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Addressing Bioavailability & Solubility Challenges

The Science & Business of Pharmaceutical and Biological Drug Development

Jim Huang, PhD
Considerations in Development & Manufacturing of Complex Injectables for Early Phase Studies

Cindy Dubin
Improving Bioavailability & Solubility: Each Molecule Is Unique

Aldo Romano
End-to-End Cell & Gene Therapy – From Development to Commercialization – Buy or Build?

IN THIS ISSUE

INTERVIEW WITH PII'S
SENIOR VP, OPERATIONS & STRATEGIC INITIATIVES
SRIDHAR KRISHNAN

PRODUCT APPROVALS 20
Kurt Sedo

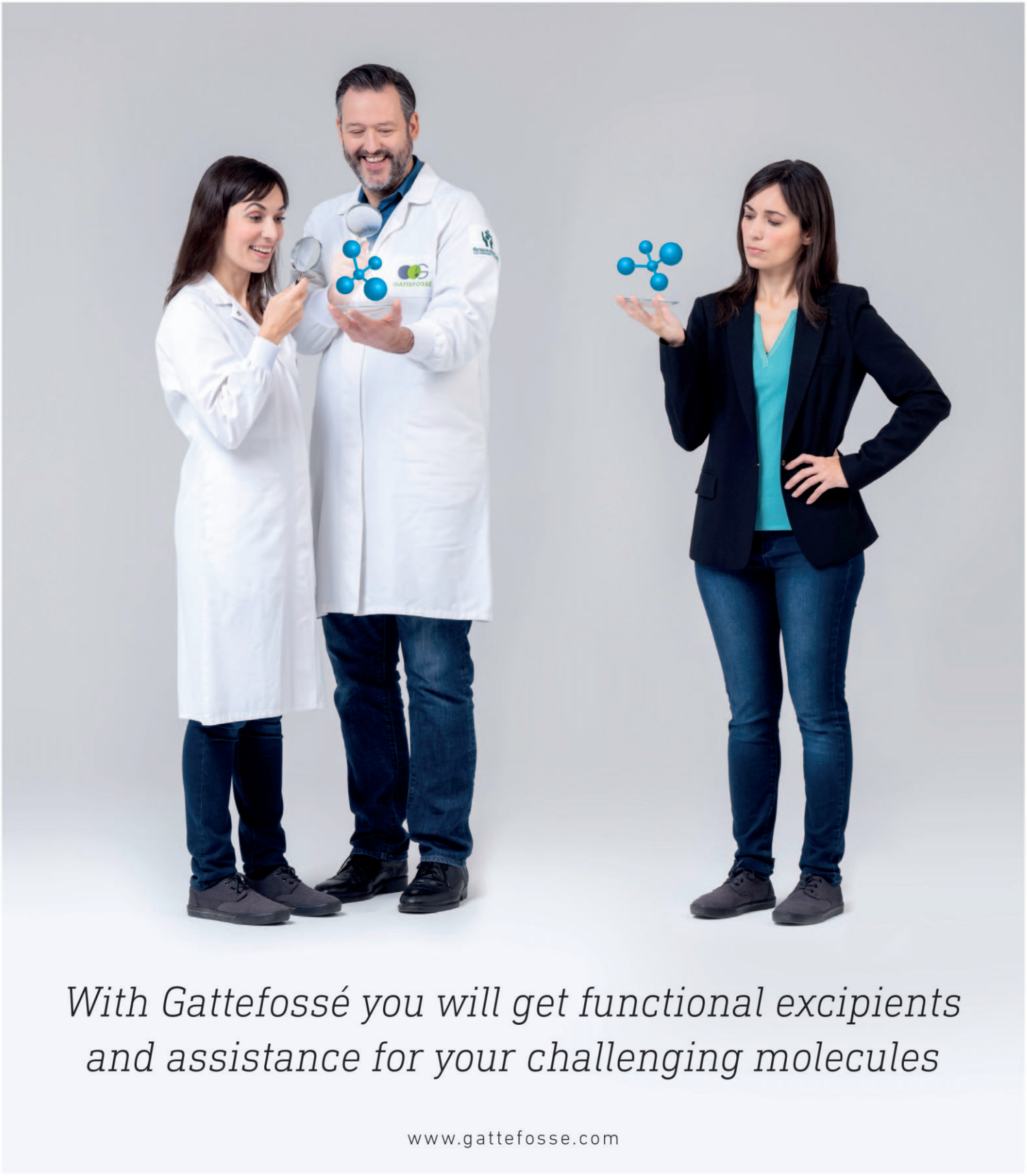
DEVELOPMENTAL DISORDERS 30
Michael Snape, PhD

IMMUNE ACTIVATORS 37
Siddhartha De, PhD
Peter Vanderslice, PhD

MICROFLUIDIC ENCAPSULATION 56
Olivia Merkel, PhD
Christoph Zimmermann, PhD

CDMO/CRO SERVICES 61
Frank Tagliaferri, PhD

DEVELOPMENT STRATEGIES 69
Josef Bossart, PhD



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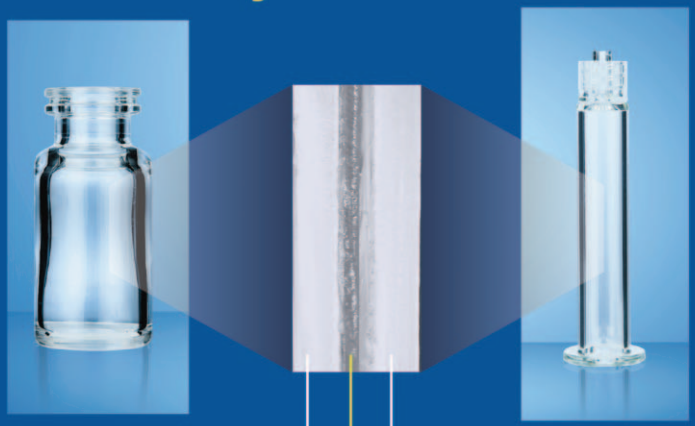

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Real World Challenges
in *Drug Delivery and Formulation*

The Objective

Need to understand the changing dynamics of clinical-stage pipelines by indication, molecule type and most advanced phase to present to senior management

The Challenge

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Buy or Build?

"In the face of these barriers, companies embarking on CGT development must make timely decisions. Do they build or do they buy? Most CGT innovators lack the expertise necessary to build capacity in-house. And most of these organizations must manage their limited capital efficiently. For some of these CGT start-ups, the build pathway is too expensive and too risky."

p.38

Table of Contents

Formulation Forum

16 **Considerations in Development & Manufacturing of Complex Injectables for Early Phase Studies**

Jim Huang, PhD, explains how a niche CDMO, which has specialized technologies in complex injectable development and adopts GMP practice with a "laboratory setting," will have greater flexibility regarding changes, timing, and cost for successful manufacture of complex injectables in early phase development of therapeutic drugs.

Global Report

20 **2020 Global Drug Delivery & Formulation Report: Part 1, a Global Review**

In part 1 of this 4-part series, PharmaCircle, in collaboration with *Drug Development & Delivery*, focuses on a global review of 2020 product approvals.

Cell & Gene Therapy

26 **End-to-End Cell & Gene Therapy – From Development to Commercialization – Buy or Build?**

Aldo Romano and Emily Moran, MBA, discuss how the life science industry must bring technology solutions to the table while at the same time securing the capacity to develop and manufacture groundbreaking cures so patients and society can tap the benefits of cell and gene therapies.

Drug Development

30 **Understanding & Targeting the Mechanism of Action of Developmental Disorders**

Michael Snape, PhD, says when identifying promising therapeutic targets for any disease or condition, including developmental disorders such as Fragile X syndrome, PMS, CDM1, and Rett syndrome, it is critical researchers work to understand the mechanism of disease and disease pathways as well as the unmet need and patient experience.

Executive Interview

34 **Pharmaceutics International, Inc: Getting Back to its Roots**

Sridhar Krishnan, Pii's Senior Vice President, Operations and Strategic Initiatives, discusses how manufacturing is not solely where Pii wanted to be, so the company made a concerted effort to get back to its CDMO roots.

Immune Activators

37 **Enhancing Cell Adhesion to Safely Improve Effectiveness of Vaccines & Cancer Immunotherapies**

Siddhartha De, PhD, and Peter Vanderslice, PhD, present their research on the use of proprietary, orally available compounds that can activate the immune system to enhance the effectiveness of vaccines as well as immuno-oncology therapies for cancer, especially in patient populations that are most vulnerable to disease.

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"The driver for novel strategies to resolve solubility issues, with a target of improving bioavailability, often stems from a requirement to get more information about the key relationships using less API, in a reduced time frame. This has led to the implementation of 'miniaturized' approaches, such as small-scale micro-dissolution testing, working with very small amounts of material and establishing accelerated stability predictive modeling that can shave months off a development timeline."

p.42

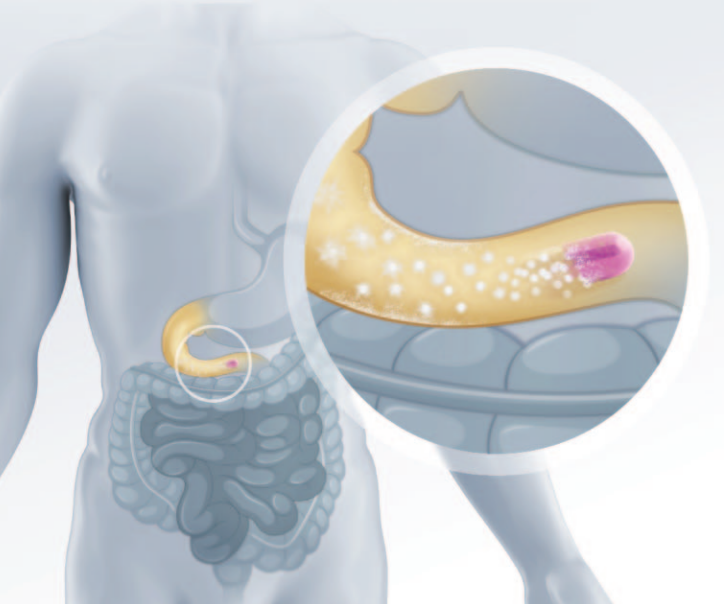

Table of Contents

Special Feature

42 **Improving Bioavailability & Solubility: Each Molecule Is Unique**

Contributor Cindy H. Dubin speaks with several innovative companies to discuss novel approaches to improving bioavailability and solubility that have one commonality: they treat each molecule as an individual.

Microfluidic Encapsulation Technology

56 **Achieving Reliable siRNA Drug Delivery for Inflammatory Diseases & Tumor Targeting by Nanoencapsulation**

Olivia Merkel, PhD, and Christoph Zimmermann, PhD student, discuss the benefits of microfluidic encapsulation technology for gene silencing applications in cancer immunology and inflammatory diseases, where siRNA can potentially be used to downregulate genes associated with these pathologies.

Executive Interview

61 **Pace Analytical® Life Sciences: Delivering Science Better; Advancing Novel Therapies Through the Clinic to Commercialization**

Frank Tagliaferri, PhD, Vice President of Pharmaceutical Development at PLS, discusses his experiences with the company and its recent growth.

Oral Mucosal Immunotherapy

64 **Oral Mucosal Delivery of Allergenic Proteins for Inducing Tolerance in Food Allergic Individuals**

William R. Reisacher, MD, highlights some of the differences between food allergy immunotherapy via the oral mucosal route versus exposure through the stomach and intestines.

Product Development Strategy

69 **ESCP, Estimating Product Performance Part 1 - Playground Physics**

Josef Bossart, PhD, introduces, in a series of short articles, a qualitative model to help understand and visualize the potential of a product with prescribers, patients, and payors. This simple model can help weed out product ideas that may at first glance seem attractive but offer little potential in the real world.

Departments

Market News & Trends.....10
Technology & Services Showcase..... 72

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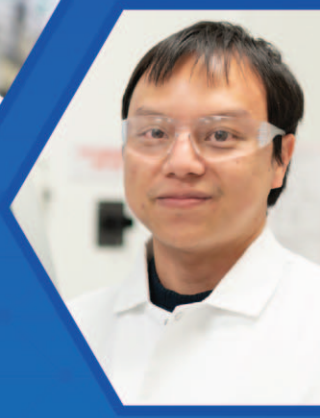

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Biomunex Pharmaceuticals Signs Strategic License & Co-development Agreement With Onward Therapeutics

Biomunex Pharmaceuticals and Onward Therapeutics SA recently announced the signing of a strategic exclusive worldwide license and co-development agreement for a proprietary bispecific antibody program, resulting from Biomunex's proprietary next-generation bi- and multi-specific antibody platform BiXAb.

Under the terms of this agreement, Biomunex will receive an upfront payment for the license deal and may subsequently receive payments upon reaching clinical, regulatory, and commercial milestones, combined with tiered royalties on global net sales. The deal also includes a strategic investment by Onward in the ongoing Biomunex Series A financing round. Other financial details were not disclosed.

Biomunex and Onward will jointly develop, in a preclinical and early clinical program, a "first-in-class" immunotherapeutic bispecific antibody, proprietary to Biomunex, in hematological malignancies. Biomunex will be responsible for the early part of the preclinical development, while Onward will be in charge of the regulatory preclinical activities and subsequently the overall clinical development of the product within various indications and geographical areas.

"This license and co-development agreement for one of Biomunex's proprietary antibodies is a major step forward in the company's development and confirms the positioning of the company as a key player in the immunotherapy field. This demonstrates once again following the 2019 deal with Sanofi, the value of our disruptive BiXAb technology in rapidly and efficiently generating new drugs in immuno-oncology. It also highlights the relevance of our business model, which combines partnerships around BiXAb technology with collaborations and licensing on its proprietary products. With this agreement, the ultimate objective is twofold: to discover and develop innovative bi- and multi-specific antibodies, offering new therapeutic options to patients and to bring an excellent return on investment and a high multiple to our investors", said Dr. Pierre-Emmanuel Gerard, Founder and CEO of Biomunex. "The revenue, as well as the planned Series A fundraising, will allow us to further accelerate our corporate growth and drive our immuno-oncology drug candidates towards clinical development."

"This deal is a great opportunity for Onward Therapeutics in establishing our product portfolio in the exciting field of immuno-oncology," added Dr. C. Grace Yeh, Chairman and CEO of Onward. "Both companies believe that this project ushers in the beginning of a productive collaboration for building innovative and robust pipelines in the future."

Onward Therapeutics is a development stage oncology company, focusing on the identification and development of innovative medicines for the treatment of cancer. The company, led by an experienced team in drug development, adopts a "Buy and Build" business model of acquiring licenses for potential development candidates and investing in their partners with platform technologies

Biomunex Pharmaceuticals is a biopharmaceutical company, based in Paris, France, and Cambridge, MA, focused on the discovery and development of breakthrough immunotherapies using its unique and proprietary BiXAb technology to create next generation bi- and multi-specific antibodies for immuno-oncology. "Plug-and-Play" BiXAb technology produces bi- and multi-specific antibodies, with minimal engineering, from any pair of monoclonal antibodies as building blocks, in a timely and cost-effective manner.

SCYNEXIS Announces Licensing Agreement & Strategic Partnership With Hansoh Pharma

SCYNEXIS, Inc. recently announced it has entered into a licensing agreement and strategic partnership with Hansoh Pharmaceutical Group Company Limited. Under the terms of the agreement, Hansoh will obtain an exclusive license from SCYNEXIS to research, develop, and commercialize ibrexafungerp in the Greater China region.

Ibrexafungerp is a first-in-class, broad-spectrum triterpenoid antifungal agent providing the therapeutic advantages of both intravenous and oral formulations. It is currently under review by the US FDA for the treatment of vaginal yeast infections, and in late-stage development for multiple indications, including life-threatening fungal infections in hospitalized patients.

"We are excited and honored to partner with Hansoh Pharma given their strong experience in the infectious disease space and their exceptional development, manufacturing and commercial capabilities in Greater China," said Marco Taglietti, MD, President and Chief Executive Officer of SCYNEXIS. "This agreement represents a major step forward for ibrexafungerp in the global market, as resistance to azoles grows and deadly fungal infections such as Candida auris continue to emerge worldwide. This partnership not only provides non-dilutive funding to our Company, but also further validates the potential of ibrexafungerp as a global antifungal franchise. We continue to seek other opportunities to monetize our global rights and leverage ibrexafungerp's long-lasting patent exclusivity."

Aifeng Lyu, PhD, President of Hansoh Pharma, added "Antifungal resistance is on the rise, posing a global health threat, and with only three classes of antifungal drugs on the market, we recognize the urgent need for more effective antifungal therapies. We believe in ibrexafungerp's potential to address this need and we are confident that with our integrated R&D, manufacturing and commercial infrastructure, we can make ibrexafungerp a significant commercial success in Greater China. We look forward to working with SCYNEXIS to bring this novel and differentiated antifungal to patients in Greater China."

Under the terms of the agreement, Hansoh shall be responsible for the development, regulatory approval and commercialization of ibrexafungerp in Greater China. SCYNEXIS will receive a $10-million upfront payment and will also be eligible to receive up to $112 million in development and commercial milestones, plus low double-digit royalties on net product sales.

Ibrexafungerp is an investigational antifungal agent and the first representative of a novel class of structurally-distinct glucan synthase inhibitors, triterpenoids. This agent combines the well-established activity of glucan synthase inhibitors with the potential flexibility of having oral and intravenous (IV) formulations. Ibrexafungerp is currently under regulatory review for the treatment of vaginal yeast infection, also known as vulvovaginal candidiasis (VVC), and in late-stage development for multiple indications, including life-threatening fungal infections caused primarily by *Candida* (including C. *auris*) and *Aspergillus* species in hospitalized patients. It has demonstrated broad-spectrum antifungal activity, *in vitro* and *in vivo*, against multidrug-resistant pathogens, including azole- and echinocandin-resistant strains.

Halberd Collaborates With GreenBioAZ for the Development of Radio Frequency Technology to Eliminate Disease Pathogens

Halberd Corporation has engaged GreenBioAZ, Inc. to conduct laboratory testing of Halberd's patent pending Radio Frequency (RF) extracorporeal treatment to eliminate infectious disease pathogens. The methodology entails treating bodily fluids with an Antibody-Metallic Moiety Conjugate (AMMC) which targets a specific pathogen such as SARS-CoV-2 during extracorporeally exposing bodily fluids to a specific Radio Frequency wavelength. This will permit the established frequency of the electromagnetic wave being converted into thermal energy and then transferred to the metallic element of the Antigen-Antibody-Metallic Moiety Conjugate (AAMMC), causing heat eradication of the SARS-CoV-2 viral particles, inside and outside of the infected human cells.

The tests are designed to verify and fine tune the parameters which will safely and effectively destroy the target Antigen Antibody-Metallic Moiety Conjugate without damaging adjacent healthy cells.

Dr. Reyes, Halberd's Chief Technical Officer, stated, "These initial tests will allow us to quickly confirm the efficacy of various components, parameters and the ideal metrics which will lead us to the next level of tests – in vivo testing."

Dr. Shawn Chen, Director of GreenBioAz, Inc., indicated, "We are pleased to collaborate with Halberd in developing a novel treatment to address the global public health threat from COVID-19, as well as other diseases. We hope to develop this new treatment based on our synergistic expertise in antibody development and extracorporeal treatment of diseases."

William A. Hartman, Chairman, President & CEO, stated, "To have Dr. Chen and GreenBioAZ conducting these initial RF tests provides a strong synergy with our work in developing antibodies targeting SARS-COV-2 antigens. These are very important steps in applying our technology to a commercially viable treatment regimen. By conjugating our highly specific antibodies with a metallic moiety, we expect to be able to accurately target the desired viral antigen for elimination through radio waves. If these tests prove to be as successful as we think they will be, and we are able to successfully identify the frequency that is tied to the target antigen, it is theoretically possible to create specific antibody-metallic moiety conjugates to target and eliminate any infectious disease! This process could revolutionize medicine in that we believe there would likely be no adverse side-effects, particularly when conducted extracorporeally."

GreenBioAZ, Inc. is a Biotechnology company using its proprietary technologies to develop monoclonal antibodies and vaccines against infectious diseases and cancer. GreenBioAz is based in Chandler, AZ.

Halberd Corporation is a publicly traded company on the OTC Market, and is in full compliance with OTC Market reporting requirements. Halberd's Articles of Incorporation prohibit the company from issuance of convertible debt which would result in dilution.

Altimmune Announces FDA Clearance of Single-Dose, Needle-Free, Intranasal COVID-19 Vaccine Candidate

Altimmune, Inc. recently announced the US FDA has cleared the company's Investigational New Drug (IND) application for its Phase 1 clinical trial of AdCOVID, a novel, single-dose, intranasal COVID-19 vaccine candidate. Altimmune expects to commence patient enrollment in the Phase 1 clinical trial in the coming week.

"We believe deployment of intranasal vaccines like AdCOVID will be essential to a successful global response to the pandemic," said Vipin K. Garg, President and Chief Executive Officer of Altimmune. "FDA clearance of the IND marks an important step in developing a safe and effective vaccine designed to stimulate mucosal as well as systemic immunity following intranasal administration. Developing vaccines that can effectively prevent transmission is a growing imperative to block the spread of disease and combat the emergence of new variants. We look forward to the data from this trial in the coming weeks."

The Phase 1 clinical trial will evaluate the safety and immunogenicity of AdCOVID in up to 180 healthy adult volunteers between the ages of 18 and 55. Volunteers will receive AdCOVID at one of three dose levels administered as a nasal spray. In addition to the primary study endpoint of safety and tolerability, the immunogenicity of AdCOVID will be evaluated by serum IgG binding and neutralizing antibody titers, mucosal IgA antibody from nasal samples, and T cell responses.

AdCOVID is a single-dose intranasal vaccine candidate for COVID-19. It is designed to stimulate a broad immune response including both systemic immunity (neutralizing antibody) and local immunity (mucosal IgA, resident memory T cells) in the nasal cavity and respiratory tract.

In published preclinical studies conducted in collaboration with the University of Alabama at Birmingham, potent serum-neutralizing antibody responses, T cell responses, and a robust induction in mucosal immunity were observed in mice following a single intranasal dose of AdCOVID. Mucosal immunity was characterized by IgA antibody and resident memory T cell responses in the respiratory tract, both of which are believed to be important in fighting infection, and importantly, transmission.

Based on data from Altimmune's other intranasal platform vaccine candidates, AdCOVID is expected to have extended stability at room temperature that would allow for cold chain-free shipment of the vaccine. If demonstrated, AdCOVID could be stored in the common refrigerators found in community-based doctors' offices and pharmacies for 2 years or more. The company believes these simple and convenient handling requirements, together with the potential ability to block SARS-CoV-2 transmission, could position AdCOVID as a leading intranasal COVID-19 vaccine.

