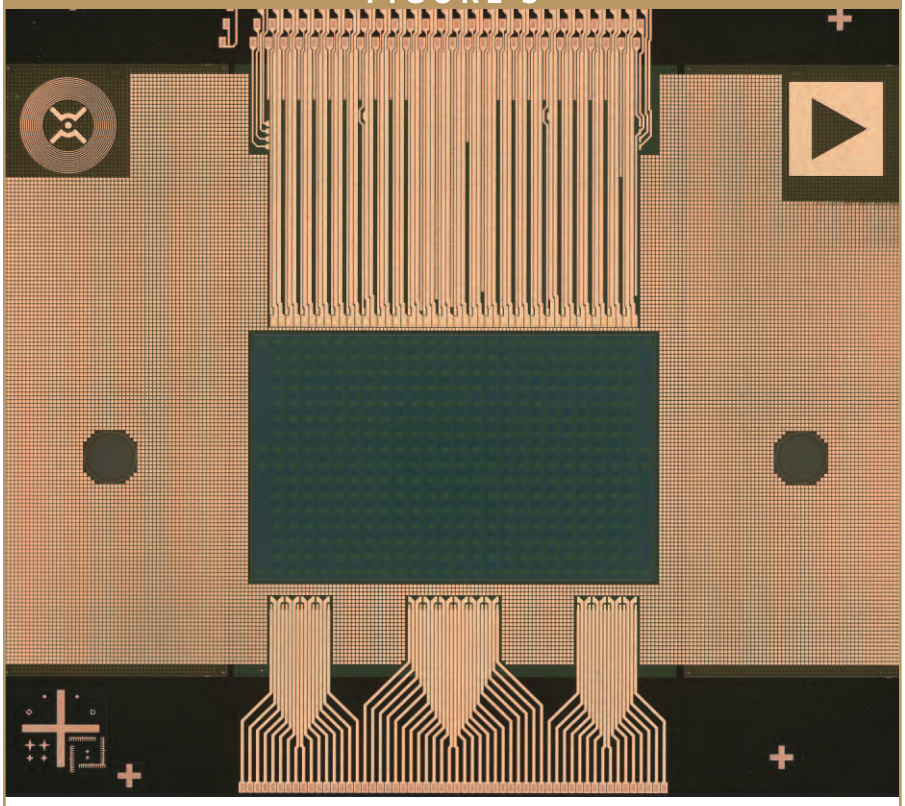
Moreover, thermal control at distinct sites combined with parallel synthesis capabilities may also facilitate the elongation of challenging DNA regions. For example, selective heating can promote the synthesis of DNA segments with high GC content, which have higher melting temperatures and are prone to secondary structure formation.[10]

## PROVIDING A LEAD TIME ADVANTAGE

The integration of thermally controlled semiconductor gene synthesis technology with a built-in error removal process into a benchtop platform will greatly expand the capabilities for the rapid synthesis of accurate gene-length DNA, reducing error rates and eliminating time-consuming post-synthesis steps.

Access to accurate gene-length DNA

## FIGURE 3



**Evonetix has developed a semiconductor chip that combines novel synthesis chemistry with thermal control to enable the synthesis of long, accurate DNA.**

poses a barrier in bioengineering research, as a centralized approach to gene synthesis can limit the DBTL cycle and conventional synthesis technologies are unable to meet the demand for long and complex sequences.

Overcoming such limitations through the development of a benchtop synthesis platform has the potential to expedite the discovery and development time for drugs and biotherapeutics, greatly accelerating the rate at which engineering biology can shape the future of healthcare.◆

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## BIOGRAPHIES

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