Altimmune is a clinical-stage biopharmaceutical company focused on developing intranasal vaccines, immune modulating therapies, and treatments for liver disease. Our diverse pipeline includes proprietary intranasal vaccines for COVID-19 (AdCOVID), anthrax (NasoShield), and influenza (NasoVAX); an intranasal immune modulating therapeutic for COVID-19 (T-COVID); and next-generation peptide therapeutics for NASH (ALT-801) and chronic hepatitis B (HepTcell).

Samsung Biologics, National OncoVenture & Eutilex Obtains IND Approval From FDA

Samsung Biologics recently announced another successful Investigational New Drug (IND) clearance from the FDA for its client to begin clinical trials on a cancer immunotherapy, furthering the company's track record as a premiere CDO service provider in the global market.

In late 2018, National OncoVenture (NOV), a government-funded virtual oncology drug development program in Korea, approached Samsung Biologics for the development of EU101(NOV1801), an anti-4-1BB therapeutic monoclonal antibody provided by Eutilex (KOSDAQ:263050). Studied by NOV and Eutilex with humanized mice, EU101(NOV1801), effectively eradicated human tumors and showed synergistic anti-tumor activity with immune checkpoint blockade.

"We are extremely glad to be notified of EU101(NOV1801)'s IND approval from the FDA," said John Rim, CEO of Samsung Biologics. "This is our third IND approval demonstrating our unparalleled quality and speed, and as our CDO business continues to expand geographically in the next decade, we look forward to assisting many more clients in bringing their products to market faster and better as the most qualified CDMO partner."

Young-Whan Park, President of NOV, added "In order to conduct the domestic and global clinical trials of EU101(NOV1801) in parallel, we plan to file another IND with the Ministry of Food and Drug Safety in Korea within the first half of this year, working together with Samsung Biologics."

Dr. Soo Young Choi, COO of Eutilex, said "In the US, we will be able to determine the Maximum Tolerated Dose (MTD) and Recommended Phase II Dose (RP2D) through Phase I of clinical trials within the first half of the year."

Samsung Biologics' CDO business has shown rapid growth and success since its business expansion, signing over 60 CDO contracts within two years to obtain three IND approvals by the FDA as well as one Clinical Trial Application (CTA) approval by the EMA. Leveraging Samsung Biologics' extensive expertise for the submission process, the successful IND was achieved with no further significant comments from the regulatory agency.

Samsung Biologics (KRX: 207940.KS) is a fully integrated CDMO offering state-of-the-art contract development, manufacturing, and laboratory testing services. With proven regulatory approvals, the largest capacity, and the fastest throughput, Samsung Biologics is an award-winning partner of choice and is uniquely able to support the development and manufacturing of biologics products at every stage of the process while meeting the evolving needs of biopharmaceutical companies worldwide. For more information, visit www.samsungbiologics.com.

Eutilex is a biotechnology company focused on the research and development of three in-house immunotherapeutic platforms, including innovative T cell receptor (TCR) T cell therapy, chimeric antigen receptor (CAR) T cell therapy and immunomodulatory antibody therapeutics, to help lead the fight against incurable diseases such as cancers. The mission of Eutilex is a dedication to develop and deliver innovative immune-oncology therapeutics to patients in desperate need.

Maravai LifeSciences Expands Its Intellectual Property Portfolio

Maravai LifeSciences, Inc. recently announced the United States Patent and Trademark Office has issued a new patent, United States Patent No. 10,913,768, to the company's TriLink Biotechnologies subsidiary.

The patent covers advancements in TriLink's CleanCap technology for the co-transcriptional capping of messenger RNAs (mRNAs). This patent is the third US patent that has issued to the company related to the CleanCap technology. Capping is an important step in the production of synthetic mRNA, which is used to develop nucleic acid vaccines and therapeutics that deliver instructions to human cells to produce proteins that may prevent or correct disease.

The technology described by United States Patent No. 10,913,768 facilitates the production of mRNAs and provides a significant improvement over legacy co-transcriptional capping methods.

"Capping is a critical process in creating viable mRNA constructs that remain biologically active without eliciting immune responses," explained Mike Houston, PhD, Chief Scientific Officer of Maravai. "By changing the capping approach and streamlining the manufacturing workflow, we deliver a novel solution, whether customers purchase CleanCap in bulk for their own mRNA development programs or utilize mRNA synthesized by TriLink BioTechnologies that already incorporates this novel capping technology."

CleanCap overcomes many drawbacks of existing approaches, enabling highly efficient, reproducible production of capped mRNA. CleanCap technology allows capping to occur in a single reaction, streamlining the manufacturing of mRNA at large scales. Reduced manufacturing time is critical for a number of emerging applications, such as the development of personalized cancer therapeutics and during rapid vaccine responses to pandemics. CleanCap also reduces the cost of mRNA manufacturing, further accelerating the adoption of new mRNA therapeutics.

Maravai is a leading life sciences company providing critical products to enable the development of drug therapies, diagnostics, and novel vaccines o support research on human diseases. Maravai's companies are leaders in providing products and services in the fields of nucleic acid synthesis, bioprocess impurity detection and analysis, and protein labeling and detection to many of the world's leading biopharmaceutical, vaccine, diagnostics, and cell and gene therapy companies.

TriLink BioTechnologies, part of Maravai LifeSciences, is a CDMO helping life science leaders and innovators overcome challenges in the synthesis and scale-up of nucleic acids, NTPs and mRNA capping analogs with scale-up expertise and unique mRNA production capabilities, including its proprietary CleanCap mRNA capping technology. TriLink continues to expand its cGMP and general manufacturing capacity at its new global headquarters to support mRNA, oligonucleotide & DNA plasmid therapeutic, vaccine and diagnostic customers. For more information, visit www.trilinkbiotech.com.

Progenity Initiates Safety & Tolerability Study of its Smart Capsule-Based Oral Drug Delivery System for GI Diseases

Progenity, Inc. recently announced the initiation of a clinical study for their Drug Delivery System (DDS) capsule, an ingestible and self-guided drug delivery device. The study will evaluate the capsule's safety and tolerability in the gastrointestinal (GI) tract of Normal Healthy Volunteers (NHV). The study will also collect the first clinical data on the ability of the DDS to auto-locate and accurately deliver a payload to the colon, a key delivery site for the treatment of ulcerative colitis.

This study will investigate the *in vivo* behavior of the DDS using the well-established method of scintigraphic characterization. Gamma scintigraphy will be used to validate the DDS GI localization as well as the drug delivery accuracy using a saline solution payload that includes radioisotopes. The DDS capsule will be evaluated in a single-dose application to approximately 12 subjects in three separate dosing cohorts. Results of the study are expected in the second quarter of this year.

"This research is a significant step in our efforts toward improving the management of ulcerative colitis, in which therapy is often challenged by the inability to achieve sufficient drug concentrations at the site of disease without incurring dose-limiting side effects," said William Sandborn MD, Chief of the Division of Gastroenterology and Director of the Inflammatory Bowel Disease Center at the University of California San Diego. "Verifying the safety and tolerability of this drug delivery system will allow us to advance this technology to potentially provide a noninvasive, oral solution for safe and effective treatment of this and other GI diseases."

Progenity designed the DDS capsule with the aim of ultimately using it to deliver bolus doses of therapeutic compounds formulated in proprietary solutions at a defined location within the GI tract. If successful, the DDS could be used to transport previously approved therapeutics directly to their intended disease target in the GI tract, thereby improving efficacy through increased localized drug concentration while potentially minimizing harmful side effects associated with systemic drug delivery. This technology offers the potential to improve treatment for patients suffering from conditions, such as ulcerative colitis (UC) and inflammatory bowel disease (IBD). Current drug treatments for these conditions suffer from less than optimal efficacies at safe doses, leading to a loss of response in the majority of patients within the first few years of treatment.

Progenity has two lead drug-device candidates utilizing the DDS technology: PGN-001, a high-concentration, liquid formulation of adalimumab, and PGN-600 a liquid formulation of tofacitinib. Both are under development for the treatment of ulcerative colitis. Using intracecal catheter preclinical colitis models, the company previously observed significant efficacy, as well as both high local tissue drug levels with localized drug delivery and reduced systemic drug exposure, compared to systemic injection.

Cue Biopharma Initiates Patient Dosing in Phase 1 Study of CUE-101 in Combination with KEYTRUDAr as First-line Treatment for HPV+ Recurrent/Metastatic Head & Neck Cancer

Cue Biopharma, Inc. recently announced the first patient was dosed in a Phase 1 dose escalation clinical trial of CUE-101 in combination with Merck's anti-PD-1 therapy, KEYTRUDA (pembrolizumab). CUE-101 is being evaluated in combination with KEYTRUDA as first-line treatment for human papilloma virus positive recurrent/metastatic head and neck squamous cell carcinoma (HPV+ R/M HNSCC).

"We are very pleased to have initiated our combination trial of CUE-101 with KEYTRUDA," said Ken Pienta, MD, Acting Chief Medical Officer of Cue Biopharma. "In our ongoing dose-escalation monotherapy Phase 1 trial, CUE-101 has been well tolerated at doses where we've observed preliminary evidence of clinical activity, and in preclinical studies we've demonstrated that the combination of CUE-101 and checkpoint blockade appear synergistic by significantly extending survival in mouse models of HPV positive cancers. These data taken together support our belief that the combination of CUE-101 with KEYTRUDA has the potential to enhance anti-tumor activity and prolong patient survival."

This Phase 1 dose escalation combination trial (NCT03978689) is being conducted in parallel at the same clinics that are conducting the ongoing Phase 1 monotherapy study of CUE-101. Due to the tolerability profile demonstrated to date in the CUE-101 monotherapy dose-escalation trial, the first dose in the combination arm is 1 mg/kg every 3 weeks (Q3W), which is also the recommended dosing interval for KEYTRUDA.

The CUE-100 series consists of Fc-fusion biologics that incorporate peptide-MHC (pMHC) molecules along with rationally engineered IL-2 molecules. This singular biologic is anticipated to selectively target, activate and expand a robust repertoire of tumor-specific T cells directly in the patient. The binding affinity of IL-2 for its receptor has been deliberately attenuated to achieve preferential selective activation of tumor-specific effector T cells while reducing potential for effects on regulatory T cells (Tregs) or broad systemic activation, potentially mitigating the dose-limiting toxicities associated with current IL-2-based therapies.

The company's Immuno-STAT (Selective Targeting and Alteration of T cells) biologics are designed for targeted modulation of disease-associated T cells in the areas of immuno-oncology and autoimmune disease. Each of our biologic drugs is designed using our proprietary scaffold comprising: 1) a pMHC to provide selectivity through interaction with the T cell receptor (TCR), and 2) a unique co-stimulatory signaling molecule to modulate the activity of the target T cells.

The simultaneous engagement of co-regulatory molecules and pMHC binding mimics the signals delivered by antigen presenting cells (APCs) to T cells during a natural immune response. This design enables Immuno-STAT biologics to engage with the T cell population of interest, resulting in highly targeted T cell modulation. Because our drug candidates are delivered directly in the patient's body (*in vivo*), they are fundamentally different from other T cell therapeutic approaches that require the patients' T cells to be extracted, modified outside the body (*ex vivo*), and reinfused.

OliX Pharmaceuticals Launches New Subsidiary to Develop mRNA Vaccines & Therapeutics

OliX Pharmaceuticals, Inc. recently announced the establishment of mCureX Therapeutics, Inc., a subsidiary focused on the research and development of messenger RNA (mRNA) vaccines and therapeutics.

Based in South Korea, mCureX will initially focus on developing mRNA-based vaccines for human diseases, including COVID-19, as well as animal diseases. mRNA-based vaccines and therapeutics contain the genetic instructions for the body to produce proteins that may stimulate and train the immune system to prevent disease or produce proteins that are deficient within a cell to treat disease. mRNA technology has been used in two COVID-19 vaccines recently granted Emergency Use Authorization by the US FDA.

"We launched mCureX to address a number of health crises, including the global public health crisis of COVID-19, leveraging our expertise in RNA-based drug discovery and development," said Dong Ki Lee, PhD, Founder and Chief Executive Officer of OliX Pharmaceuticals. "mRNA represents a rapidly advancing and nimble platform that complements our existing RNAi drug development platform as we develop therapeutics and vaccines for urgent and unmet medical needs in Korea and beyond."

Dong Won Shin, PhD, Chief Technology Officer of mCureX, will oversee the R&D department. Dr. Shin has over 20 years of experience in mRNA and oligonucleotide chemistry, serving most recently as director of oligonucleotide chemistry at OliX Pharmaceuticals and senior staff scientist at TriLink BioTechnologies. He developed the foundational technology of mRNA 5′-capping, which is used in the COVID-19 mRNA vaccines developed by Pfizer and BioNTech.

The company also announced the addition of Anton McCaffrey, PhD, to mCureX's Scientific Advisory Board, which will provide guidance on the subsidiary's research and development strategy and emerging pipeline. Dr. McCaffrey has over 2 decades of experience in oligonucleotide therapeutic research and development, having served as Senior Director of R&D biology at TriLink BioTechnologies and as assistant professor at the University of Iowa.

OliX Pharmaceuticals is a clinical-stage pharmaceutical company developing therapeutics against a variety of disorders by down-regulating expression of disease-causing genes, based on its own proprietary RNAi technology. The Company's core RNAi platform, asymmetric siRNA (asiRNA), is a unique gene silencing technology based on RNA interference (RNAi), which is considered as the most efficient gene-silencing technology. Based on asiRNA technology, OliX has developed cell-penetrating asiRNA (cp-asiRNA), a therapeutic RNAi platform to effectively target locally administrable diseases, such as hypertrophic scar, dry and wet age-related macular degeneration (AMD), subretinal fibrosis, idiopathic pulmonary fibrosis (IPF), and neuropathic pain. OliX has also developed another therapeutic RNAi platform, GalNAc-asiRNA, to target a variety of liver diseases. For more information, visit https://www.olixpharma.com/eng/.

Formulation Forum

Considerations in Development & Manufacturing of Complex Injectables for Early Phase Studies

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BACKGROUND

A complex injectable is a product that has a complex active ingredient, complex formulation, complex route of delivery, or complex drug device combinations. Complex parenteral products include delivery systems, such as sterile suspensions, liposomes, lipid nanoparticles, emulsions, polymeric microspheres, pellet implants, etc, for administration of drug via the injection, ophthalmic, subcutaneous, implant route, etc. Complex injectables have gained increasing attention due to their advantage in applications in both acute and chronic diseases treatments. Particularly, suspensions and lipid-based nanoparticles are being increasingly utilized due to their ability to increase drug loading to improve bioavailability/stability and to enable long-acting injectable of poorly soluble drugs and biologicals.

However, the development and manufacturing of those injectables is extremely complex, requiring careful design of formulation and process, in-depth characterization of products, confirmation of safety and efficacy per FDA regulation, and high-quality standards in manufacturing, packaging, distribution, and storage.

Production of the first clinical trial materials for a new pharmaceutical dosage form is a significant milestone event in the development of a pharmaceutical product. As a product transitions from pre-clinical development to the clinical development phase, the manufacturing process takes on a much greater role in the overall success of the project. This transition is particularly difficult for emerging pharmaceutical companies whose expertise typically lies in the biology and chemistry, and less on the engineering, regulatory, and quality aspects of manufacturing the drug product. This critical milestone is made even more challenging when the drug product is intended to be a sterile injectable dosage form.

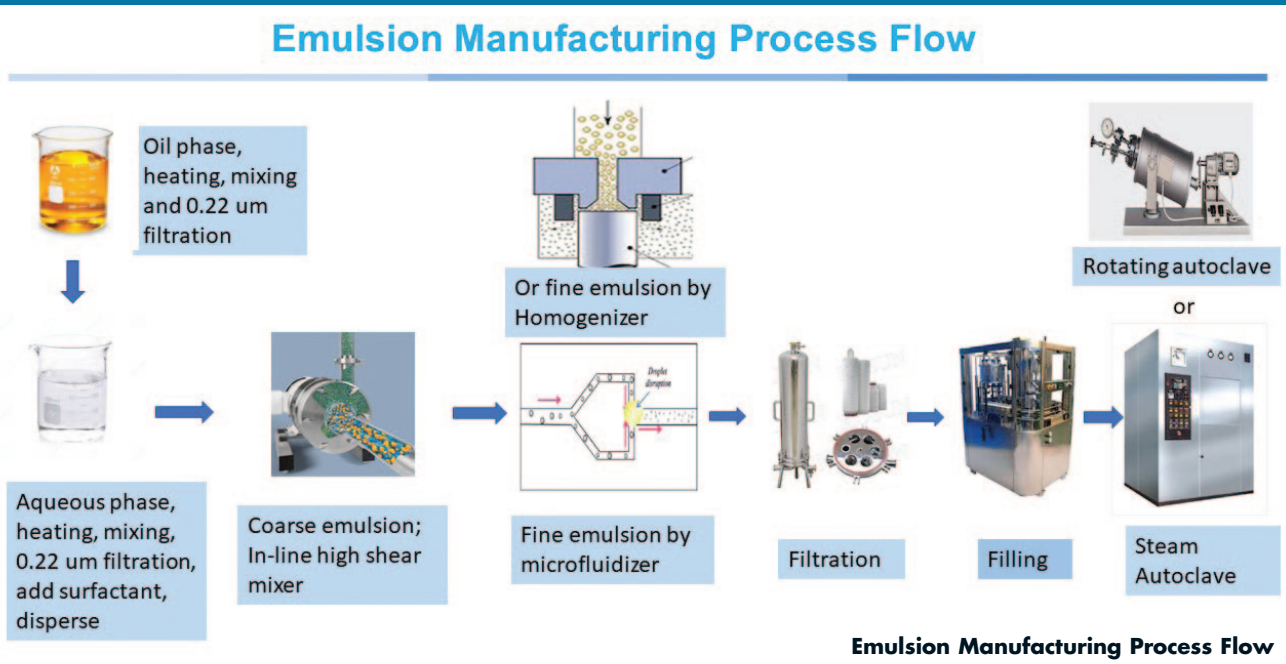
COMPLEX INJECTABLE DOSAGE FORM DEVELOPMENT

Suspensions/Nanosuspensions

The primary consideration for suspensions is physical stability, ie, they tend to settle over time and to change in particle size distribution. Physical stability in suspensions can be controlled by (1) the addition of flocculating agents to enhance particle "dispersability," (2) the addition of viscosity enhancers to reduce the sedimentation rate in the flocculated suspension; and (3) selection of appropriate stabilizer and surfactant. The addition of the flocculating agent, at some critical concentration, negates the surface charge on the suspended particles and allows the formation of floccules, or clusters of particles, that are held loosely together by weak van der Waals forces. Because the particles are linked together only loosely, they will not cake and may be easily re-dispersed by shaking the suspension. Viscosity enhancers are typically hydrocolloids (natural, semisynthetic, or synthetic) used in a concentration that overcomes the suspended particle's tendency to settle.

A couple of methods have been used to prepare parenteral suspensions. First, aseptically combining sterile powder and vehicle involves aseptically dispersing the sterile, milled active ingredient(s) into a sterile vehicle system; aseptically milling the resulting suspension as required, and aseptically filling the milled suspension into suitable containers. Second, in-situ crystal formation by combining sterile solutions. In this method, active ingredient(s) are solubilized in a suitable solvent system, a sterile vehicle system or counter solvent is added that causes the active ingredient to crystallize, the organic solvent is

FIGURE 1



Emulsion Manufacturing Process Flow

aseptically removed, the resulting suspension is aseptically milled as necessary, and then filled into suitable containers. Sterility of those suspensions can be achieved by an aseptic process or by a terminal sterilization if those suspensions have enough thermal or irradiation stability upon the sterilization process.

Lipid-Based Nanoparticles

Lipid-based nanoparticles, such as lipid complexes, lipid nanoparticles, and liposomes, are formed from lipids and other excipients in aqueous medium. Lipid nanoparticles can be produced by dispersion of lipids in water via mechanic energy, such as high-pressure homogenization or microfluidics. This results in the formation of lipid nanoparticles. Further extrusion may be utilized to refine the particle size distribution to a narrower range. Lipid nanoparticles can entrap both hydrophilic and hydrophobic drugs, and drug release can be targeted to specific sites. Typically, the particle sizes of lipid nanoparticles range from 50 to 200 nm. To make lipid nanoparticles suitable for therapeutic applications, their size distribution has to be controlled, which can be realized via mechanic energy or by extrusion membranes with defined pore size. In order for lipid nanoparticles to be processed by 0.22-micron sterile filtration, its particle size distribution has to be well below 200 nm; otherwise, an aseptic process needs to be utilized.

Emulsions/Microemulsions

Emulsions can be characterized as macroemulsions, microemulsions, or nanoemulsions. Macroemulsions are typically opaque in appearance because the average particle size of the hydrophobic droplet in a macroemulsion is typically > 500 nm and thus scatters light. Microemulsions and nanoemulsions are obtained when the size of the droplet is typically in the range of <500 nm. The distinction between microemulsions and nanoemulsions relates to their thermodynamic stability. Microemulsions are thermodynamically stable due to the use of sufficient co-solvents and co-surfactants to prevent Ostwald ripening. Nanoemulsions contain much less of the stabilizing co-solvents and co-surfactants, and as such, are metastable and more susceptible to Ostwald ripening.

The emulsion can be prepared via high-energy and low-energy methods. A high-energy method includes high-pressure homogenization, micro-fluidization, and ultrasonication normally used to make emulsions; whereas low-energy methods, including the phase inversion emulsification method and the self-emulsification method, are utilized to prepare microemulsions. Terminal sterilization will be used if the emulsion has good thermal stability, otherwise aseptic process or filter sterilization will have to be exploited. In order to enable 0.22-micron sterile filtration, the emulsion's particles size distribution needs to be below 200 nm, and its viscosity should be low enough for formulation to flow freely through the filtration membrane.

Polymeric Nano/Microparticles

To realize long-acting attributes, the polymeric macro/nanoparticles can protect the drug from burst release and degradation, thus achieving prolonged drug delivery and a

longer shelf-life. For therapeutic purposes, the most commonly used polymers include polyethylene glycol (PEG), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), alginate, chitosan, and gelatin base. Polymeric microspheres can be developed based on hydrophobic materials that facilitate controlled release of the therapeutic. This is achieved via slow degradation of the microsphere's polymer backbone that subsequently leads to kinetically driven release of the drug. Long-established polymer microspheres that have had significant success are based on incorporation of leuprolide (a testosterone-inhibiting drug) into polylactide (PLA) and polylactide-co-glycolic acid (PLGA) microspheres. Sterility of polymeric microparticles can be achieved via aseptic process or by terminal sterilization if the product has enough thermal or irradiation stability upon the sterilization process.

GMP CONSIDERATIONS OF COMPLEX INJECTABLES

Regulatory Requirements

On September 15, 2008, the FDA made effective an amended rule that applies to small molecule drugs and biologics, including vaccines and gene therapy products. The note in the Federal Register of July 15, 2008 (Volume 73, No. 136) announced an adaptation of 21 CFR 210 and 211: investigational medicinal products solely intended for use in Phase 1 are to be exempted from complying with the "final rule" under FD&C Act 505(i) (21 U.S.C 355(i)). The text stresses that the cGMP requirements of 21 CFR 211 are applicable only to Phase 2 and Phase 3 drugs. This makes a solid rationale for Phase 1 clinical trials production to focus on safety of manufacture rather than qualification of processes at this point of drug development.

FIGURE 2

Ascendia's scientist Jose Parra is making nanoemulsions using a microfluidizer in a cleanroom.

In addition to product quality, sterility and non-pyrogenicity are of utmost importance for Phase 1 sterile manufacture. The injectable product can be terminally sterilized in its packaging or manufactured aseptically. Aseptic manufacturing of sterile products is still seen as a last resort that is only acceptable if all methods of terminal sterilization in the final sealed container fail. In the US, the FDA's 2004 publication Guidance for Industry Sterile Drug Products Produced by Aseptic Processing describes the expectations of the FDA for the validation of aseptic processing in a more detailed manner. This guidance updates the 1987 guidance primarily with respect to personnel qualification, cleanroom design and isolators, air supply system, integrity of container closure systems, process design, quality control, environmental monitoring, and review of production records.

Facility, Environment & Equipment

In early phase trials, the quantity of the drug substance available is often very small. Matching the equipment scale and material handling expertise with the product batch size is essential in order to ensure a successful, cost-effective outcome. Fill and finish CMOs that manufacture high-volume commercial products typically lack the equipment and personnel to manage a developing product that requires low volume and flexibility in scheduling. Companies that specialize in small-volume early stage products have staff experience in rapid small-scale manufacturing campaigns. A smaller support staff generally has greater flexibility with regard to changes and timing. The lead time for changes at a smaller CDMO should be less than larger CMOs. Although larger CMOs have much greater capacity, they tend to be more rigid and generally have defined systems in place that are not easily changed and longer lead time.

The environment in which the aseptic processing takes place may require use of class 100 clean room suites, laminar flow hoods, biosafety cabinets, isolators, and restricted access barrier system (RABS). It is important to have suitable air flow during compounding and manufacturing. All of the air delivered to a cleanroom should pass through HEPA filters. The aseptic process should also include

environmental monitoring to ensure microbiological control over the product.

The equipment used for the sterilization needs to be monitored with calibrated temperature probes and sterilization cycles that are documented and incorporated into the batch record. The product's contact surface of manufacturing equipment needs cleaning verification following production. Items that cannot be sterilized must be disinfected before brough into the cleanrooms, and the entire area should be disinfected after processing.

Production Process & Validation

The production process should ensure the sterility and low endotoxin level of the product. If filtration is used as a method of sterilization, filters are bubble point tested to ensure integrity. To achieve the aim of a sterile product by an aseptic process, several aspects have to be considered and processes validated. In the end, process simulation with media fill is the key validation measure and allows the final evaluation of the appropriateness of the entire process. Process validation includes checks on the process by means of process simulation tests using microbial growth media (ie, media fill tests). The validation covers filling of media, environmental monitoring, and incubation and evaluation of the filled vials. Microbial control is critical as a part of cGMP for sterile products. Proper aseptic technique, properly functioning equipment, and adequate cleaning processes are to be demonstrated using media fills. Media fills are conducted routinely in order to establish that the process, environment, and controls are capable of producing a sterile drug product via an aseptic process.

Quality Control

The Quality Control department is responsible for the release of the finished drug product per specification and review of the manufacturing batch record to ensure that procedures are followed, investigations are performed if any, any subsequent corrective actions are incorporated, and that all testing performed during the process meets specifications so that the product is demonstrated to be sterile and meet product quality attributes. Micro testing for the finished product includes sterility testing per USP <71>, endotoxin testing per USP <85>, and bioburden testing per USP <61>.

Laboratory Controls

Laboratory tests used in manufacturing (eg, testing of materials, in-process material, packaging, drug product) should be scientifically sound (eg, specific, sensitive, and accurate), suitable and reliable for the specified purpose. The main purpose of laboratory testing of a Phase 1 investigational drug is to evaluate quality attributes, including identity, strength, potency, and purity, etc. The extent of analytical procedures and methods validation necessary will vary with the phase of the IND. The main goal of performing "staged" validation in early drug development is to provide test procedures that are reliable, able to support clinical studies, and evaluate the safety of the product.

Calibration and maintenance of laboratory equipment at appropriate intervals according to established written procedures is required. Personnel verify that the equipment is in good working condition when samples are analyzed (eg, system suitability). Initiation of a stability study using representative samples of the Phase 1 investigational drug to monitor the stability and quality of the Phase 1 investigational drug during the clinical trial (ie, date of manufacture through date of last administration) should be performed under ICH temperature, humidity, and light storage conditions.

Personnel & Training

All personnel involved in the manufacture of clinical trial material are to be trained in cGMP aseptic techniques. The procedures established should be clearly written and followed throughout the entire manufacturing process. Personnel are the main source of contamination of cleanrooms with microorganisms. The education and training of the personnel, the garments, the dress procedures, the rules for entry, and the behavior inside the clean rooms are important factors. Very important for aseptic manufacturing processes are the detailed SOPs of the CDMO, such as aseptic operation, gowning, room cleaning, as well as personnel and room environmental monitoring procedures.

SUMMARY

Complex injectables have gained increasing popularity in their applications in both acute and chronic disease treatments for small molecules and biologicals. However, production of the early phase clinical trial materials for complex injectables is a challenging task. A niche CDMO, which has specialized technologies in complex injectable development and adopts GMP practice with a "laboratory setting," will have greater flexibility regarding changes, timing, and cost for successful manufacture of complex injectables in early phase development of therapeutic drugs. ◆

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2020 Global Drug Delivery & Formulation REPORT

Part One of a Four-Part Series

Part 1: A Review of 2020 Product Approvals

Part 2: Notable Drug Delivery and Formulation Product Approvals of 2020

Part 3: Notable Drug Delivery & Formulation Transactions and Technologies of 2020

Part 4: The Drug Delivery and Formulation Pipeline

By: Kurt Sedo, Vice President Operations, PharmaCircle LLC

Introduction

If the Pharmaceutical Industry ever needed a reminder of how important resilience and formulation capabilities were, last year provided convincing evidence. The emergence of COVID-19 has placed a heavy burden on society and the industry. This was particularly evident with the two COVID-19 vaccines that were approved in record time for Emergency Use in the US, BioNTech and Pfizer's Comirnaty and the Moderna COVID-19 Vaccine. While both products reported outstanding clinical results, they have put heavy demands on distribution systems and end-user storage. Comirnaty requires storage at -70°C during transportation and storage. Moderna's vaccine's demands are somewhat less demanding, -20°C, but still strain current infrastructure. These demands are particularly problematic with a need to ship what will eventually be billions of doses.

Formulation should be coming to the rescue as soon as the third quarter of 2021 when it is hoped that lyophilized presentations of both vaccines might be available. There is also a hope that some of the newer vaccines in development, and probably approved for emergency use by the time this article is published, will require only a single dose, cutting transportation issues in half, and perhaps less-stringent temperature management demands.

In the world of Drug Delivery, there were no first products approvals using breakthrough technologies as there was in 2019 with the approval of Novo Nordisk's Rybelsus. Rybelsus was, however, approved in both Europe and Japan in 2020, and its label was expanded in the US. Halozyme's Enhanze technology was also well represented in 2020, with new drug approvals for Janssen's Darzalex FasPro and Genentech's Phesgo, new formulations of previously approved products that provide for the convenience of simple and quick subcutaneous injections rather than hours-long infusions. These two product improvements not only benefit patients and caregivers, but also the companies by quite likely extending their periods of market exclusivity.

Despite all of the challenges of 2020, the regulatory authorities didn't seem to miss a beat. Approvals were at much the same level as 2019. Most, if not all, of the submissions approved in 2020 would have been filed prior to the pandemic, meaning the pressure was on the regulatory authorities to appropriately reconfigure their resources with all of the challenges of social distancing.

What isn't yet apparent is how product development was impacted by the pandemic. Some companies certainly would have had resources reallocated to COVID-19 vaccines and therapeutics development. Social distancing and the application of medical resources to treating COVID-19 patients would have impacted all companies. The FDA also had to deal with restrictions on travel to conduct preapproved inspections.

Looking at the approval data on the following pages it's hard to imagine that the pandemic had any impact on the pharmaceutical industry. Next year's data may tell a different story.

Overall 2020 US NDA and BLA approvals were similar to 2019's numbers

Table 1. FDA Therapeutics Approval Numbers by Classification2 (2019 and 2020)

		2020	2019
BLA (CDER*, CBER*)		**26**	**26**
CDER	Biologic, 351(a) & 351(k)	21	22
	- 351(a) (Innovator)	18	12
	- 351(k) (Biosimilar)	3	10
CBER	Biologic Therapeutics, 351(a)	5	4
NDA (CDER)		**122**	**117**
Type 1	New Molecular Entity	44	39
Type 2	New Active Ingredient	2	7
Type 3	New Dosage Form	25	26
Type 4	New Combination	7	8
Type 5	New Formulation or New Manufacturer	24	32
Type 7	Previously Marketed, Unapproved	0	1
Type 1/4	New Molecular Entity and New Combination	2	1
Type 3/4	New Dosage Form and New Combination	3	0
Other Type	Other Type or Not Specified	11	N/A
Medical Gas	Medical Gas	4	3
ANDA (CDER)	Abbreviated New Drug Approvals (Generic, Multisource)	**903**	**962**

Source: PharmaCircle Pipeline & Products Intelligence and FDA Products Modules

*CDER (Center for Drug Evaluation and Research), CBER (Center for Biologics Evaluation and Research)

- 2020's 26 Biologic approvals, 351(a) and 351(k), matched 2019's total. CBER and CDER Innovative BLA 351(a) approvals, were up almost 50%, while biosimilar 351(k) approvals, dropped by 70%.
- Biosimilar approvals in 2020, 3 in total, fell well short of 2019 (10 approvals) and 2018 (9 approvals).
- 2020's 46 non-biologic novel drug approvals, including single active and combination products, were above the 40 approved in 2019.
- New Dosage Form approvals (Type 3 and Type 3,4) totaled 28, a little ahead of 2019. These products incorporated previously approved actives (PAA), often with the benefit of improved convenience or a focus on pediatric patients.
- New combination approvals incorporating PAA and novel actives totaled 12 in 2020, a bit ahead of 2019 and well short of 20 approvals in 2018.
- New Formulation or New Manufacturer, Type 5, approvals totaled 24 in 2020, well short of 2019's 32, and 2018's 40 approvals.
- There were a large number of products, 11, in 2020 that were not classified by the FDA. This was a mixed bag of approvals that included new presentations with lower sodium content, Xywav, and expanded patient population approvals, as well as a couple of prescription to OTC switches.
- ANDA approvals were down about 6%, with Tentative Approvals representing almost 17% of all ANDA approvals.

Table notes: *Some multisource injectables are approved through the NDA rather than the ANDA regulatory process and can unintentionally skew the new drug approval figures. Type 5 approvals, often injectable multisource products, are not considered in the analyses presented on the following pages.*

Injection Route products continue to represent a significant proportion of new product approvals

Table 2. 2020 Approvals by Administration Route

Route of Administration	US (n=132)	Europe (n=238)	Japan (n=66)
Buccal / Sublingual	1	3	1
Inhalation	2	10	2
Injection (All)	58	83	31
Instillation/Implantation	-	1	-
Nasal	3	-	1
Ophthalmic	4	13	2
Oral	56	97	27
Rectal	-	1	-
Surgical Insertion	2	5	-
Topical	3	17	2
Transdermal	1	1	-
Vaginal	2	4	1

Source: PharmaCircle Pipeline & Products Intelligence module

*CDER (Center for Drug Evaluation and Research), CBER (Center for Biologics Evaluation and Research)

- The approvals in Europe include both EMA and country level approvals for non-generic products. Not surprisingly, products using the Oral route were the most common followed by Injection (All). The Inhalation figures are skewed a little by the EMA practice of granting separate approvals for different brands of the same product. The topical figures are remarkably high in part because of country-level approvals for slightly differentiated formulations using previously approved actives. This is the same reason for the relatively high number of product approvals using the Ophthalmic route.
- The US approval population is consistent with earlier reports and represent new molecular entities and new novel formulations of previously approved actives. With this product set, Injection (All) noses ahead of Oral. Inhalation is an increasingly a less-favored administration route. In some cases, systemic injectables are being developed and approved to treat pulmonary conditions previously treated by inhalation.
- In terms of relative numbers, Japanese product approvals largely parallel the US, with Injection leading Oral.
- Nasal and Transdermal delivery continues to be associated with a very limited number of new approvals. For both Nasal and Transdermal delivery, the issue is largely related to the limited number of molecules suited for what is essentially "transdermal" delivery. The most interesting newer molecules pose increasingly significant demands on all delivery systems by virtue of drug size, limited lipophilicity, and stability challenges.

Table notes: *The figures above include all formulations approved for each product. In a few cases, two or more formulations were approved for a single product. Some products were categorized in two columns, for example, Injection and Ophthalmic, for a product delivered intravitreally. The figures above do not include Type 5 Approvals (FDA), Generics (All), or Biosimilars (All).*

Renewed industry interest in formulation-enhanced products will depend on new technologies

Table 3. 2020 Approvals by Drug Delivery Category

Route of Administration	US (n=132)	Europe (n=238)	Japan (n=66)
Inhalation			
- Devices (Integral*)	1	7	2
- Formulations	1	3	-
Injection			
- Device, Injection Systems (Integral*)	2	13	9
- Device, Pre-Filled Syringes	3	10	4
- Formulations	8	16	6
- Conjugates	13	3	2
- Viral Vectors	-	1	1
- None	29	44	11
Implantation			
- Formulations	-	1	-
Nasal			
- Devices	2	-	-
- Formulations	1	-	-
Ophthalmic			
- Device and/or Pre-Filled Syringes	-	2	-
- Formulation	4	8	1
- None		3	1
Oral			
- Formulations	12	16	6
- None	45	79	20
Topical			
- Formulations	2	4	-
- None	1	11	2
Vaginal			
- Formulations	1	1	1
- None	1	3	-

Source: PharmaCircle Pipeline & Products Intelligence Module

- Good enough seems to be the working approach for most oral products approved in 2020. Formulation-enhanced oral products continue to command a limited share of approvals. With the most obvious oral drug enhancement products already approved, and new molecular entity oral products being approved with optimized pharmaceutical therapeutic and convenience properties, the opportunity for reformulations has dropped. The obvious next formulation advancement, converting chronic therapy injectables to oral dosing, awaits the development of the necessary technology. Novo Nordisk's Rybelsus is but a first step at validating the possibility and potential.
- Injection systems, an increasingly popular option to encourage the use of outpatient injectables, represented a relatively small proportion of total 2020 Injectable approvals. To some extent, this can be attributed to the approval of many new biologic products targeting rare diseases that require only a single injection or infusion, or injections on a monthly or longer interval. For these products outpatient friendly approaches are much less important than efficacy and safety considerations.

Table notes: *The figures above include all formulations approved for each product. In a few cases, two or more formulations were approved for a single product. Some products were categorized in two columns, for example, Injection and Ophthalmic, for a product delivered intravitreally. The figures above do not include Type 5 Approvals (FDA), Generics (All), or Biosimilars (All).*
** - Integral refers to devices that are integrally associated with a product. Examples would include auto-injectors and dry powder inhalers.*

Simple Dosage Forms, Solutions and Tablets, were the norm in 2020

Table 4. 2020 Approvals by Dosage Form

Route of Administration	US (n=132)	Europe (n=238)	Japan (n=66)
Inhalation			
- Inhalation Powder	1	5	2
- Inhalation Suspension	1	4	-
Injection			
- Emulsion	2	1	-
- Gel	-	-	1
- Lyophilized Powder for Solution or Suspension	13	9	5
- Powder for Solution or Suspension	-	4	1
- Solution	37	52	20
- Suspension	5	15	4
Nasal			
- Powder	-	-	1
- Solution	1	-	-
- Spray Solution or Metered	1	-	-
Ophthalmic			
- Implant	2	-	-
- Ointment	-	1	-
- Solution	2	8	-
- Suspension	1	1	1
Oral			
- Buccal or Sublingual	-	3	1
- Capsule	12	17	1
- Capsules, Soft Gel or Liquid Filled	1	5	-
- Film	1	2	-
- Liquid	1	-	-
- Lozenge	-	1	-
- Tablet or Powder for Solution or Suspension	4	3	-
- Sachet, Granules	3	3	2
- Solution	5	8	1
- Suspension	3	1	1
- Syrup	-	3	-
- Tablet	27	54	23
Topical			
- Cream	1	1	-
- Gel	-	3	1
- Lotion	1	-	-
- Ointment	1	1	1
- Patch	-	2	-
- Solution	-	8	-
- Shampoo	-	1	-
- Sponge	1	-	-
- Spray	-	1	-
Other			
- Rectal Suppository	-	1	-
- Transdermal Patch	-	1	-
- Vaginal Insert, Gel, Ring, Suppository or Tablet	-	4	1

Source: PharmaCircle Pipeline & Products Intelligence module

- Dosage Form approvals in 2020 largely paralleled the relatively conservative Route of Administration and Drug Delivery Category approvals with a heavy emphasis on simple Oral Tablet, Oral Capsule, and Injection Solution Dosage Forms.

Table notes: *The figures above include all formulations approved for each product. In a few cases, two or more formulations were approved for a single product. Some products were categorized in two columns, for example, Injection and Ophthalmic, for a product delivered intravitreally. The figures above do not include Type 5 Approvals (FDA), Generics (All), or Biosimilars (All).*

Approvals by Molecule Type in 2020 largely paralleled 2019 approvals

Table 5. 2020 Approvals by Molecule Type

Molecule Type	**US** (n=126)	**Europe** (n=235)	**Japan** (n=66)
Antibody	17	11	6
Carbohydrate	1	4	-
Cell Therapy	1	2	-
Gene Therapy	-	4	1
Natural Product	-	3	-
Oligonucleotide	1	-	1
Peptide	2	5	5
Plasma Derived	-	1	-
Polymeric	-	4	1
Protein	8	17	8
mRNA	2	1	-
SiRNA	1	3	-
Small Molecule	92	178	44
Stem Cell	-	-	-
Vaccine or Virus	1	1	-

Source: PharmaCircle Pipeline & Products Intelligence module

- The US and Japan approval figures best represent the current trend with respect to Molecule Types. The European data includes a number of country-specific approvals that largely represent reformulations of previously approved actives, generally small molecules.
- Macromolecule product approvals, with the exception of Small Molecule and Natural Product approvals, accounted for 27% (34/126) of US approvals, a little bit lower than 2019's 31%. Japanese Macromolecule approvals represented 33% (22/66), a slight drop from 2019's 35% (21/60).
- Both the US and Japan figures are higher in terms of Macromolecule approvals if one considers only new molecular entity approvals. Many of the Small Molecule approvals represent new Dosage Forms and Formulations of previously approved actives targeted to specialty populations, notably pediatric patients.
- Antibody-related approvals in terms of total number were higher in 2020 in all regions, except for Japan where they were flat.

Table notes: *The figures above include all formulations approved for each product. In a few cases, two or more formulations were approved for a single product. Some products were categorized in two columns, for example, Injection and Ophthalmic, for a product delivered intravitreally. The figures above do not include Type 5 Approvals (FDA), Generics (All), or Biosimilars (All).*

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1. Summary reports of 2020 approvals in all three territories are available at http://www.pharmacircle.com/info.
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CELL & GENE THERAPY

End-to-End Cell & Gene Therapy – From Development to Commercialization – Buy or Build?

By: Aldo Romano and Emily Moran, MBA

INTRODUCTION

The exponential growth of the cell and gene therapy (CGT) market has been driven by the convergence of several economic and scientific developments. The rapid growth in computing power combined with the use of artificial intelligence has resulted in a greater understanding of the human genome and its role in the origin of disease. Scientists have now identified more than 6,000 life-threatening diseases that are the result of a single genetic defect.

The convergence of these technologies and computing power amplifies the transformative promise cell and gene therapies can offer to millions of patients. If manufacturing obstacles and capacity constraints can be resolved, the growth potential for these innovative and life-saving treatments can be achieved. The industry desperately requires development and manufacturing capacity to meet the growing demand.

In addition to the advances in innovation, other factors are driving the demand for manufacturing capacity, including both the macroeconomic and geopolitical environment. Venture capital, IPOs, and M&A volume in the life sciences are at all-time highs. Supply chain constraints experienced before and during the pandemic have led to the on-shoring of manufacturing supply. The pandemic has resulted in vaccine development that has led to extreme demand for manufacturing capacity.

The lack of process development and manufacturing capabilities within many of the companies developing these therapies represents another major challenge. Accelerated timelines put pressure on academic institutions, biotech start-ups, and pharma companies to decide early in the clinical process the best path forward to manufacture, and ultimately commercialize, their CGT product candidates. This decision is often referred to as the choice to "buy" – partnering with a Contract Development and Manufacturing Organization (CDMO) or to "build" – building the development and manufacturing capacity in-house. The latter can present significant financial and resource burdens.

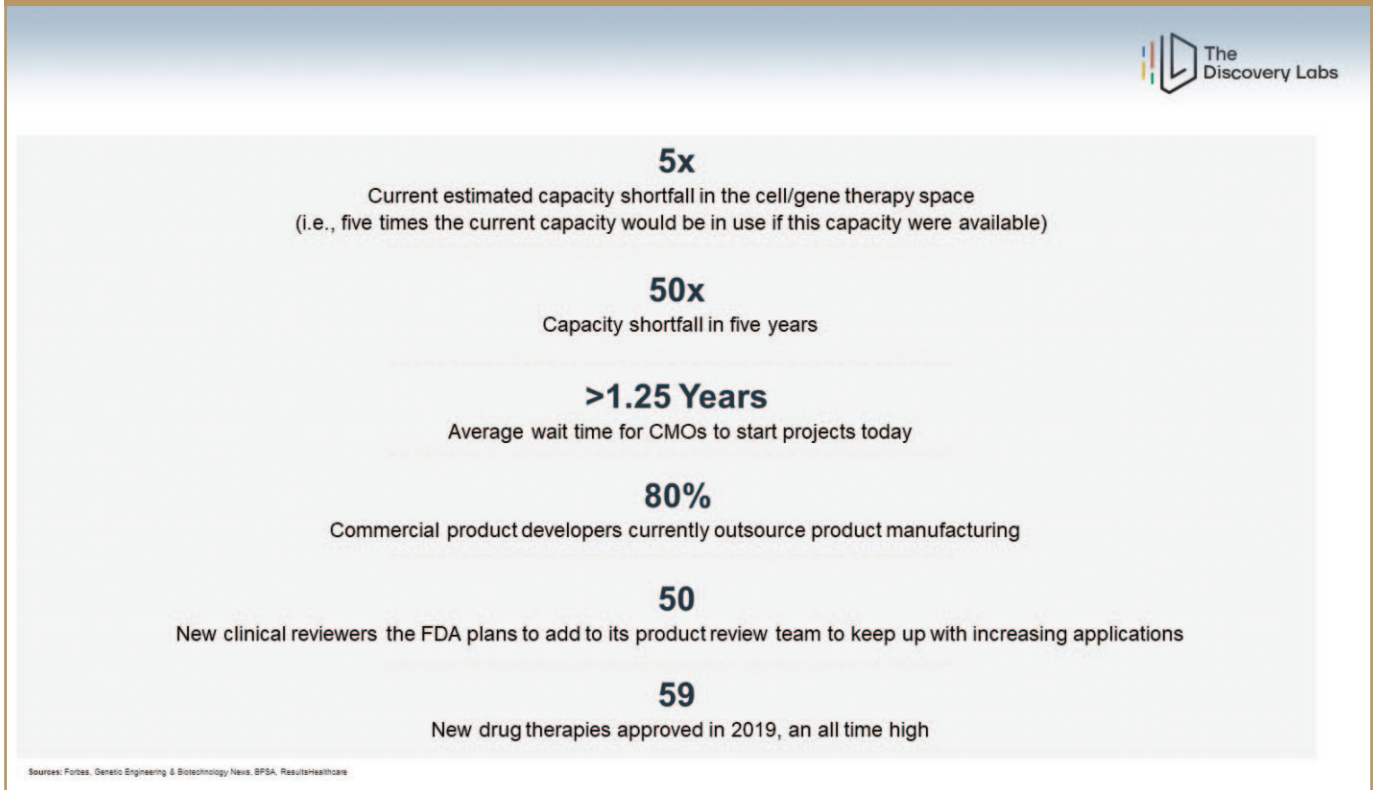
CELL & GENE THERAPY DEVELOPMENT – OPPORTUNITIES/BARRIERS

The CGT industry is growing exponentially. According to the Alliance for Regenerative Medicines (ARM), there are currently 1,078 regenerative medicine and advanced therapy clinical trials ongoing worldwide as of the middle of 2020. At the same time, the US FDA expects to receive 200 investigational new drug applications in 2020 alone. It has been estimated that by 2025, the FDA will be approving 10 to 20 CGT products per year. Further, the agency's eventual goal is to add approximately 50 clinical reviewers charged with overseeing the clinical investigation, development, and review process for these therapies.[1]

Due to the expected increased clinical success of gene therapies, robust pipelines, and the growing list of potential diseases CGTs can target, the gene therapy segment is expected to reach revenues of more than $11 billion by 2025. In oncology alone, the CGT market is expected to reach $9.5 billion by 2025. The market is expected to grow at a compounded annual growth rate of more than 24% between 2019 and 2024.[2] According to PhRMA, there are 362 CGT drug candidates in clinical development in the US in 2020, an increase of more than 25% since 2019.[3]

As more innovators identify novel CGT targets, the demand for CGT development and manufacturing capacity grows. This increasing demand has created a backlog that has delayed new

FIGURE 1



therapies from reaching patients in need.

Compounding the capacity constraints, manufacturing of CGT products is complex. Processes and materials needs could differ from one product candidate to another, creating a challenge in setting production standards. An overall lack of options, whether internally at an innovator company or with an outsourced CDMO provider, represents the largest barrier to entry. There are multiple levers to reduce these barriers, such as adopting a platform process and/or partnering with a CDMO early in product development to ensure an industrialized and GMP-minded approach is taken early in development.

DETERMINING A PROCESS PLATFORM & MAKING CAPITAL INVESTMENTS

Most CGT programs are being developed in an academic or small-scale manufacturing environment and lack standardized, easily scaled process platforms for commercial supply. As the process is scaled and more batches are produced, complexities or gaps are identified and expensive, time-consuming rework is often required. This can set back clinic dates and delay patient trials.

These risks can be mitigated by partnering with CDMOs with regulatory and commercial experience early, and adopting a robust, repeatable platform process or technology that supports safe, GMP manufacture and provides for seamless scale up and scale out.

PROCESS DEVELOPMENT & TECHNOLOGY TRANSFER

Conducting tech transfer from an R&D lab into a GMP environment is extremely challenging for early stage CGT companies. CGT development and lack of standardization makes tech transfer complex, challenging, and time-consuming. Adhering to a scalable and commercially viable platform process early in the product's life cycle ensures that tech transfer is seamless during the later stages of development when larger batches and more patient doses are needed.

This requires an early stage, in-depth program assessment, and a strong plan for establishing critical attributes to monitor during the development process. Ensuring the PD team working on the scale up has a strong understanding of GMP manufacturing and regulatory guidelines.

TABLE 1

Buy Versus Build (Internal versus External) Decision:	Discovery Labs Differentiators Include:
Speed - When a product needs to move, buying (outsourcing) is a key option.	DL's size and existing infrastructure allows for speed and capacity.
Supply - Redundancy and dual supply of a product (CDMO/build) is attractive and critical for regulatory and supply chain mitigation.	DL offers build and buy hybrid solutions.
Outsourcing - Outsourcing mitigates the upfront spend and de-risks investment while moving funds to product development.	DL provides maximum optionality for its clients.
Experience - CDMOs have vast experience and resources to accommodate the highly complex needs of biotech companies.	DL provides turn-key space and services.
	Experienced management team
	Cellicon Valley location offers a talent rich environment with a surplus of biopharma professionals in the Greater Philadelphia region.

Development programs must balance progressing to meet clinical timelines while maintaining a risk register with appropriate countermeasures as scale-up and scale-out challenges are determined.

CAPACITY & SUPPLY CHAIN

Another important piece of the CGT development and manufacturing puzzle is capacity. Part of the capacity gap is the profound lack of suitable space. But not just any space – the right kind of space. CGT innovators need rapid access to lab and GMP space specifically designed to provide process development, plasmid and viral vector production, analytical testing, and cell banking. The ideal capacity solution should also offer the ability for on-site expansion.

Fundamentally linked to the capacity gap is the increasing supply chain barrier. Confounded by the COVID-19 global pandemic, CGT developers must contend with these barriers or partner with CDMOs with advanced supply chain expertise. The Center for Breakthrough Medicines (CBM), a CDMO focused on CGT development and commercialization, mitigates the diverse and fragmented elements of the supply chain through strategic partnerships with vendors and sophisticated enterprise resource planning (ERP) software and automation technology to properly "demand plan" for the needed raw materials and consumables, including bioreactor bags, serum, buffers, and media.

To avoid production delays, the CBM offers a single-source, end-to-end solution that eliminates these supply chain challenges. Under one roof, CBM offers production for plasmids, viral vector, cell therapies as well as a fully loaded analytics lab for raw materials and in-process and release testing. CBM also maintains the ability to prepare in-house buffers as well as component preparation and sterilization to simplify the supply chain. These holistic considerations reduce tech transfer, execution, and release time significantly for programs.

BUY OR BUILD?

In the face of these barriers, companies embarking on CGT development must make timely decisions. Do they build or do they buy?

Most CGT innovators lack the expertise necessary to build capacity in-house. And most of these organizations must manage their limited capital efficiently. For some of these CGT start-ups, the build pathway is too expensive and too risky. Consequently, many CGT companies seek partners with a robust infrastructure and the process expertise.

Ideal CDMO partners should be able to deliver a full complement of end-to-end services. Best-in-class CGT CDMOs can provide discovery support, process and analytical development, plasmid DNA manufacturing, viral vector manufacturing, cell processing, testing, and specialized clinical services all under one roof. CBM fills the capacity gap by bringing significant experience, technology, and GMP space to this complex marketplace.

Best-in-class CGT CDMOs should also offer value-added services, including supply chain expertise and cold chain storage solutions. Strong existing supply chain relationships provide rapid access to equipment, raw materials, and disposables. Close partnerships with suppliers and robust processes ensure delivery of materials that have long lead times. CBM's size allows the supply chain and ecosystem to coexist on one site.

CDMOs also offer partners hybrid solutions, especially in the manufacturing and commercialization stage. Some innovators are avoiding choosing one path over another and taking a dual-track approach to manufacturing by using both their own facilities and those of a CDMO to ensure they have the needed capacity and a redundant supply chain. However, CGT innovators must consider the onboarding and training of scientists, operators, and support personnel needed to run the facility once at full scale with repeatable right-first-time execution; utilizing a CDMO avoids this risk.

With the hybrid model, a client can easily reserve manufacturing capacity in anticipation of commercialization potentially saving precious time in making these

life-saving therapies available to patients who need them the most.

COVID WILDCARD

Companies in the cell and gene therapy market must be aware of significant headwinds caused by the global COVID-19 pandemic. COVID-19 has caused considerable supply chain disruptions. In particular, the reduced availability of active pharmaceutical ingredients and other raw materials is widespread.

In addition, significant manufacturing capacity, especially final fill and finish, have been reserved globally for when a vaccine is developed. The Coalition for Epidemic Preparedness Innovation (CEPI) has identified manufacturers with capacity to produce 4 billion doses a year. With nine vaccines in development, CEPI plans to have two or three manufacturing plants for each vaccine.[4]

GROWTH POTENTIAL

As Moore's Law continues to contribute to the expanding pipeline of cell and gene therapies, the potential for growth in this market seems to be endless. And, as these therapies develop, we are witnessing more global regulatory authorities fast-tracking approvals for these revolutionary new treatments. The growing evidence that monogenic defects can be corrected for previously uncurable rare and orphan diseases is unimaginable.

For patients and society to tap the benefits of cell and gene therapies, the life science industry must bring technology solutions to the table while at the same time securing the capacity to develop and manufacture these groundbreaking cures. Developing advanced biologics, engineered cells, and viral vectors is vital in moving toward effective, personalized, restorative, and regenerative medicines. In addition to complex manufacturing, a fragmented and constrained supply chain must be managed. Meanwhile, the need for flexible state-of-the-art processes and production technologies continue to grow. ◆

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BIOGRAPHIES

Aldo Romano is VP of Commercial Development at The Discovery Labs and has over 20 years of Bioprocess, Cell, and Gene Therapy experience in capital equipment and disposable process technologies. He was previously Business Development Manager at GE Healthcare Lifesciences, where he was responsible for growing their Enterprise Solutions business. Prior, he served at Advanced Scientific (now part of Thermo-Fisher) as a Healthcare Sales Manager in their OEM medical technology contract manufacturing unit. He began his career at Pall Corporation, where he worked as a Director of Sales focused on the growth and adoption of single-use technologies into the Biotech market. He earned his Bachelor's degree in Biology from Binghamton University.

Emily Moran is Senior Director, Viral Vector Manufacturing, at The Discovery Labs. She is an experienced leader in cell and gene therapy and biologics manufacturing – with a focus on commercial readiness, industrialization, and manufacturing stabilization. She has experience in viral vector manufacturing, aseptic processing, upstream and downstream technology, supply chain and demand planning, quality auditing, and facility and organization while employing lean manufacturing standards. Prior to The Discovery Labs, she was the Head of Viral Vector Manufacturing for Lonza in Houston, TX, as well as Sanofi Pasteur. She earned her Bachelor's degree in Biology from Ursinus College and Duke University and her MBA in Project Management from the New York Institute of Technology.

DRUG DEVELOPMENT

Understanding & Targeting the Mechanism of Action of Developmental Disorders

By: Michael Snape, PhD

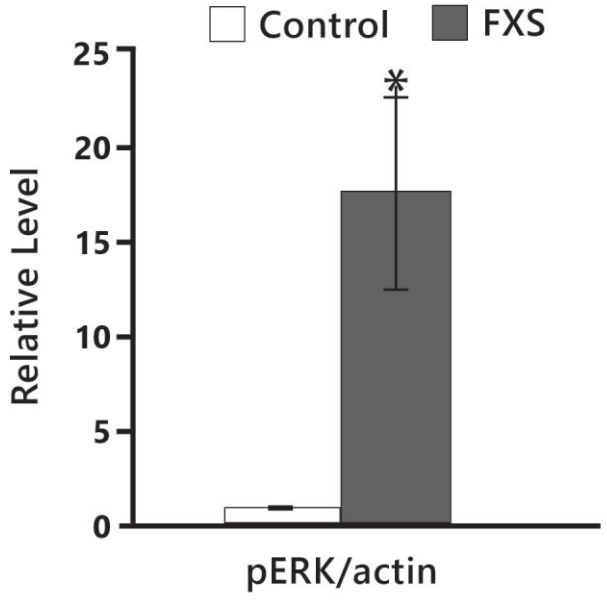
INTRODUCTION

In many cases, developmental disorders that impact thousands of people around the world are neglected due to a lack of precedent in research, limited understanding of the mechanism of action of onset and progression, and the inability to carefully assess preclinical and clinical data. However, recent advancements in research have provided many important insights that are helping drug developers and academic research centers to consider new approaches to treatment for these patients. In many cases, research has demonstrated that some therapies previously studied in preclinical or clinical-stage research in oncology and other indications may have applications in the treatment of diseases and conditions associated with developmental delays. By applying a range of innovative clinical trial assessment tools and methodologies, researchers are advancing development programs involving drugs with established safety and efficacy profiles.

THE RAS-ERK PATHWAY: ADDRESSING DESTRUCTIVE DYSFUNCTION

There is substantial research indicating that the RAS-ERK signaling pathway in the body's cells is dysregulated in cancerous tumors, usually caused by mutations in the RAS or BRAF genes. Based on this understanding, many efforts in oncology research and drug development have worked to target components of the RAS-ERK signaling cascade. But a long-standing body of neurobiological research suggests that RAS-ERK pathway activity also plays a critical role in synaptic plasticity (the ability of synapses to strengthen or weaken over time) and therefore may also play a role in the coding of information in the brain that influences neurodevelopment. As a result, researchers are now looking at the use of drugs that can inhibit the RAS-ERK pathway to treat genetic encephalopathies, a series of rare or ultra-rare disorders frequently associated with developmental delay, learning difficulties,

FIGURE 1



Activation of the RAS-ERK pathway in the brain in patients with Fragile X syndrome as indexed by pERK levels (* = statistically significant at p < 0.05 level; FXS = Fragile X syndrome) (from (Wang et al 2012 J Neurochem. 121 672-679).

autism spectrum symptoms, and epilepsy.

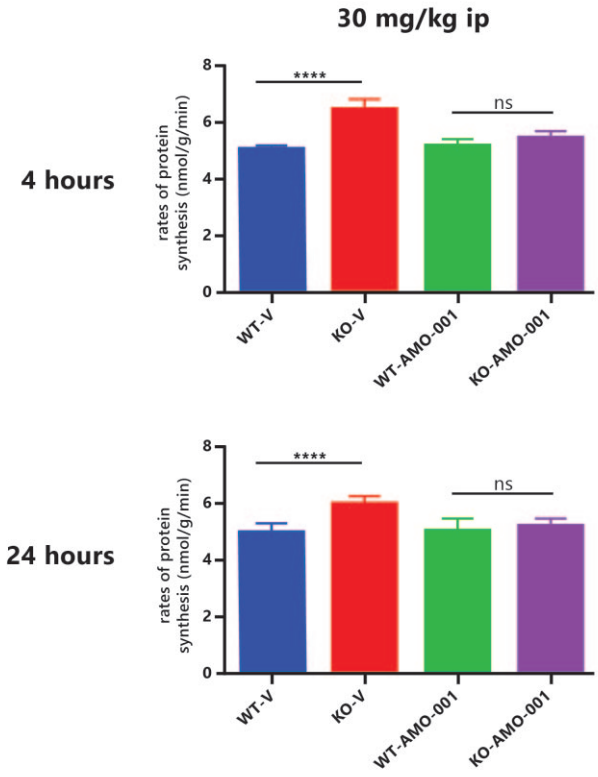
Through most of the last century, relatively little data was available related to the neuropathology and neurochemistry of childhood-onset pediatric disorders associated with developmental delay, such as Phelan-McDermid syndrome, congenital myotonic dystrophy, and Rett syndrome. To better understand the underlying biology of these and other developmental disorders and the role of the ERK signaling cascade, in the early 2000s members of the team now at AMO Pharma initiated a collaboration with a research group at Case Western University to begin investigating the status of the RAS-ERK cancer pathway in postmortem brain tissue samples from young individuals with autism and Fragile X syndrome (which is the largest single inherited cause of intellectual disability and the leading genetic cause of autism) and in brain tissue from a genetically manipulated mouse model of Fragile X syndrome. The data showed that the RAS-ERK pathway was aberrantly activated in the brains of people with autism and Fragile X syndrome. This increase in RAS-ERK activity was seen in neurons and in the glial cells that support neuronal function. (It is now known and commonly reported that abnormal behavior of glial cells is seen in multiple genetically determined neurodevelopmental disorders.) Additional research efforts using a genetic knockout mouse model of Fragile X syndrome suggested that inhibiting RAS-ERK pathway activity could also potentially prevent susceptibility to seizures.

These results, coupled with existing data, indicated that the RAS-ERK pathway may control the production of new synapses in the brain that are associated with learning processes in individuals with certain genetically determined neurodevelopmental disorders. Stimulated by these findings, researchers at AMO Pharma were intrigued with the concept of using RAS-ERK pathway inhibitors designed in the oncology setting to try to change the underlying biology and core symptoms of neurodevelopmental disorders.

AMO-01: RAS-ERK PATHWAY ACTIVATION & SYNAPTIC FUNCTION

When AMO Pharma was founded in 2015, the company had identified and acquired a promising investigational anti-cancer therapy now known as AMO-01 that is engineered to inhibit activation of the RAS gene and ERK pathway. Early stage clinical data showed that this drug could inhibit RAS-ERK activity in cancer

FIGURE 2



AMO-01 normalizes synapses in vivo in Fmr1 KO mouse (** = statistically significant at p < 0.0001 level; WT = Wild Type or control mouse; KO = Fragile X syndrome Fmr1 knockout mouse; V = Vehicle or control injection.**

patients at doses that were shown to be safe and well tolerated. More importantly, data showed that the drug could penetrate the brain. In fact, its entry into the brain was shown to be enhanced when glial cells in the brain are activated (which occurs in Fragile X Syndrome, for example).

The AMO Pharma team further examined the effects of AMO-01 and its potential as a RAS-ERK inhibitor to treat Fragile X syndrome and Phelan-McDermid syndrome (PMS), a similar ultra-rare developmental disorder characterized by cognitive deficits, autism, and severe, often intractable epilepsy. In vitro and in vivo testing showed that AMO-01 could normalize the synapse abnormalities seen in neurons derived from knockout mouse models of Fragile X syndrome and PMS and reverse behavioral abnormalities, including sensori-motor behavior, anxiety, social behavior, cognition, seizures, and the mouse equivalents of self-care and activities of daily living. These clinical benefits were seen in mice within hours of receiving a one-time administration of AMO-01 and even persisted 5 to 10 days later.

PMS is a devastating disease for which there are no therapies available and current standard of care involves addressing symp-

toms on an as-needed basis. These mouse model data raised the possibility of human studies in which a single dose treatment with AMO-01 could result in a "remission" of symptoms associated with neurodevelopmental disorders.

Encouraged by these findings, AMO Pharma is supporting a Phase 2 clinical study in PMS being conducted at Mount Sinai School of Medicine and Texas Children's Hospital. The Phase 2 open-label study is assessing the safety, tolerability, and efficacy of AMO-01 following a single-dose administration in adults and adolescents with PMS who have also been diagnosed with epilepsy. PMS patients who have epilepsy often experience worsening seizures in terms of severity and frequency and, in many cases, have difficulty controlling their seizures with traditional epilepsy medicines.

AMO-02: ROUTES TO NEUROGENETIC DISORDERS VIA INCREASED GSK3 BETA ENZYME ACTIVITY

AMO Pharma is also advancing an investigational medicine called AMO-02 for the potential treatment of congenital myotonic dystrophy (CDM1), a genetically determined form of muscular dystrophy associated with trinucleotide expansion repeat in the patient genome, adjacent to the DMPK gene. This DNA expansion repeat is coded to form an expanded repeating RNA that is believed to be the causal agent in congenital myotonic dystrophy. Recently published data show that administration of AMO-02 initiates the breakdown of this toxic RNA.

Myotonic dystrophy (MD) is the most common form of muscular dystrophy, affecting about one in 8,000 people worldwide. The congenital onset form (CDM1) affects approximately one in 40,000 children and is the most serious and life-threatening. CDM1 typically presents at birth and causes severe symptoms of the central nervous system (CNS), including significant physical and cognitive impairment, symptoms of autism, difficulty sleeping, thinking and problem solving, and problems with speech, hearing, and vision. CDM1 patients may also experience muscle weakness and muscle myotonia.

There are currently no approved treatments for CDM1. Patients are typically treated with drugs to address individual symptoms and receive additional support through special education, speech therapy, and physical therapy. Research at AMO Pharma is focused on the potential correlations between reduction in mutant RNA repeats (caused by a mutation in the DMPK gene) and reduction of synapse and tissue dysfunction associated with CDM1.

In transgenic mouse models and ex vivo tissue samples in patients with CDM1, AMO-02 was shown to be effective in reducing toxic RNA repeats. Based on this therapeutic approach, a Phase 2 proof-of-concept study found that treatment with AMO-02 provided a range of clinical benefits for the majority of CDM1 patients (13 out of 16, mean age 22 years old) at 12 weeks, with the largest benefit seen in patients treated with the highest dose (1,000 mg/day). Significant improvements were seen in cognitive function, fatigue, and the ability to perform activities of daily living as well as in certain neuromuscular symptoms. Co-occurring symptoms of autism also improved in several patients. AMO-02 is now being evaluated in a pivotal randomized, multicenter, double-blind, placebo-controlled Phase 2/3 study in

FIGURE 3

Patients with PMS often experience seizures that are not easily controlled by traditional epilepsy medications, leading some to undergo invasive procedures with the hope of reducing the intensity and frequency of seizure activity.

children and adolescents (aged 6 to 16 years old) with CDM1. The trial is being conducted at 10 treatment centers across the US, Canada, and the UK and plans to enroll a total of 56 patients. Patients will be assessed on a range of measures of CNS features and muscle function associated with CDM1. Pending positive results from the pivotal trial, the data will support a future submission for marketing authorization of AMO-02 in treatment of CDM1.

AMO-04: GLUTAMATE MODULATORS & TREATING RETT SYNDROME

AMO Pharma is also advancing a program in Rett syndrome, a rare neurological disorder that is typically first recognized in infancy and seen almost always in girls. It is often misdiagnosed as autism, cerebral palsy, or non-specific developmental delay, and occurs in one of every 10,000 female births. Rett syndrome is caused by mutations in the X-linked methyl-CpG-binding protein 2 (MECP2) gene, which can alter normal functioning of the neurotransmitter glutamate in the brain. Glutamate is the major excitatory neurotransmitter in the CNS and glutamate pathways are linked to many other neurotransmitter pathways. Glutamate receptors are found in neurons and glial cells throughout the brain and spinal cord.

In 2017, AMO Pharma licensed a promising therapy, now called AMO-04, which functions as a glutamate modulator and has the potential to treat neurological symptoms associated with Rett syndrome. In preclinical studies, AMO-04 was shown to normalize multiple aspects of a MECP2 knockout mouse model of Rett syndrome. This debilitating rare disease can lead to a wide range of disabilities, ranging from mild to severe, often including problems with cognitive, sensory, emotional, motor and automatic function. These symptoms may lead to challenges with speech, learning, mood, movement, cardiac function, chewing, swallowing, and digestion. Many patients also experience a regression in communication skills and a loss of purposeful hand function, and some also experience seizures, hyper- and hypo-ventilation, apnea, autism, cognitive deficits, scoliosis, and sleep disturbances. AMO-04 received Orphan Drug Designation from the FDA in 2018 and is currently being evaluated in Phase 1 clinical trials.

SUMMARY

In identifying promising therapeutic targets for any disease or condition, including developmental disorders such as Fragile X syndrome, PMS, CDM1, and Rett syndrome, it is critical that researchers work to understand the mechanism of disease and disease pathways as well as the unmet need and patient experience. While the RAS-ERK signaling pathway has been known to play a role in certain types of cancer, we now have further established a connection between this pathway and the development of certain neurogenetic disorders. Research thus far indicates that this approach has the potential to reverse the basic causes of the neurodevelopmental disorders. Advances in research are also driving a new focus on the treatment of these often-neglected disorders with therapies engineered to target the mechanism of action of the disease rather than focusing on symptom management. ◆

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BIOGRAPHY

Dr. Michael Snape is Chief Scientific Officer at AMO Pharma. He is a neurobiologist who has spent 27 years in the pharmaceutical industry working for large and emerging biopharmaceutical companies before co-founding AMO Pharma in 2015. His principal experience lies in the area of demonstrating clinical proof-of-concept with novel targeted therapeutics in rare diseases. He is a named inventor in numerous patents and is a scientific advisor to several patient foundations.

Drug Development
EXECUTIVE

Sridhar Krishnan

Senior VP, Operations & Strategic Initiatives

Pii

Pii

Pharmaceutics International, Inc
Challenges Frame Opportunities

Pharmaceutics International, Inc: Getting Back to its Roots

Pharmaceutics International, Inc (Pii) has continuously evolved throughout its history as market and patient needs emerge and fade. There have been times when Pii developed and manufactured its own products and times when Pii delivered these services to drug sponsors. Pii started in 1994 as a contract drug development solution provider, focused primarily on solid formulations. Throughout the early- to mid-2000s, the company expanded its manufacturing capabilities at its Hunt Valley, MD, campus, which now includes four cGMP- and FDA-inspected facilities, four integrated aseptic filling suites, and 70 manufacturing suites. However, as Sridhar Krishnan, Pii's Senior Vice President, Operations and Strategic Initiatives, recently told *Drug Development & Delivery*, manufacturing is not solely where Pii wanted to be, so the company made a concerted effort to get back to its contract development and manufacturing outsourcing (CDMO) roots. As a CDMO, Mr. Krishnan says Pii can deliver on its passion for helping drug sponsors solve challenging issues associated with complex formulations, drug products that are difficult to manufacture at scale, or formulations that pose high risks to quality and safety, like oncology drugs, highly potent compounds, non-aqueous parenterals or steroids.

Q: Based on this evolution, how do you now describe Pii's business model?

A: Our model is simple: We respond to patients and market changes in real time. Clients working with large CDMOs have a predetermined game plan looking out 2 to 4 years. We don't delineate a schedule to an activity, and we are not selective about onboarding based on a predetermined schedule. We work in real time for the patient and the client. Additionally, we are flexible and agile, providing solutions to complex problems that you cannot get from other large CDMOs. Our flexibility allows us to take a drug from cradle to grave; from small to large batches. And, because our model is responsive to patients, healthcare providers, and market changes, it is absolutely a sustainable model. Sustainability is the construct for everything we do. The outcome of sustainability is increased capacity. Capacity is, of course, our ability to develop and manufacture drug therapies: how many projects we can effectively manage at any given time. Sustainability is applied to every resource and staff area we have available at Pii. We continuously develop leaders throughout Pii, increasing our capacity to apply more leadership to problem-solving and delivering better results faster for our clients and patients.

Q: Exploring this concept of sustainability a bit further, how might this impact getting a drug to market faster?

A: When I think about sustainability, it's all about reducing lead time for a drug to come to market. There are several dimensions to sustainability at Pii. These are having a strong foundation in a safe environment for our staff, business continuity, crisis management, corporate social responsibility beyond our finances, and operational excellence for continuous improvement. These layers work together to make us a flexible CDMO and create capacity creatively to reduce lead times. Growing capacity comes in different forms, such as leadership capacity, scientific capacity or manufacturing capacity.

Q: Can you provide an example of how Pii helped a client reduce its lead time?

A: When a CDMO accelerates the timeline to deliver results, it adds agility to the healthcare supply chain, making it more responsive to drug sponsors and patients. An example of this is Cure HHT, a foundation representing a group of patients and their families, suffering from Hereditary Hemorrhagic Telangiectasia. A pharmaceutical company discovered side effects during clinical trials that might mitigate or even cure the rare hereditary blood disorder, but due to a variety of unexpected circumstances, the program was never completed. However, a clinician who had worked on the original project began working with Cure HHT to re-establish the project. An IND application was filed, but the FDA rejected it for lacking adequate testing and controls. The molecule, a BCS Class II, had originally been developed as 200mg and 400mg tablet formulations for a different indication. The client believed that a 25mg dose was needed for the rare disease, but they required a fully developed, tested, and properly documented formulation and regulatory support to properly file an IND application with the FDA. The most significant challenge was the work needed to be done in an extremely short timeframe. Pii's development, analytical, CMC experience, and ability to work collaboratively for rapid solutions overcame the challenges and filed the IND 14 days after starting the project.

Q: What does it mean to "reimagine" drug development?

A: When I think about reimagining drug development, I think of it in the context of the current pandemic. The whole thing has opened up a huge debate as to how companies can rethink their capabilities and strategies. Drugs needed to be developed and distributed. To reimagine something, especially something as complex as bringing a scientific concept to commercialization, you must start with the question, "what if?" COVID-19 has delivered a devastating blow to humanity. But the pandemic has also served as the catalyst for "what-if" questions. The drug development process has been reimagined as something that can be done in months rather than years. And this success has been achieved by more than just money. Organizational structures, processes, FDA responsiveness, clinical trial recruiting, and protocol execution were transformed in response to the urgency for a vaccine and treatments. The COVID vaccines development effort caused us to reorganize to co-develop a drug and coordinate with clients to make sure to reduce dependencies on sources, such as ingredients. The silver lining to the challenges of the pandemic is adoption of new accelerating technology to reduce commercial development time, bringing important products to patients faster. Also, we are managing knowledge and analyzing data more effectively and efficiently with integrated data acquisition systems. I am excited to apply the lessons learned from this rapid vaccine development and mRNA technology and apply them to other diseases and healthcare challenges. So, because of COVID-19,

we are reimagining everything – from technology to ways of working together to be more agile and flexible.

Q: Will the speed of developing a COVID-19 vaccine make it difficult to return to traditional development timelines?

A: When you learn to run, it is difficult to go back to walking. Before 2020, we only talked about what it would take to significantly reduce the time to bring a drug to market. Now that it has been achieved, many of the reasons given for the time it has traditionally taken to develop a drug therapy are no longer valid. It will be difficult for us all to accept going in reverse. Also, the credit for these recent COVID vaccine achievements goes to the people in this industry. I work with incredibly gifted people, their tremendous knowledge and experience directs their efforts. Even when the answers are not clear, they are able to navigate challenges with scientifically sound solutions.

Q: Based on what you just described, how will the role of a CDMO change? And more specifically, how will Pii adapt to that change?

A: Traditionally, CDMOs have been considered service providers. However, I see the role of CDMOs as collaborators in the development process and the entire pharmaceutical ecosystem, or supply chain. Rather than a contractual relationship, the CDMO is a partner that takes the time to understand what outcomes the drug sponsors are seeking and how patients will be better served when a drug therapy is successfully delivered to them. This requires business continuity between the CDMO and the drug sponsors, and when this is achieved, amazing results can be achieved faster. At Pii, we believe challenges frame opportunities, and so our culture is one that embraces, even seeks, complex development and manufacturing programs. We enjoy working with complex formulations, and this has given us the knowledge and skills to solve problems associated with them. It all comes down to bringing a drug to market safely and efficiently, but still ensuring compliance. To quote a former colleague: "Compliance is not an option." ◆

IMMUNE ACTIVATORS

Enhancing Cell Adhesion to Safely Improve Effectiveness of Vaccines & Cancer Immunotherapies

By: Siddhartha De, PhD, and Peter Vanderslice, PhD

INTRODUCTION

7 Hills Pharma, of Houston, TX, is focused on the use of proprietary, orally available compounds that can activate the immune system to enhance the effectiveness of vaccines as well as immuno-oncology therapies for cancer, especially in patient populations that are most vulnerable to disease.

The company is one of seven start-up companies in the US, Canada, and Europe participating in the Johnson & Johnson (J&J) Innovation – JLABS Blue Knight collaboration with the Biomedical Advanced Research and Development Authority (BARDA) to help battle the COVID-19 pandemic.

BARDA is a component of the Office of the Assistant Secretary for Preparedness and Response in the US Department of Health and Human Services. The Blue Knight collaboration is designed to identify and accelerate development of innovative therapies and diagnostics to address global health security threats. It is anticipated that 7 Hills Pharma and the other six participating companies combined will receive approximately $500k and mentorship from J&J and BARDA.

SARS-CoV-2, the virus that causes COVID-19, first appeared in late 2019 and erupted into a global pandemic infecting more than 100 million people and killing more than 2 million as of the end of January 2021, according to the Johns Hopkins University Coronavirus Resource Center. In the US, 25.8 million people have been infected and more than 430,000 have died.

In response to the urgent need to battle the pandemic, 7 Hills Pharma launched an evaluation of its lead immunostimulant, 7HP349, an oral small-molecule integrin activator, for use with COVID-19 vaccines. 7HP349 already was in development for use with vaccines for influenza.

Both vaccine programs target elderly populations whose immune systems have weakened with age. Age-related immune deficiencies are the reason 50% of older adults do not respond to protection from influenza vaccinations, and the same will likely occur with COVID-19 vaccinations.

7HP349 activates a class of receptors called integrins that mediate cell-to-cell adhesion, which is necessary for generating immune responses, including those required for vaccine effectiveness. This process, however, deteriorates with age. 7HP349 directly activates integrins in an effort to overcome age-related cell adhesion deficiencies. Integrin-mediated cell adhesion is essential for any immunotherapy, including immuno-oncology treatments.

7 Hills Pharma launched Phase 1 safety trials of 7HP349 in October 2020. Phase 2 trials of the immunostimulant with COVID-19 and influenza vaccines and immuno-oncology drugs are expected to begin in 2021.

7HP349 HARNESSES THE POWER OF IMMUNOTHERAPY

The immune system is made up of several cell types, such as lymphocytes, neutrophils, and macrophages, which work together to protect the body from infectious diseases and cancer.

Upon recognition of a foreign agent or malignancy in the body, these immune cells are pivotal for generating a cascade of

protective defenses to clear the body of the unwanted invaders. The immune system orchestrates this response with integrin-mediated cell adhesion between cells that present the immune system with antigen signatures derived from the infectious agent or malignancy and defending cells that communicate, coordinate, and migrate toward the site of the infectious agent or malignancy to fight and eliminate it.

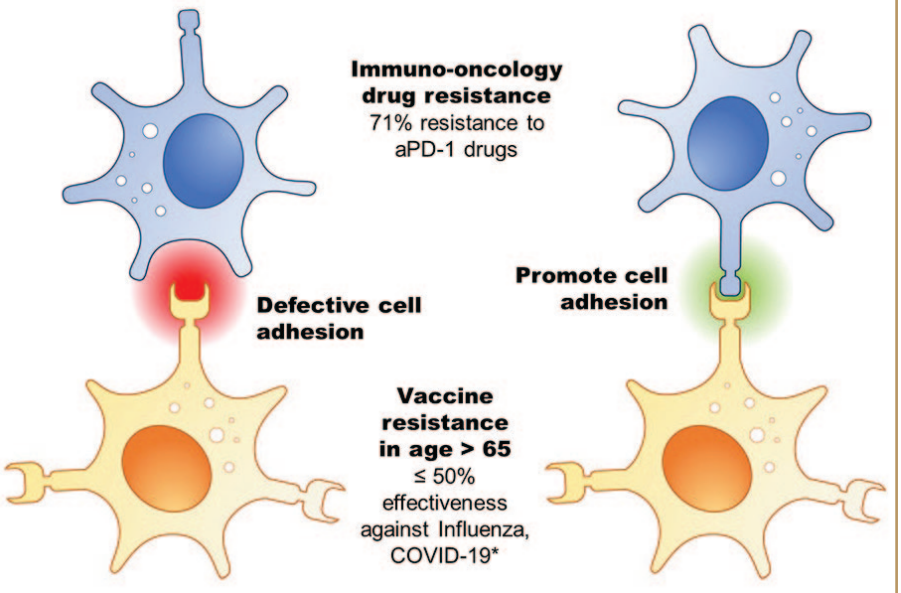
Whether using immunotherapies to target infectious agents or cancerous tumors, enabling cell adhesion is essential to harness and direct the power of a strong immune response to the diseases (Figure 1). As immune system cells age, they become less capable of mounting responses over time.

7 Hills Pharma's investigational new drug (IND) application for 7HP349 has been activated by the US FDA. A Phase 1 randomized, placebo-controlled clinical trial to evaluate the safety, tolerability, and pharmacokinetics of 7HP349 was initiated in October 2020. Phase 2 clinical trials with vaccines for COVID-19 and influenza, as well as with immune checkpoint inhibitors for the treatment of melanoma and other solid tumor cancers are expected in 2021.

INCREASING VACCINE ANTIBODY PRODUCTION TO PROTECT THE MOST SUSCEPTIBLE POPULATIONS

7 Hills Pharma has in-licensed a library of more than 600 first-in-concept, small molecules developed by the Texas Heart Institute that activate integrins α4β1 and αLβ2, which are essential for cell adhesion. These receptors are critical for stimulating both humoral and cellular immunity and can be targeted to strengthen the antigen-specific immune responses of any immunotherapy.

FIGURE 1



Promoting cell adhesion improves immunotherapy.

7HP349 and its related compounds are the only immunostimulants of both α4β1 and αLβ2, generating the potential for significantly better immunity. These integrins have been clinically established as therapeutic targets for inhibition to treat inflammatory diseases and autoimmune disorders, but 7 Hills Pharma is the first to activate these same integrins for enhancing immune responses.

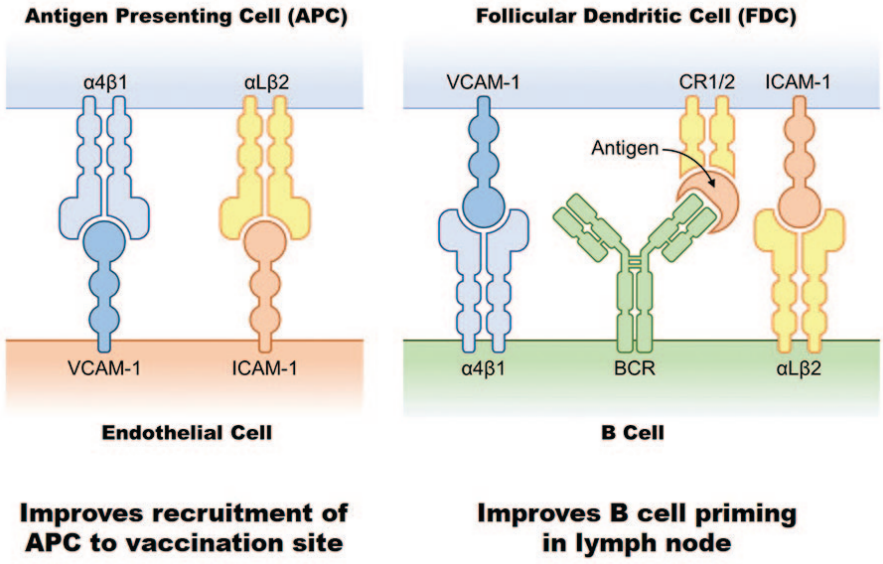
Although kick-starting immune responses may suggest risks of harmful overstimulation, the company's robust preclinical research has established a positive safety profile for 7HP349. 7 Hills Pharma studied the effects of its immunostimulant in autoimmune mouse models. Addition of 7HP349 at concentrations much higher than therapeutic doses did not exacerbate autoimmune side effects.

7HP349 is an allosteric activator that binds directly to α4β1 and αLβ2 on immune cells, such as B cells (Figure 2) and T cells (Figure 3), to strengthen and prolong adhesion to the endogenous ligands, VCAM-1 and ICAM-1, on antigen presenting cells. These specific cell-to-cell interactions are responsible for generating antibody production, T cell activation, and T cell memory, all of which are critical requirements for a vaccine to be effective against infectious diseases.

In preclinical studies, 7HP349 has demonstrated effectiveness in augmenting vaccine responses against multiple infectious agents, including a protein subunit vaccine for SARS-CoV-2, an inactivated virus vaccine for influenza, a DNA vaccine for Trypanosoma cruzi (Chagas disease), and a live attenuated vaccine for Mycobacterium tuberculosis (Tuberculosis).

7 Hills Pharma is the first to conceive of using integrin activators to improve vaccines. There are no direct competitors. Although some vaccines are strong enough to elicit an immune response on their own, most are used with admixed vaccine adjuvants or immune stimulants. The unique mechanism of action of the company's proprietary compounds suggests broad applicability to any existing vaccine and

FIGURE 2



7HP349 improves humoral responses to vaccinations.

the fact that such compounds are dosed systemically, independent of the vaccine, means there would be no need to reformulate existing vaccine stockpiles.

Preclinical studies suggest 7HP349 can be used as an immunostimulant for any vaccine with the potential not only to augment its effectiveness, but also accelerate immune system response, allow for dose sparing, and reduce or eliminate the need for boosters.

The compound has demonstrated the potential to be the first systemic drug used to improve vaccine effectiveness without any change in standard of care for administering the vaccines. By reducing or eliminating the need for booster shots, 7HP349 also could address concerns about a global shortage of COVID-19 vaccines that require each person to receive two doses.

BREAKING DOWN RESISTANCE TO IMMUNO-ONCOLOGY DRUGS

The major challenge posed by cancer always has been its ability to evade detection by the body's immune system, leaving patients without a primary defense against the disease. Immuno-oncology drugs are designed to unmask the malignant cells and expose them to attack by the immune system's killer T cells.

Immuno-oncology drugs such as PD-1 checkpoint inhibitors have become the frontline standard of care for advanced melanoma and other solid tumors. But the treatment's lack of widespread effectiveness is caused by primary and adaptive resistance, resulting in ineffective migration of killer T cells to the site of tumors.

One of the most important predictors of cancer-related mortality for numerous solid tumor cancers is the failure of immune cells to access the tumor. In preclinical research, 7HP349 reversed resistance to checkpoint inhibitors in resistant mouse tumor models.

These preclinical studies demonstrate that 7HP349 augments the effectiveness of immune checkpoint inhibitors and immunogenic doses of local ionizing radiation.

7HP349 not only enhances trafficking of the T cells into tumors, but also may improve the immune response to tumor antigens resulting in enhanced T cell activation similar to the immune response to foreign antigens generated by vaccinations.

If successful, 7HP349 has the potential to change the standard of care treatment for solid tumor cancers targeted not only by checkpoint inhibitors, but also by other forms of immuno-oncology therapies such as CAR-T cell therapy.

PIONEERING PATENT ESTATE

Due to the novel biology of this class of compounds, 7 Hills Pharma has received broad patent protection from the US Patent and Trademark Office (USPTO) on four patents covering the use of the company's integrin activators with any therapeutic antibody, any checkpoint inhibitor for immuno-oncology, and any vaccine.

The technology was co-invented by scientists at 7 Hills Pharma and the Texas Heart Institute. The company's four patents are:

- No. 10,342,866, which covers the composition claims of any integrin activator and any therapeutic antibody.
- No. 10,709,781, which covers composition claims of any integrin activator and any immune checkpoint inhibitor.
- Nos. 10,709,780 and 10,716,849, which cover the composition claims of

"In response to the urgent need to battle the pandemic, 7 Hills Pharma launched an evaluation of its lead immunostimulant, 7HP349, an oral small-molecule integrin activator, for use with COVID-19 vaccines. 7HP349 already was in development for use with vaccines for influenza. Both vaccine programs target elderly populations whose immune systems have weakened with age. Age-related immune deficiencies are the reason 50% of older adults do not respond to protection from influenza vaccinations, and the same will likely occur with COVID-19 vaccinations."

any vaccine, including one of the company's novel integrin activators, and an antigen and method claims for administering the vaccine compositions, respectively.

The issuance of these patents indicates that the USPTO recognizes the fundamental role of integrins and the unique functionality of the company's integrin activators in allowing such broad claims. The patent coverage may enable 7 Hills Pharma to become an essential partner for immuno-oncology and vaccine developers.

In addition, the company is developing 7HP349 for improving umbilical cord blood (UCB) transplants with a $1.9 million Phase 2 STTR (R42) grant from the National Heart, Lung, and Blood Institute of the US National Institutes of Health (NIH).

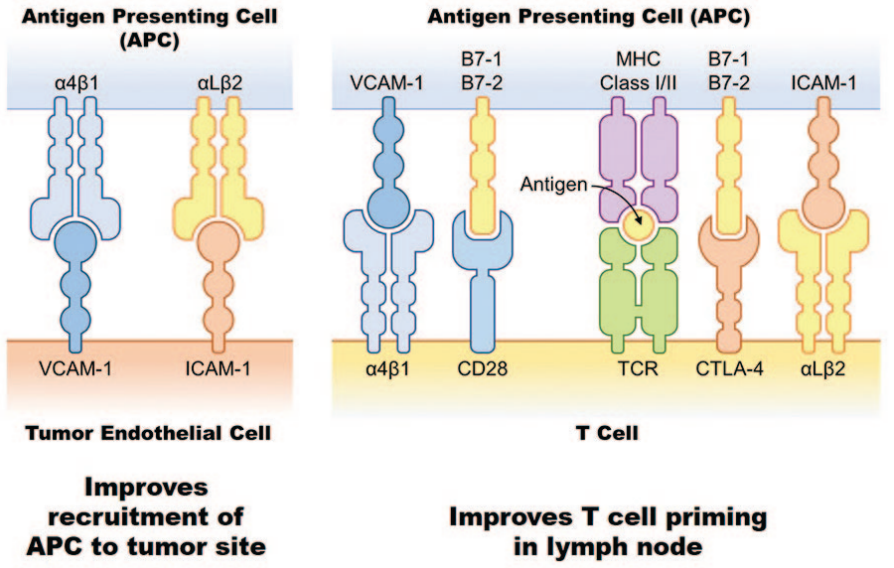
The UCB program is aimed at making cord blood transplants a more viable alternative to bone marrow and peripheral blood as a source of stem cells for transplant in treatment of blood cancers and genetic diseases. The major advantage to the use of cord blood cells is that a close donor-patient match is not required. The problem, however, is that it contains fewer stem cells than bone marrow and peripheral blood, which means it takes longer to reconstitute patients' immune systems, leaving them vulnerable to infections. In preclinical studies, 7HP349 has demonstrated it can overcome this shortcoming by reducing the time of immune system reconstitution.

SUMMARY

Integrin-mediated cell adhesion is essential for establishing the immune system's cell-to-cell interactions necessary to mount the body's natural defenses against infectious diseases and cancer.

The immune system has the potential to independently respond to insults, but in most cases, help is required. Vaccines are designed to train the immune system to

FIGURE 3



7HP349 improves T cell responses to immuno-oncology drugs.

recognize specific infectious agents. Similarly, immuno-oncology drugs unmask cancers that evade the immune system.

Despite the availability of such immunotherapies, a large number of people do not respond to treatment, primarily due to a weakened or resistant immune response. 7 Hills Pharma is developing oral small molecule compounds, administered separately and systemically, to give immunotherapies the necessary assistance to improve effectiveness for more people.

The company's integrin activators are the first of their kind to be deployed as immunostimulants for vaccines against COVID-19 and influenza and for immune checkpoint inhibitors against malignant melanoma and other solid tumor cancers.

7 Hills Pharma received a 2-year, $2-million Small Business Innovation Research (SBIR) grant from the US National Institutes of Health to support Phase 1 safety trials of 7HP349. Phase 2 trials of the immunostimulant to augment the effectiveness of COVID-19 and influenza vaccines, as well as checkpoint inhibitors, are expected to begin in 2021.

The company is seeking partners to pursue all three indications (aPD-1 resistant tumors, geriatric seasonal influenza, and geriatric COVID-19) to establish 7HP349 proof-of-concept as a safe, systemic immune activator and seek partnerships with vaccine and immuno-oncology producers for what could be a game-changer for immunotherapies. ◆

To view this issue and all back issues online, please visit www.drug-dev.com.

BIOGRAPHIES

Dr. Siddhartha De is a pharmacologist by training and is the Senior Director of Development at 7 Hills Pharma, where he has been deeply involved with the preclinical development of 7HP349 and its preparation for clinical readiness. He earned his PhD in Biochemistry in 2001 from the University of Mississippi School of Medicine, during which he elucidated the role of different H1 histone variants in the architecture of chromatin in the cell nucleus, and the functional consequence of this chromatin organization on nuclear processes, such as transcription, replication, and recombination. After his PhD, he worked at the University of Virginia for his post-doctoral research work, where he focused on the role of estrogen and androgen receptors in cancer, and at Luna Innovations to kick-start different Department of Defense (DoD)-funded programs to address homeland defense and counter bioterrorism. For the next several years, he worked for the drug discovery and development operations at Eurofins Advinus, where he headed in vitro Discovery Biology and the CNS therapeutic area, and led several programs to clinical candidacy. Additionally, he has worked in Project Management and Business Development, representing outlicensing strategy, partnerships, and contract services.

Dr. Peter Vanderslice is the Director, Biology of the Molecular Cardiology Research Laboratory at the Texas Heart Institute in Houston, TX, and is a 7 Hills Pharma Co-founder, investor, and an inventor of the company's technology. He has spent over 25 years leading teams focused on the development of small molecule compounds that bind and modulate the function of integrins, selectins, and chemokine receptors. He has authored numerous peer-reviewed publications, reviews, and book chapters focusing on the biological function and therapeutic targeting of cell adhesion molecules. Much of his professional career has been in the pharmaceutical industry, where he gained extensive experience with each stage of the pipeline from discovery to progression into clinical trials. As Senior Director of Drug Discovery at Encysive Pharmaceuticals, he led teams developing therapeutics for autoimmune and inflammatory diseases. Three such programs resulted in compounds entering clinical trials. He joined the Texas Heart Institute in 2008 and has continued to discover and characterize integrin-targeted small molecules as potential therapeutics. These include a family of integrin activators, licensed to 7 Hills Pharma, that function as immune stimulants. He earned his PhD in Biochemistry from the University of Texas at Austin and trained as a Parker B. Francis post-doctoral fellow in the Cardiovascular Research Institute at the University of California, San Francisco.

Special Feature

Improving Bioavailability & Solubility: Each Molecule Is Unique

By: Cindy H. Dubin, Contributor

The virtual 2020 Global Drug Bioavailability Enhancement Summit this past December showcased novel technologies and platforms aimed at addressing bioavailability and solubility challenges. These included techniques like electrospinning, mesoporous silica technology, physiochemical optimization, amorphous nanoparticle engineering, and pharmacokinetic modeling. And the end goal is the same: Save time and money while accelerating formulation development.

"The driver for novel strategies to resolve solubility issues, with a target of improving bioavailability, often stems from a requirement to get more information about the key relationships using less API, in a reduced time frame," says

Helping companies with solubility challenges, from candidate selection through to commercial launch (Quotient Sciences).

Andrew Parker, PhD, Director Open Innovation, Small Molecules, Oral and Speciality Drug Delivery, Catalent. "This has led to the implementation of 'miniaturized' approaches such as small scale micro-dissolution testing, working with very small amounts of material and establishing accelerated stability predictive modeling that can shave months off a development timeline."

This annual *Drug Development & Delivery* report introduces readers to some novel approaches to improving bioavailability and solubility that have one commonality: they treat each molecule as unique.

Adare Pharma Solutions: Proprietary Solutions Enhance Bioavailability

While most of the bioavailability-enhancing technologies are directed toward achieving immediate-release after oral administration, Adare has devoted significant efforts in going beyond immediate release.

"We realized early on that the magnitude of pH dependency of solubility could be as high as several log orders," says Jin-Wang Lai, PhD, senior director in R&D, Adare Pharma Solutions. "Such dramatic change in solubility poses great challenges in the design of an extended-release dosage form using Adare's Diffucap®-coated pellet technology."

Where drug solubility is inadequate to generate the necessary driving force for drug release, it creates a typical 'no release, no bioavailability' situation, explains Dr. Lai. One approach to overcome such a pH-dependent solubility issue in the case of an alkaline drug is to use an organic acid as the drug core or an acid layer prior to using it as the substrate for the drug layer, which is then followed by a rate-controlling membrane. "As water permeates into the organic acid element creating a solution of organic acid, the alkaline drug is dissolved due to the acidity generated by the dissolved acid as it moves through the drug layer and out of the coated pellets." This microenvironment pH approach was granted a US patent.

A second approach to overcome solubility/bioavailability is to enhance solubility by adapting solid dispersion/solid solution technology to the Diffucap concept. For the coated pellet technology, the drug layer typically is sprayed as a solution or a suspension onto the pellet cores. The amount of polymer binder used is usually low relative to that of the active pharmaceutical ingredient (API), in about 1/10 weight ratio. The polymer selected serves only as a vehicle for the API to be attached onto the pellet core. When enhanced solubility is necessary, the binder polymer is strategically selected based on its ability to form a solid dispersion/solid solution with the drug, usually through a solvent system.

"Adare leveraged its solvent-handling capability, which is a natural extension of Adare's Microcap® technology, using cyclohexane to overcome the solubility challenge through the well-established solid dispersion/solid solution approach," says Dr. Lai. A US patent was recently granted for this approach.

Another related technology developed by Adare for the purposes of bioavailability enhancement is "Biorise®." The Biorise technology is uniquely characterized as a material state between a solid dispersion/solid solution and nanocrystals. Biorise can be achieved through a media-grinding process or a solvent process, and can achieve immediate release.

In addition to Diffucap, Microcap, and Biorise, Adare is equipped with a spray dryer, a hot-melt extruder, and a lipid-based melt congealing technology called Optimμm®. He says these technologies are designed to be used together for greater flexibility in designing release profiles for customers.

Adare also announced the launch of a small non-GMP laboratory for the exploration of various technologies that may be suitable for solubility/bioavailability enhancement. "The new laboratory space brings customizable, flexible systems that will expedite formulation and process development services," he says.

Ascendia Pharmaceuticals: Tech Platforms for Tough-to-Formulate Compounds

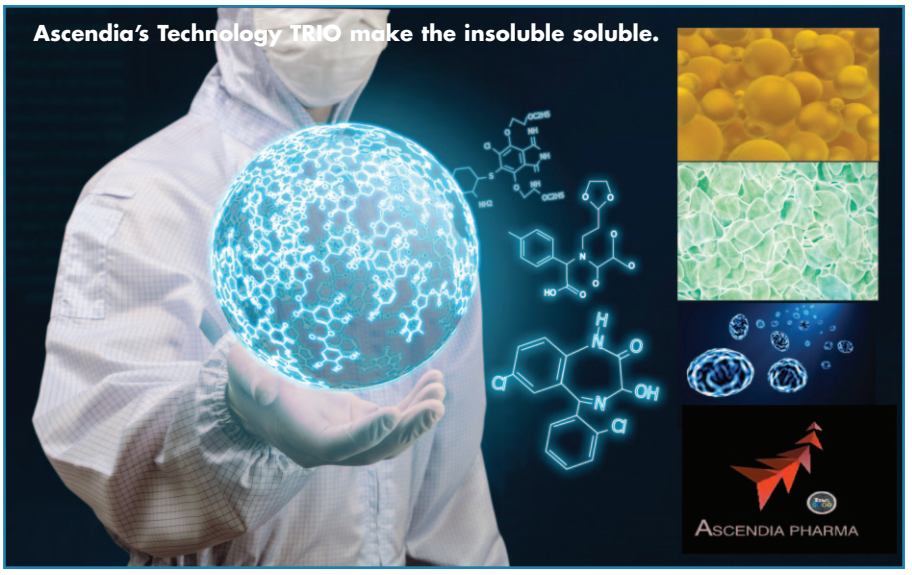
The number-one impediment factor to improve bioavailability and solubility is the intrinsic properties of the compounds, particularly for compounds with low permeability through the GI membrane, i.e. BCS IV compounds. "We may improve the solubility of these compounds, however, due to intrinsic low permeability of those compounds to penetrate the intestine membrane before being absorbed into the body, challenges remain to

improve the oral bioavailability of those compounds," says Jim Huang, PhD, CEO, Ascendia Pharmaceuticals.

Dr. Huang says Ascendia achieves systemic absorption of For BCS IV compounds in several ways: to utilize permeation-enhancers, including lipid, surfactant, and other GRAS excipients using its technology platforms to open tight junction or enhance absorption through GI membrane or lymphatic routes after oral administration; to explore intranasal route of administration to overcome the GI barrier and potentially to overcome the blood brain barrier or GI membrane barrier; and to develop injectable or topical dosage forms that can achieve high drug loading and achieve high systemic bioavailability.

Ascendia routinely utilizes its platform technologies, NanoSol (nanosuspension), AmorSol (amorphous nanoparticles), and EmuSol (nanoemulsion) to solve over 90% of difficult-to-formulate compounds by enhancing bioavailability and solubility of compounds for different routes of administration.

As an example, Ascendia recently received a request to formulate a compound just out of the discovery labs with no preformulation or animal PK data, with a time window of three months and limited API supply. Dr. Huang explains that Ascendia was able to perform essential preformulation studies to understand the compound's properties related to solubility and *in vitro* performance; utilize Ascendia's Technology Trio (EmulSol, NanoSol, and AmorSol) to screen the prototype formulations within the three-month window; and obtain a multiple-fold increase in oral exposure in animal models, utilizing multiple formulation principles that enabled a smooth and rapid transition of the project in the IND-enabling Tox study.



BASF Pharma Solutions: New Module Estimates Oral Bioavailability Based on Computed Molecular Properties

The number-one requirement for improving bioavailability and solubility of a drug is understanding the physiochemical properties of drugs in relation to human physiology, says Nitin Swarnakar, PhD, Global Technical Marketing, BASF Pharma Solutions. Drugs categorized as poorly soluble (mainly BCS class II) can show either dissolution rate-limited or solubility-limited absorption, and the final formulation considerations and requirements are different, depending on the driving reason for the drug not fully dissolving in GI conditions. Drugs classified as BCS class III and IV exhibit permeability-limited absorption, where there is poor absorption due to Pgp efflux, drug first-pass metabolism, the API's physiochemical properties, and other factors. Here, the final formulation strategy must assist in addressing the low permeability.

"Therefore, before a successful and robust formulation can be developed, a mechanistic understanding of the reason for poor bioabsorption is critical to overcome the solubility and bioavailability problem," says Dr. Swarnakar. "In order to select the right strategy to improve drug delivery, it is first paramount to know the cause for poor solubility of an API. Once you identify the cause, you can best identify your formulation's dosing, delivery route, dosage form, and processing requirements."

To help formulators understand their APIs and facilitate the right strategy approach, BASF Pharma Solutions recently launched the *Developability Classification of Active Ingredients* module in its virtual pharma assistant ZoomLab™ platform. This module gives formulators the ability to simply enter a few physical properties about their API, and, in return, the module gives recommendations on developing a formulation for the API, includ-

ing additional formulation work or dosing adjustments that might be required to achieve the desired solubility and bioavailability. By combining user-entered information on solubility, permeability, and dose, the module estimates the oral bioavailability based on computed molecular properties and provides formulation options according to the assigned DCS. These recommendations can cover an adjustment on particle size, maximum dose, and more. "In this way, a significant amount of pre-formulation work can be overcome via time- and money-saving digital formulation recommendations," says Lindsay Johnson, PhD, Global Technical Marketing, BASF Pharma Solutions.

In addition to BASF's virtual pharma assistant like ZoomLab, the technical team supports a wide range of formulation technologies. "Many pharmaceutical companies have collaborated with BASF to improve drug solubility using functional inactive excipients such as Kolliphor® ELP, Kolliphor® RH 40, Kolliphor® HS 15, Soluplus®, and Kollidon® VA 64," says Dr. Johnson. "Our experts are trained to help improve efficacy, safety, and patient compliance through a range of technologies, including hot-melt extrusion, lipid-based drug delivery formulation, spray drying, nanomilling, and drug layering. These technologies can provide drug product development strategies to innovator companies, and new regulatory application pathways for generic companies, to overcome the solubility and bioavailability challenges of the starting API."

A Candoo formulation scientist performs coating of tablets.

Candoo Pharmatech Company Inc.: Accelerating Molecules to Medicines

Poorly water-soluble drugs often demonstrate inadequate and/or inconsistent *in vivo* exposure in nonclinical and clinical studies, leading to suboptimal therapeutic performance together with significant food effect. Many promising compounds can be terminated prematurely if their bioavailability results are not interpreted properly from formulation and biopharmaceutics perspectives.

As a technology-driven contract research organization (CRO) in Canada providing formulation and drug delivery solutions to small and large molecules, Candoo Pharmatech Company Inc. believes the number-one impediment to improving bioavailability and solubility of drug candidates is lack of a clear-cut formulation strategy. Each molecule is unique. To accelerate molecules to medicines, Candoo applies a risk-based formulation strategy successfully to the development of many new chemical entities in nonclinical and clinical stages. This involves three steps: measure and understand the physicochemical and stability profiles, biopharmaceutical properties, and target doses of lead compound; evaluate and identify the risks for adequate oral absorption, figuring out the absorption-limiting factors; and design a compound-specific formulation and manufacturing process to get sufficient bioavailability with a control strategy in place.

"Candoo's risk-based formulation strategy aligns nonclinical and clinical formulations, maximizes bioavailabil-

ity right the first time, and expedites first-in-human (FIH) studies," says Yongqiang Li, PhD, Chief Executive Officer, Candoo.

For a lead compound, having a thorough understanding of three biopharmaceutical properties – permeability, biorelevant solubility, and target dose – and their interplay, is essential to this strategy, he says. If oral absorption is dissolution-limited, micronization or nanosizing of the active compound can achieve optimal therapeutic effect. Otherwise, if oral absorption is solubility limited, enabling and solubilization formulations such as amorphous solid dispersions are necessary to enhance solubility and bioavailability of the compound.

As an example of this strategy, a client wanted to develop a tablet formulation using compound CD-8. However, incomplete, variable, and contradicting bioavailability results were reported in the nonclinical studies. CD-8 is a crystalline neutral compound with a relatively high permeability in Caco-2 cells (P_{app}= 1.2 x 10^{-4} cm/sec) but low solubility (98 μg/mL) in fasted state simulated intestinal fluid (FaSSIF, pH6.5) and high solubility (790 μg/mL) in fed state simulated intestinal fluid (FeSSIF, pH5.0).

Candoo evaluated the factors affecting oral absorption of CD-8, reviewed previous formulations and animal studies, and discovered that target dose, right formulation, and control strategy were critical to address the observed bioavailability and variability issues, explains Dr. Li. Initial doses were below 50mg/tablet. Candoo micronized the compound (D_{90}<10μm) and formulated the tablets with good dispersing and wetting agents. Control strategy on particle size of CD-8, formulation, and process were developed to ensure reproducible *in vivo* absorption. Linear and satisfactory absorption profiles were obtained. However, the micronization approach failed when the dose was increased to 100mg/tablet.

Consequently, Candoo developed the HPMCAS-based amorphous solid dispersion formulation and achieved adequate bioavailability for the compound. "This demonstrates that Candoo's risk-based formulation strategy can ensure sufficient absorption and margins for promising compounds, enable nonclinical studies to be applicable to clinical development, and accelerate the transformation of more novel molecules into medicines," says Dr. Li.

Catalent: Identify the Relationship Between Physicochemical & Biopharmaceutical Properties

"It is difficult to completely deconvolute the solubility challenge from the bioavailability challenge as both have mutual and complex interdependencies with other factors such as molecule permeability, kinetic solubility constraints, pH variation along the gastrointestinal tract, food effects, and first-pass metabolism," explains Andrew Parker, PhD, Director Open Innovation, Small Molecules, Oral and Speciality Drug Delivery, Catalent. "Generally, the main challenge for any new molecule is understanding the dictating relationships that will dominate final exposure in humans."

During the course of pre-clinical and early clinical evaluation of a new chemical entity (NCE), the inter-relationships of physicochemical and biopharmaceutical properties of the NCE and their impact and dependencies on final bioavailability should be considered, he continues. This is often complex for a NCE, but addressing modeling (both *in silico* and *in vitro*); coupled with targeted characterization and analytical testing means that relationships can be better understood, and the correct formulation strategy can be identified and implemented based on a working understanding of the critical relationships at play.

Physiologically-based pharmacokinetic (PBPK) modeling of early animal data is pivotal to help understand the overall picture and how difficult the challenge is. Factors such as whether a bioavailability enhancement approach is required to improve solubility, what the starting dose should be for first time in-human administration, and what initial formulation strategy to use should be considered. "Regarding the latter, considerations can include overcoming issues such as adverse pH-dependent solubility profiles or better processing of the NCE material to make it more amenable for dosing, such as size reduction or co-processing with other excipients to prevent aggregation, which is associated with a lack of potential exposure," says Dr. Parker. "From this and the initial clinical testing, the relationships can start to be further resolved."

The most common customer scenarios that Catalent has observed are requests for creating a development

Early development capsule filling at Catalent's facility in Nottingham, UK, one of the company's Centers of Excellence for small molecule development.

strategy for preclinical toxicology and first-in-human studies for poorly soluble drugs, says William Wei Lim Chin, PhD, Manager, Global Scientific Affairs, Catalent. Usually, the major challenges revolve around limited budget and limited amounts of API.

"We would typically perform a developability assessment to find out where the compound lies in the developability classification system (DCS) and estimate its solubility-limited absorbable dose (SLAD)," Dr. Chin explains. "If the intended dose is above the SLAD, it suggests that the limited intrinsic solubility of the drug is the limiting factor for absorption, therefore bioavailability enhancement through formulation would be considered."

The suitability of each type of formulation approach would then be assessed, coupled with PBPK modeling based on *in vitro* and *in silico* data to rule out first-pass effect and predict human exposure. The result of this assessment is a recommendation of a path forward to reach toxicology and pharmacokinetic studies, and ultimately, first-in-human clinical studies.

"This recommendation is unique to different drugs, which means the bioavailability-enhancing formulation options depend on biopharmaceutical properties, stability, and processability of the drug," says Dr. Chin.

Cyclolab Ltd.: Replacing Outdated Inactives with Cyclodextrins in Parenteral Formulations

A major challenge for drug developers is coping with the lipophilicity of active pharmaceutical ingredients. Among the various strategies of formulating drug substances in a patient-friendly way (i.e. developing solvent- and surfactant-free compositions), introduction of colloidal carriers (typically 30-200nm), such as liposomes, may be a feasible option. However, intravenous use of such nanostructures is often associated with the potential risk of anaphylaxis.

On the contrary, cyclodextrins (CDs) falling in the size range of the smallest colloids (1nm in diameter) do not typically possess this drawback, explains Dr. Istvan Puskas, Research Chemist, Cyclolab Ltd. CDs may form complex with hydrophilic domains of hard-to-formulate drug molecules. By covering the lipophilic domains of the guest molecules, a non-covalent and reversible physical binding occurs, resulting in enhanced water solubility. In practice, water-insoluble drug molecules were already successfully incorporated into hydrophilic CD complexes in various marketed compositions, enabling the injection of the required dosage quantity in aqueous vehicle without cosolvent.

Hydroxypropyl betadex (HPBCD) and Betadex sulfobutyl ether sodium (SBECD) are types of cyclodextrins that offer the most versatile administration routes. The success of CD complexation strategy may be exemplified by the formulation of different lipophilic drugs with the help of these two CD types. Voriconazole is marketed in various products wherein the solubilization is achieved in lyophilized amorphous matrices consisting of either SBECD or HPBCD. Mostly innovative drugs and those of weak base character (remdesivir, ziprasidone, amiodarone, aripiprazole and delafloxacine) are formulated with SBECD, says Dr. Puskas.

Both types of cyclodextrins are also applied to replace some outdated, toxic, irritative inactive ingredients. "For example, SBECD-containing busulfan formulation does not contain toxic excipient N,N-dimethylacetamide and allergizing polyethylene glycol, which are both present in the traditional marketed product," he says. "SBECD can replace irritating polysorbate 80 in docetaxel prepara-

tions. Cyclodextrins may prevent the formation of toxic degradation products compared to classical solubilizing agents, ensuring a higher patient safety profile. Melphalan in SBECD complex shows superior chemical stability over its traditional propylene glycol-based formulation, and similarly due to the presence of SBECD, fosphenytoin owns significantly longer shelf life as well."

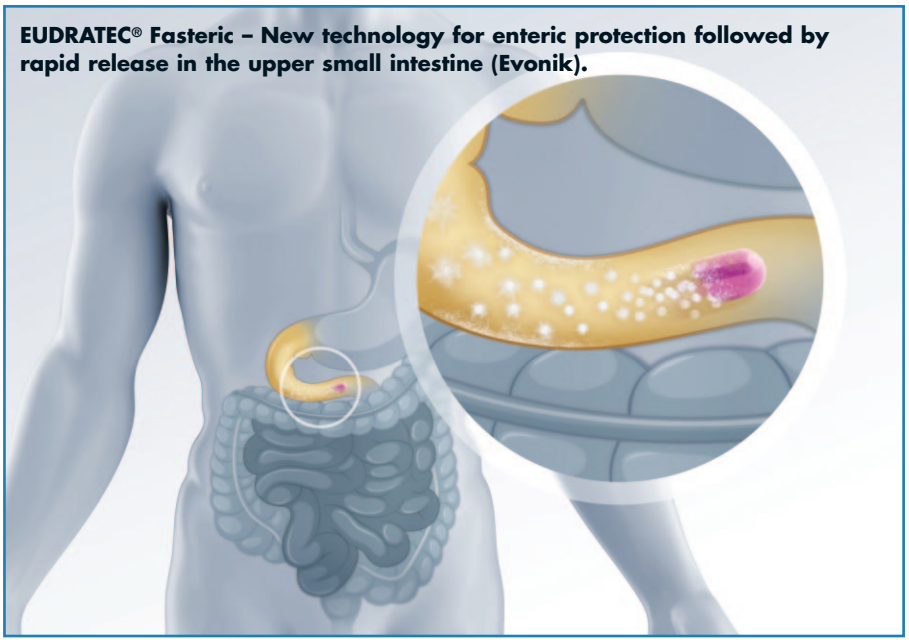
Reduction of threats related to therapeutic intervention will remain the utmost focus of developers in the pharmaceutical industry, which requires the use of the best tolerable auxiliary materials, says Dr. Puskas. "Selection of cyclodextrins for such a purpose pays off in enhanced safety profile without compromise in solubilizing functionality."

Evonik: Toolbox for Delivering New Modalities

COVID-19 has taught us how fast the world can completely change, and that technological progress is more important than ever before. The same is true for drug discovery. "We need a continuously expanding toolbox that can better address new modalities," says Dr. Jessica Mueller-Albers, Strategic Marketing Director for Oral Drug Delivery Solutions at Evonik.

Small molecules continue to become more complex and show reduced solubility. Increasing demand for mRNA therapeutics, antibody drug conjugates, gene therapies, and other specialized or personalized drug products also requires more sophisticated formulation strategies.

She says: "This huge variability in new modalities makes a one-size-fits-all approach to improve bioavailability and solubility impossible. So, a key market goal must be to create or enhance transport mechanisms to precise locations in the human intestinal tract or other sites for more efficient and effective delivery."



EUDRATEC® Fasteric – New technology for enteric protection followed by rapid release in the upper small intestine (Evonik).

Some of the challenges resulting from this paradigm shift are the high molecular weight, the presence of numerous hydrogen bond donors and acceptors, and multiple negative charges that can complicate the ability to access the intracellular site of action, she explains. For example, oligonucleotides have a short half-life and are quickly eliminated. However, physiological functions are not only a barrier to effective delivery but also an opportunity for enhanced nanoparticle design. Advanced liposomes, self-nanoemulsifying drug delivery systems, and nanoparticles enabled with targeted delivery in the gastrointestinal tract are promising emerging technologies with some first-proven success in the market.

Technological progress is also a key element in product development to expedite the formulation of new modalities. Pharmaceutical companies are looking for partnerships during the early phase of development to get access to the newest and most innovative solutions and technologies that can optimize targeting and delivery outcomes for their API. "One example of these new technologies is EUDRATEC® Fasteric, a formulation technology for enteric protection followed by rapid release in the proximal region of the small intestine, the duodenum," says Dr. Mueller-Albers. "Some drugs, such as cancer drugs or immune-suppressants, must minimize exposure to P-glycoprotein transporter, which increase towards the distal region of the intestine."

Mesoporous particles are also a promising vehicle for controlled release and targeted delivery due to their unique mesoporous structure that enables stabilization and functionalizing. This remains a highly effective approach for solubility enhancement and drug targeting, she says.

Hovione: Emerging Platforms Address Extreme Compounds

The industry has a wide toolbox to address bioavailability and solubility issues. Promising technologies may require more time and studies, but provide the opportunity to deliver compounds that would be considered unviable in the recent past.

Multiple platforms have emerged in pharmaceutical development to address bioavailability and solubility challenges. Amorphous solid dispersions by spray drying have become an industry standard, says João Henriques, Scientist, Group Leader, Hovione, R&D Drug Product Development. "This growth is generating a wealth of data that not only provides confidence in the platform, but also creates the foundation for empirical-driven formulation approaches," he says. "The use of prior data allows correlating molecular descriptors of low solubility molecules with formulations that have been successfully developed as solid dispersions. The correlations aid in preliminary prototype definition and evaluating of the likelihood of success of such a formulation approach."

In addition to statistical methods, the use of first-principle models that incorporate thermodynamics of mixing, diffusion, and kinetics of solvent evaporation provide valuable information for *in silico* screening and excluding non-viable formulations. "Both strategies have their advantages and disadvantages and can be used complementary to each other as a valuable tool for formulators to define the best prototypes to screen and reduce development time and material consumption while delivering optimized formulations that provide the required performance, are stable, and commercially viable," he explains.

While the formulation of most DCS2b compounds is presently a well-known and understood challenge, there remains a wide space for solutions to address some extreme compounds that either require significant amounts of stabilizers to maintain the amorphous form or that are not amenable to spray drying with reasonable cost of goods due to low solubility in organic solvents, he states. Alternative production methods of solid dispersions, such as co-precipitation, can address the low organic solubility issue. "Emerging platforms, such as impregnation with mesoporous silica, present an opportunity for molecules that cannot be stabilized in the amorphous form with common stabilizers at reasonable ratios," says Mr. Henriques. "API-loaded silica has been shown to improve amorphous stability of compounds with a high tendency to recrystallize. Additional studies and clinical programs may help establish this technique as a standard alongside lipid formulations and solid dispersions in improving bioavailability and solubility."

"One of the recurrent challenges we face is related to accelerated approvals from the FDA with Fast Track and Breakthrough designations on low solubility compounds," says Mr. Henriques. "This is common for oncology programs that show promising early-stage results and may have reduced clinical study requirements."

Accelerated approvals put an increased amount of pressure on all Chemistry, Manufacturing, and Control (CMC) activities, which must be compressed and de-risked, he says. The use of enabled formulations further increases the complexity of this activity. "Adequate risk assessment and management tools are required because reformulations may compromise all clinical timelines," says Mr. Henriques. "Right-first-time formulation in this case is a significant advantage, and all formulation activities must have a strong sense of the manufacturability and scalability early to ensure seamless transition from clinical to commercial scales."

Lubrizol Life Science Health: Proprietary Technologies for Amphiphilic Molecules

Some biologics require high concentrations to be effective, even though they may be soluble. Formulating at these high concentrations can lead to issues with solubility or viscosity, requiring advanced delivery technologies such as the formulation of nanoparticles or microparticles. These engineered particles flow well even at high concentrations.

Formulation strategy is determined by looking at the physicochemical characteristics of a given active pharmaceutical ingredient (API). For crystalline API compounds with high melting points, a go-to technology for Lubrizol Life Science Health (LLS Health) is nanomilling, which reduces the size and increases the dissolution rate.

"Depending on lipid solubility, nanoemulsions or our proprietary LyoCell® technology can be useful approaches," says Dr. Robert Lee, Presi-

dent, LLS Health, CDMO Division. "LyoCells are better able to accommodate amphiphilic molecules (i.e., those with both a hydrophilic and a hydrophobic region) and are compatible with both small molecules and biologics."

Assessing the feasibility of nanomilling provides clients with a cost-effective and efficient way to determine whether particle size reduction will work for their API, he continues. Nanomilling is well suited for formulating APIs with poor aqueous solubility and has been used in a range of dosage forms, including liquids, capsules, tablets, and injectables. "Because this process is in an aqueous vehicle, it bypasses the problems associated with the large volumes and flammability of organic solvents used in other methods; it is also scalable and commercially validated," he says.

Nanomilled API particles — nanoparticles — can be formulated as liquids, lyophilized powders, or oral solid dosage forms. Solid-lipid nanoparticles (SLNs) are submicron lipid particles that are used as an API delivery vehicle, including for hydrophobic and high molecular weight APIs. "Their cost and recognized safety make them an attractive alternative," says Dr. Lee.

Polymeric nanoparticles are a relatively new addition to the solubility arsenal and have been used with serum albumin to make such drugs as Abraxane®. Additionally, other particulate systems comprised of inorganic compounds — silica, magnetite, gold — in a formulation matrix or coating that incorporates chemotherapeutics or other APIs can improve bioavailability.

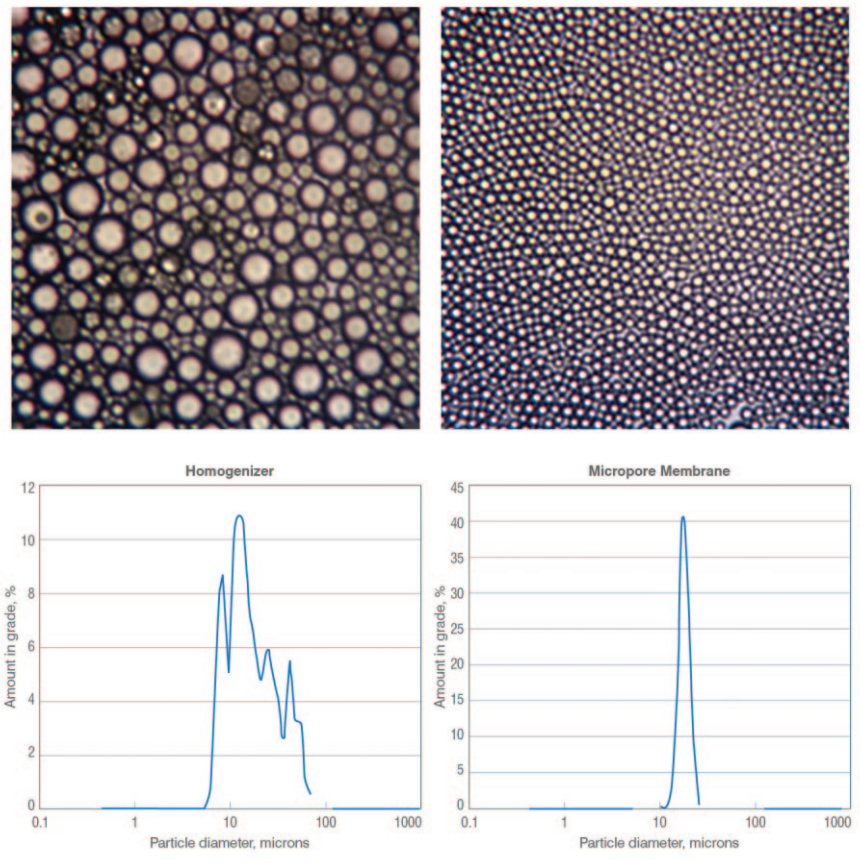
LLS Health has also developed SATx technology, an interpretation of antibody drug conjugates in which the surface of a nanoparticle loaded with a chemotherapeutic agent is decorated with biologics or targeting molecules, such as monoclonal antibodies. The SATx technology has been successfully applied to vaccines, says Dr. Lee."

Micropore Technologies Inc: Improved Encapsulation Controls Bioavailability & Solubility

Injectable drug formulations are designed to deliver drugs directly into a cellular environment, bypassing both the acid environment of the stomach and primary metabolism by the liver, to optimize viable therapeutic effects. Formulation of the API within a predictable sustained-release delivery system can optimize bioavailability at the desired therapeutic dose over a defined period with enhanced patient compliance and improved therapeutic outcomes.

Despite their advantages, since the approval of Lupron (1989) as the first sustained-release injectable drug using PLGA, surprisingly few similar drugs have been authorized by the FDA compared to the thousands of oral formulations approved over the same timeframe, says Tom Murphy, Vice President Sales & Business Development, Micropore Technologies Inc. This, he says, illustrates the scale of the challenge in the development of injectable formulations for clinical use.

There are several issues to con-



The difference in particle size distribution between a rotor stator homogenizer and an Micropore AXF membrane emulsification unit (Micropore Technologies Inc).

sider in the development of the desired bioavailability characteristics from an injectable formulation. "One of the most important is the control of microsphere size to achieve a balance between predictable drug release over the desired time, a good patient experience, and an optimized manufacturing process," says Mr. Murphy. "Uniform microsphere size in any formulation is one of the determinants for controlling bioavailability and solubility of an API."

Because there is no viable sterilization method after the manufacture of microspheres, he says, the entire process needs to be undertaken in aseptic conditions. The simpler the process, the better the compliance and the fewer the non-conformities. "The manufacture of microspheres using traditional homogenization techniques enable the production of high volumes, but at the cost of a wide particle size distribution requiring significant downstream processing and significant yield losses," he says. "Microfluidic techniques offer particle size control, but this precise processing technique is challenging to scale up and suffers from process reliability issues."

Membrane emulsification, at an industrial scale, is a relatively new process offering the high-volume potential of homogenization with the particle size control advantages of microfluidics, he explains. "The technology is available to robustly and reliably delivering a predictably narrow size distribution (coefficient of variation <20%) at a tunable size between 5-500µm through compact precision engineered, GMP compliant devices. The simplicity and flexibility of this new generation of membrane emulsification devices is such that developments can be transferred seamlessly from lab bench for pre-clinical formulation development, through pilot scale, and into GMP manufacturing, opening rapid development opportunities to explore the best way to optimize bioavailability for formulations."

He adds that the gentleness of membrane emulsification enables the production of enhanced microspheres using a double emulsion-based processes with microsphere size variability eliminated, high encapsulation efficiencies, and the preservation of the biological activity of sensitive materials, such as proteins, to be maximized.

Mr. Murphy says: "Together, recent advances in biopolymers and membrane-based manufacturing technologies now enable formulation of injectable drug products to be tailored at will to achieve a target solubility and bioavailability, delivering significantly improved therapeutic outcomes against the target disease."

Pharmaceutics International Inc: Amorphous API Yields Desired Bioavailability

A client approached Pharmaceutics International Inc (Pii) with a micronized poorly soluble drug in combination with excipients that was displaying an incomplete and very slow-release profile. The product needed to be developed as a tablet dosage form. The combination drug and excipient was dissolved in a solvent and spray dried to provide amorphous material. However, the spray dried material was hygroscopic, static, and flowed poorly. Sundeep Sethia, PhD, Senior Director of Research and Development, Pii, explains that for better flow, the material was roller compacted with binder and glidant/lubricant and then milled. The material was then final blended with lubricant and compressed in the tablet dosage form. Precaution was taken to ensure humidity controls during processing and desiccants were used for storage of finished product to ensure stability.

"The amorphous API in the tablets yielded the desired pharmacokinetics profile and much improved bioavailability compared to the micronized API tablets," he says.

Creating an amorphous solid dispersion of an API, along with excipients, using the spray drying and/or HME process increases surface area, and thereby the rate of dissolution, he says. This approach is easily scaled-up based on the properties of drug molecule characteristics, such as solvent solubility and temperature sensitivity. He warns, however, that physicochemical characterization of the solid dispersion on stability and its effect on critical quality attributes (CQAs) must be thoroughly understood.

Micronization is another approach that speeds rates of dissolution. Nanoparticles via media milling, mesoporous silica technology, and physicochemical optimization can increase bioavailability. "Of these, nanoparticles have shown notable success resulting in numerous com-

mercial products," says Dr. Sethia. Media/wet milling using a grinding mill or high-pressure homogenizer can provide particle size in the 100-200nm range, which can be stabilized by surfactants and delivered as a suspension or dried to powder from using spray drying to yield conventional tablets and capsules dosage form.

He concludes: "Each of the above approaches, along with a disciplined system for formulation and process development and robust analytical methods, can achieve improved bioavailability expeditiously."

Quotient Sciences: Integrated Approach Accelerates Drug Development

Solving bioavailability and solubility challenges to support successful drug delivery is an ever-enduring challenge (and opportunity) for pharmaceutical formulation scientists. Along with well-established approaches to improving each, there are many emerging platform technologies, providing options in the toolbox. In many ways, though, availability of such formulation and process technology approaches does not present the primary barrier to improving universal solubility and bioavailability challenges. Instead, a key challenge is the continued lack of predictive, clinically relevant models to guide formulation selection early enough in the development process – such that money and time are not unnecessarily expended, and avoidable risks not taken.

"With many examples of misleading nonclinical and *in vitro* predictability out there, robust predictive models would afford the ability to understand and adapt for successful clinical outcomes from the outset," says Dr. Sarah Stevens, Vice President of Drug Development Sciences at Quotient Sciences. "Quotient Sciences embodies science-led decision making, therefore not relying simply on potentially unreliable predictive models. A combination of unique development approaches provides the most expeditious means to improve potential bioavailability and solubility challenges."

Quotient, she says, deploys technologies such as particle size reduction, lipid-based formulation mechanisms, HME, SDD, etc., but more pertinently, drives early formulation selection by cutting through industry silos and integrating across a range of capabilities to accelerate the drug development process. One example is Quotient's Translational Pharmaceutics® platform, which integrates drug product manufacturing and clinical testing and ensures a continuum among lead compound selection, formulation development, and clinical assessments. "This reduces formulation development timelines and money, and mitigates risk in the development pathway by utilizing real-time clinical data to improve bioavailability and solubility," says Dr. Stevens.

This integrated approach requires finely tuned project management and processes to ensure the most expeditious path to evaluate new molecules and formulations in the clinic. Prior to any clinical assessment, Quotient deploys a number of approaches based around the Developability Classification System (DCS) to deeply understand molecule properties – physicochemical characterization, the use of biorelevant methods and physiologically-based modelling/simulation to best position the formulation strategy for success. Quotient uses real-time product manufacturing and clinical testing to make, dose, and test new dosage forms within a 14-day cycle time, using arising clinical PK data to adjust formulation compositions.

As an example, she explains how Quotient rapidly screened a range of formulation types, including a micronized API, spray-dried dispersions, and a lipid-based formulation using both biorelevant media and the integrated clinical manufacturing and testing platform.

"Nonclinical data was unable to provide clarity on which formulation strategy would be optimal to address PK issues seen with a simple first-in-human suspension system," says Dr. Stevens. "Our ability to make decisions based on human PK data allowed identification of a powder-in-capsule formulation containing micronized drug, which demonstrated improved exposure, linear PK, and a reduced food effect, saving time and money for our client. This example supports my assertion that the lack of predictive, non-clinical or biorelevant models is the real barrier to success in the development of bioavailability/solubility enhancing formulations."

Recipharm: Conventional Technologies Are More Time- & Cost-Effective

The most important factor preventing the improvement of solubility issues and advances in bioavailability is low-potency active pharmaceutical ingredients (APIs), says Torkel Gren, Senior Director, Technology Officer & Strategic Investments at Recipharm.

Low potency, he says, works against solubility enhancement in two ways. First, higher concentrations of API are required, if the dose is high. Secondly, smaller quantities of solubility enhancer can be added to the dosage form, if the dose is high. "All of this means that it is hard to improve the solubility of low-potency formulations, limiting the ability to improve bioavailability and therapeutic effect."

Recipharm has been exploring solubility improvements in formulations for pre-clinical and first-in-human studies. "We have found it useful to perform theoretical evaluation before testing prototypes representing different approaches, followed by an optimization phase," says Mr. Gren. "Usually, time and stocks of drug substance are very limited, so it helps to use experimental design and work in small scale."

This approach also helps to avoid using more advanced or complex technologies, unless they are necessary. Recipharm's approach is to use conventional technologies whenever possible, as this will usually be more time- and cost-effective, he says. More importantly, it will make continued development and tech transfer into commercial manufacturing less challenging, maximizing the likelihood of the project succeeding.

In a current project, Recipharm is supporting a customer with a formulation for safety studies. By applying its own screening platform, Recipharm has been able to rapidly develop an appropriately optimized formulation solution, combining pH adjustment and complexation by cyclodextrin. Stability was limited, but this was managed by using a frozen solution, which is an appropriate approach for preclinical phase and Phase 1, he explains.

"For clinical Phase 1 and 2, lower doses were required, and we designed a tablet formulation with acceptable bioavailability by controlling drug particle size and adding surface active agents," says Mr. Gren. "The work with particle size distribution was facilitated by the fact that we were also manufacturing the drug substance on behalf of the customer."

Mr. Gren admits that this approach may appear low-tech but, in this case, conventional methods were sufficient and significantly reduced the timeline for development. In addition, Recipharm created a drug product that will be easy to scale up for Phase 2 and commercial scale, and can be manufactured at a reasonable cost, he adds. ◆

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MICROFLUIDIC ENCAPSULATION TECHNOLOGY

Achieving Reliable siRNA Drug Delivery for Inflammatory Diseases & Tumor Targeting by Nanoencapsulation

By: Prof. Olivia Merkel, PhD, and Christoph Zimmermann, PhD student

ABSTRACT

Nanoencapsulation is a highly efficient way to transport small interfering RNA (siRNA) therapies to targets across the cellular membrane, protecting the material from degradation prior to endocytosis. It is important that these therapies are delivered in a controlled and reproducible manner and, as the particle size affects the rate of uptake and clearance, consistent production of monodisperse particles is essential. The following discusses the benefits of microfluidic encapsulation technology for gene silencing applications in cancer immunology and inflammatory diseases, where siRNA can potentially be used to downregulate genes associated with these pathologies.

INTRODUCTION

The development of novel therapeutics for diseases that are difficult to treat or incurable is a major area of research, and RNA therapeutics are playing a growing role in new treatments. siRNA is a type of double-stranded, non-coding RNA molecule – about 20-25 nucleotides long – that can be used to downregulate the expression of genes via the RNA interference (RNAi) pathway. siRNA molecules have a 2-nucleotide overhang on the 3′ end of each strand and are bound by a multiprotein component complex known as RISC (RNA-induced silencing complex). The antisense single-stranded siRNA component guides the RISC complex to the target mRNA and aligns it. The catalytic RISC protein then cleaves the mRNA, inducing its degradation.[1]

RNAi offers one of the most promising areas for developing new treatments for a number of diseases since the discovery of siRNA in 1998.[2] Using siRNA in this pathway opens up exciting possibilities in a breadth of applications, with each molecule capable of inactivating several target RNA molecules in a sequence-specific manner; siRNA doses of approximately 1-2 mg/kg[2] can reversibly knockdown target protein production *in vivo* by up to 90%.[3] With multiple siRNA treatments already in clinical practice, this method is gaining even more popularity, with ongoing research into chemical modifications aimed at improving qualities such as target cell delivery, siRNA potency, and serum stability, while reducing immunostimulation and off-target effects.[4]

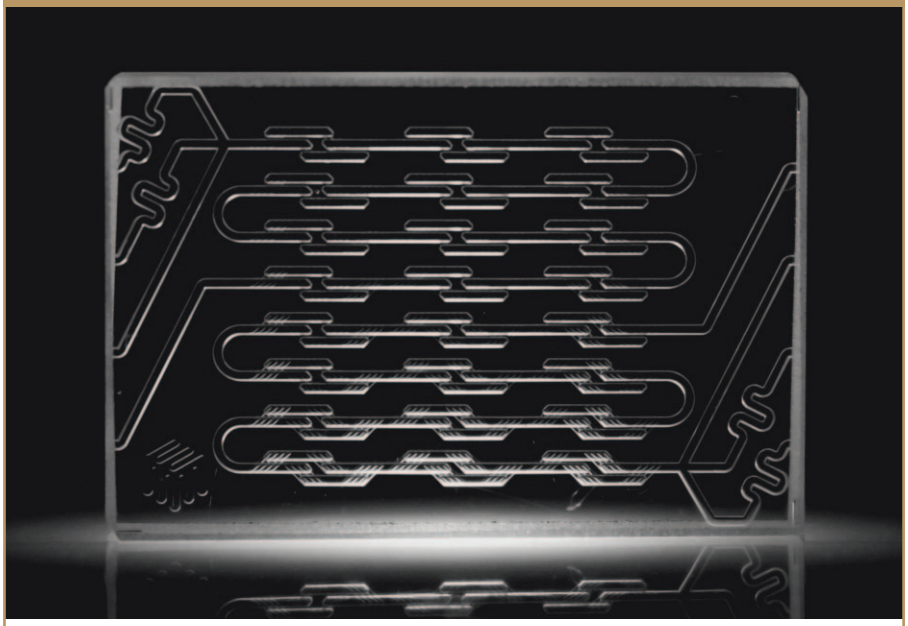
THE FOCUS OF CURRENT RESEARCH

siRNA treatment is highly selective, and can be targeted at any gene involved with a specific disease. This makes it appealing for diseases with a genetic element or that are currently incurable by conventional drugs, for instance, cancer.

Multiple ongoing projects are looking to control general targets in certain diseases, such as the downregulation of TNF-alpha

(TNFα) in active macrophages in rheumatoid arthritis, or the suppression of GATA-3 in T cells in asthma. siRNAs have also been developed to target oncogenes, as well as molecules critical for tumor-host interactions and tumor resistance to chemo- or radiotherapy.[1,5] Treatment can even be personalized; a patient's cancer can be sequenced to check for mutations to help discover the best siRNA sequence to use. For instance, EGF receptor mutations often cause chemo-resistance in lung cancer treatment with tyrosine kinase inhibitors, so these mutations can be targeted using siRNA. However, much of this research has been *in vitro*, using cell cultures – overcoming *in vivo* delivery difficulties is vital for widespread clinical use.

FIGURE 1



Dolomite Microfluidics micro mixing chip used for nanoencapsulation to reliably and consistently produce monodisperse nanoparticles for targeted delivery.

EFFECTIVE DELIVERY OF SIRNA THERAPEUTICS

Efficient delivery of siRNA treatments poses a huge barrier to wide-scale clinical use, as siRNAs must reach the cytoplasm of a cell to achieve post-transcriptional silencing. Although this represents one less barrier than those faced by plasmid delivery, which must reach the nucleus, efficiency of the delivery of free siRNA to target cells is commonly low as macromolecules cannot passively diffuse across cell membranes, and cells lack specific receptors for active nucleic acid uptake. Additionally, a large amount of siRNA is often degraded by nucleases in biological fluids.

There are several possible methods of *in vivo* delivery, including viral vectors, cationic lipids, polymer nanocarriers, and inorganic nanoparticles, each with their own benefits and limitations for therapeutic use. Research in the Merkel lab at the Ludwig Maximilian University of Munich is focused on novel non-viral and targeted nanosized siRNA delivery systems, using microfluidic mixing chips (Dolomite Microfluidics) for nanoencapsulation, to reliably and consistently produce monodisperse nanoparticles for targeted delivery of these therapeutics.

WHY NANOENCAPSULATION?

Nanoencapsulation is proving a highly efficient way to deliver siRNA to targets across the cellular membrane, protecting it from degradation prior to being taken up intracellularly by endocytosis. The actual sequence of the siRNA doesn't have a big impact on particle characteristics, but it is important to deliver the therapy in a controlled and reproducible manner; particle size has a huge impact on *in vivo* work, affecting the rate of uptake and clearance.

Self-assembling polymeric nanoparticle systems are often prepared using a batch reactor technique. These polyplexes form by electrostatic interaction when using polyamine containing polymers that are mainly positively charged at physiologic pH. When mixed with negatively charged nucleic acids, nanoprecipitation occurs, resulting in the formation of polyelectrolyte complexes. This can be achieved by simply pipetting the polymers and nucleic acids together; however, such uncontrolled methods of mixing generate polydisperse nanoparticles with a broad size distribution. While this polydispersity often does not impact on *in vitro* research, it has a more relevant effect on *in vivo* models, where the production of monodisperse particles is key to delivery and uptake, and hence the success of any therapeutic.

In some circumstances, genes that are not the exact therapeutic target can also be affected by an siRNA sequence, causing off-target effects. The polymers and lipids used in nanoparticles can cause a variety of these side effects, such as complement activation or immunostimulation, and although some may be of benefit –

depending on which immune pathways are activated – this can pose further difficulties. Treatment may also need to be specific to particular subpopulations of cells. For example, when treating rheumatoid arthritis by regulating TNFα, particles must be designed with properties that ensure they are only taken up by activated, and not resting, macrophages. Similarly, only activated, and not naïve, T cells should take up particles for asthma treatment, to prevent general immune suppression.

MICROFLUIDICS AS A METHOD FOR NANOENCAPSULATION

Microfluidic-based production of siRNA nanoparticles offers the reproducibility and consistency required for reliable drug delivery. Using microfluidics helps to regulate particle assembly; microfluidic devices allow control of the interaction of the cationic polymer with the anionic nucleic acid far better than batch methods, increasing colloidal stability. The constant ratio at which the polymer comes into contact with the nucleic acid increases particle uniformity, resulting in smaller, monodisperse particles with a much better polydispersity index and less batch-to-batch variability at scaled-up batch sizes. Additionally, microfluidic formulation of particles has been shown to yield more efficient gene knockdown *in vivo* than that from particles formulated in a batch reactor. This has been demonstrated in a lung model, where smaller particles with a lower polydispersity had much better gene-silencing efficacy of a housekeeping gene.[2] It has also been noted that larger particles are phagocytosed more easily by macrophages, while smaller particles are a lot more efficient in epithelial uptake. A microfluidic production system can therefore improve a number of particle properties, and increase production scalability, which is a crucial consideration for any clinical trial.

THE IMPORTANCE OF MONODISPERSITY FOR NANOPARTICLE CHARACTERISTICS

Different characteristics and behaviors are preferential for different applications and can be controlled by the choice of nanomaterial. There is now an ever-expanding variety of particle formulations, taking advantage of different characteristics to improve delivery or carrier identity to achieve gene regulation. Nanomaterials that can be modified via PEGylation are frequently used to improve circulation time and decrease renal clearance for particles that are administered intravenously. PEG coatings help to shield the surface from aggregation, opsonization, and phagocytosis, all of which contribute to a longer systemic circulation time.[6] Particles must also be altered to give the best possible chance of escaping the endosomal and lysosomal pathways. It is often the best route to look at hybrid materials and form particles from a combination of lipids and polymers. In this way, the best properties of polymers for shielding the particles can be combined with the fusogenic properties of lipids for the best intracellular delivery and endosomal escape. Using microfluidics enhances tweaking of particle characteristics, ensuring that particles are monodisperse and reproducibly produced, unlike batch methods that lead to the creation of irregular particles with different characteristics.

THE SIGNIFICANCE OF PARTICLE ASSEMBLY & SCALE-UP

Particle assembly has also been observed to play a key role in the efficacy of treatment, and the ability to efficiently scale this up is essential for widespread clinical adoption. The triblock copolymer polyethylenimine-polycaprolactone-polyethylene glycol (PEI-PCL-PEG) system can be modified to link targeting moieties, such as folic acid, to the micelleplex it forms with siRNA, and so microfluidic production of PEI-PCL-PEG nanoparticles enables rapid, scaled-up production of micelleplexes, while maintaining small and uniform particle distributions.[7] These micelleplexes have been found to have superior delivery efficiency in comparison to polyplexes, due to their amphiphilic properties that aid cellular internalization and potentially endosomal escape.[7] The system has been used to target folate receptor alpha (FRα), a tumor marker that is overexpressed in over 85% of all ovarian tumors, which shows real promise for potential treatment; folic acid can be attached onto the delivery vehicle, thereby mediating cell-specific siRNA delivery within tumors.

THE EFFECT OF MICROFLUIDIC PRODUCTION ON ADMINISTRATION

The administration route is another vital property for delivery of siRNA nanoparticles, as it is crucial that each treatment is taken up preferentially by the target cells. For instance, in order to reach

"There are several possible methods of *in vivo* delivery, including viral vectors, cationic lipids, polymer nanocarriers, and inorganic nanoparticles, each with their own benefits and limitations for therapeutic use. Research in the Merkel lab at the Ludwig Maximilian University of Munich is focused on novel non-viral and targeted nanosized siRNA delivery systems, using microfluidic mixing chips (Dolomite Microfluidics) for nanoencapsulation, to reliably and consistently produce monodisperse nanoparticles for targeted delivery of these therapeutics."

specific cell populations within the lung or to deliver nucleic acids into the brain, targeting ligands can be attached to the surface of the delivery vehicle. These targeting moieties have a strong affinity with the target cells and tissues, and offer promise to detect distant targets, such as mobile immune cells, circulating tumor cells, or metastases.

Therapeutic nanoparticle administration routes include intravenous, transdermal, pulmonary, and intraocular options. Pulmonary delivery – inhalation of the particle formulation through the mouth so that the inhaled pharmacological agent reaches the lower airways – is one option, and may benefit treatments targeting local conditions in the lungs, such as asthma. Alternatively, intravenously administered nanoparticles are reported to accumulate in the liver – an ideal situation if the therapeutic target is in the liver. However, for targets located elsewhere, systemic administration isn't always the best option to achieve the highest percentage of particle delivery, as the first-pass effect may reduce the amount of a drug delivered to a patient's general circulation to small percentages of the total dose administered. Microfluidics offers a way to improve this situation, as the clearance by macrophages of smaller particles made using microfluidic assembly has been shown to be less efficient, decreasing the first pass effect.

TREATMENT LENGTH

As treatment is reversible, the length of effect of any siRNA depends on the biologic half-life of the target gene. For genes coding for a protein with a long half-life, often the effect of the siRNA silencing is delayed as an abundance of protein is still present. This means that treatment frequency can be anywhere from daily to weekly. But new methods are being developed all the time to improve dosing schemes, such as gel implants that degrade under certain physiological conditions – for instance, temperature or pH – to create a release-on-demand system for the siRNA particles. This can be applied to rheumatoid arthritis, where gels have been developed that are stable at physiologic pH and are expected to stay in the synovial cavity of the joint.[8] When the pH drops due to inflammation, the gel starts to degrade, releasing particles to help fight inflammation. It is important for these particles to be monodisperse, so that siRNA release is controlled. However, much more research *in vivo* is needed to understand how long the implant would release an effective dose of the therapeutic.

SUMMARY

Optimizing particle characteristics, including formulation reproducibility, particle size, siRNA protection and release, toxicity, immunogenicity, and bioactivity, is critical for better *in vivo* results and to develop clinically relevant treatment forms, such as powders for inhalation with increased shelf-live.

Nanoencapsulation is proving to be a superior method for delivery of siRNA *in vivo*, and microfluidic methods allow precise control that is vital for efficient and effective production of smaller, uniform particles, ensuring a reliable and reproducible rate of uptake and clearance. This time-saving method offers improved batch-to-batch reproducibility, reducing the need for repeat experiments due to poor particle production, and production can be more easily scaled up for the clini-

cal environment. Microfluidic encapsulation of siRNAs is therefore an efficient method for achieving reliable delivery of therapeutics for a whole variety of diseases. It is vital that research continues to improve and explore further applications for this technology, and it will be exciting to see where this leads in the future. ◆

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BIOGRAPHIES

Dr. Olivia Merkel has been a Professor of Drug Delivery at LMU Munich since 2015. She is a registered pharmacist, having earned a Master's degree in Pharmaceutical Technology in 2006 and a PhD in Pharmaceutical Technology in 2009, and has received numerous awards, including an ERC Starting grant, the APV Research Award, and the Carl-Wilhelm-Scheele-Award. She is the author of over 85 articles and book chapters, and has served as a reviewer for NIH (2014-2015) and SNF (since 2018). She is an Editorial Board member for JCR, EJPB, Molecular Pharmaceutics, and three other journals, as well as being the President of the German Controlled Release Society.

Christoph Zimmermann has been a PhD student in Professor Olivia Merkel's group since February 2019. He is a registered pharmacist and earned a Bachelor's degree in Management and Technology from the Technical University of Munich in 2013. He is a member of the German Pharmaceutical Society.

Drug Development EXECUTIVE

Frank Tagliaferri, PhD

VP, Pharmaceutical Development & General Manager

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Our investment in state-of-the-art facilities and highly trained experts emphasizes our commitment to advancing therapies and delivering positive customer experiences across all phases of pharmaceutical and biopharmaceutical development. From early stage research and development, to clinical trials materials production and GMP manufacturing support, our 400+ scientists are dedicated to providing the best and most reliable pharmaceutical development services. Pace Analytical® Life Sciences (PLS) is well-equipped to handle almost any project regardless of scope or complexity. We recently interviewed Frank Tagliaferri, PhD, Vice President of Pharmaceutical Development at PLS to discuss his experiences with the company and its growth.

Q: I see you have worked in many places in industry, why in a CRO/CDMO now? What makes this compelling?

A: While the work itself is important, there are obvious scientific limitations and a narrowed therapeutic space in which to make meaningful contributions. This contrasts with a CRO/CDMO like PLS, where we contribute to the development of therapies for a wide range of therapeutic areas employing an almost limitless number of technologies. To accomplish such a breadth of work, we have created an organization with a diverse team of scientists with backgrounds and expertise in a variety of areas. This includes traditional small molecule development, biologics - such as proteins and monoclonal antibodies (MAbs) - and the accelerating gene therapy space, where we operate on the cutting edge to develop and support the commercialization of a number of oligonucleotide-containing products (mRNA, siRNA, DNA, etc). The tempo, the rigor, the challenge, and the interaction with a broad cast of experts from around the globe make the environment at PLS dynamic, inspiring, and in retrospect, a more satisfying experience.

Q: Tell us about the work you and your teams at PLS do and how the five sites position the company as a leader in the market.

A Essentially, we at PLS provide contract development/manufacturing and contract research (CDMO/CRO) services to help improve human health. We are supporting innovators in the Pharmaceutical /Biopharmaceutical /Biologics markets to support their early phase drug development and on through commercialization and late-phase manufacturing.

Our teams here in Woburn, MA, provide early phase, IND-enabling services to help innovative new candidate therapies progress through the pre-clinical and early clinical stages. We start with thorough characterization of novel molecules and proceed to pre-formulation and formulation development services, including lyophilization development, along with robust and stage-appropriate analytics. We do all of this in support of small molecules, biologics (proteins, peptides, antibodies, antibody drug conjugates), and gene therapies (such as mRNA- and siRNA-containing products and their varied array of delivery modalities such as LNPs, AAVs, VLPs, etc). Our most recent acquisitions of Emerson Resources in Philadelphia, PA, and Bio-Concept Laboratories in Salem, NH, have added tremendously to our experience, capabilities, and expertise in pre-formulation and formulation development. It also adds the capability to manufacture sterile, ophthalmic, and oral solid/liquid clinical trial materials. Specialized technologies like lyophilization and spray drying provide options to enhance performance and support clients from early pharmaceutical development through Phase 3a; providing tablets, capsules, solutions, suspensions, semi-solid, and other dosage forms. Bio-Concept has gained significant experience in ophthalmic formulation and manufacturing to support Phase 1, 2, and 3 clinical trials materials. Their specialization in fill-finish manufacturing of sterile aqueous products has added tremendously to our comprehensive service offerings at PLS.

Once the early stage development is completed, our two full-service, FDA-registered, cGMP-compliant laboratories (Minnesota and Puerto Rico) provide commercialization and late-phase support to ensure the purity, potency, safety, efficacy, and shelf-life of manufactured pharmaceutical, biopharmaceutical, and gene-therapy products. There we support Raw Materials Clearance Programs, Finished Product Release Testing Programs, ICH Stability Storage & Testing, Method Development & Validation, Extractable/Leachable/Elemental Impurity studies, Reference Standard Programs, and provide Microbiology Testing Services.

Q: Over the years, there has been an increase in the use of CDMO/CROs, and PLS has grown significantly. In what ways have you seen the company change, and what are your thoughts on its future?

A: Of course, the market is dynamic and ever-changing, and we are always looking at all areas in order to evaluate where we can add the most value for our partners. Our most recent acquisitions have expanded our capabilities to include clinical trial materials. We saw a tremendous amount of innovation here, and the clinical trials market is more active than ever before with a wider range of candidate molecules. We are now able to offer a smooth transition from discovery, through tox, and into dosing studies that may present additional demand in the market for support of clinical trial materials.

We have also seen a lot of recent growth in the gene therapy space, where the technologies are not only constantly evolving, but the availability of skilled CROs in this space is more limited. Thus, while work here is expanding, we continue to see strong investments and new developments in small molecules, antibodies, and ADCs. We are watching the markets closely, and because of our ability to allow smooth transition, we are optimistic about opportunities that may be present for us in the near future.

Q: There has been a lot of consolidation in the CRO/CDO/CDMO market in recent years. How does that affect your partner experience, and how does that separate PLS from the competition? What are the advantages of partnering with PLS?

A: PLS has a history of strong organic growth and some by acquisition. The number of returning clients with follow-on programs is a strong indicator we are delivering a positive client experience. Our success rate in meeting critical timelines with well-founded formulations that can advance accompanied by well-characterized analytics has become our reputation. We provide a collaborative environment in which our clients are heavily involved in the science, they retain the level of control they desire, and positive outcomes are reached in a timely manner and within a reasonable budget.

Our senior management teams have been stable and in place for a long time, and that has allowed us to deliver excellent service without interruption. We have successfully created an environment that attracts, retains, and challenges our world-class staff. Many have decades of industry experience, and we continue their development through extensive scientific cross-training in a place that they can make a difference in the

outcomes of human health. Our continued investments in facilities, latest technologies, and ongoing developments have resulted in many long-term strategic partners as well as many new opportunities.

Q: In the market today, what is the best way to interact with a CRO/CDMO to truly maximize the potential in the partnership? What are the pitfalls? How does PLS adapt to problems they have not seen before?

A: Each partner relationship is unique, and we respect the needs of each client to manage their programs in a way that fits their company culture and team dynamic. We have a construct here that is flexible and adds value in ways that work for our clients. We can drive programs and provide support while remaining engaged and mindful of the program goals. That said, in my experience, the way to get the most from a CRO/CDMO is to be as open and engaged as you possibly can with the details, goals, and targets of your development program. Get to know the individuals on your CRO/CDMO team, understand their backgrounds and strengths, and share the true challenges ahead of you. In this way, the teams come together to share goals, collaborative energy, and scientific rigor in ways that produce positive outcomes. In this industry, most of us are scientists at heart. We understand the business needs and disciplines, but what gets teams clicking is the scientific challenge and the pursuit of successful outcomes. It is a bit of the old axioms that you get out what you invest, but I believe it's really true in this case.

Q: We all hope to emerge from our early phase tox studies with a molecule showing true potential. How does PLS help their partners with the next steps toward commercialization?

A: As we know, there are many reasons why progress may be thwarted at any of many points along the journey. Sometimes it is for good reasons inherent to the molecule, and there is not much we can do except to recognize it early and end it before too much time and energy is invested. Many times, the advantages that can be gained are in the favorable selection of strategies and direction. The efficient study designs that yield the most information quickly and economically, while sufficiently reducing risks, are those that are successful in reaching milestones and progressing further toward positive outcomes. Again, this is where the close personal involvement of our experienced technical program managers working closely with our client teams can be quick, nimble and efficient. I keep coming back to the teams and the scientific rigor, but it is what makes the difference.

Q: You've been in this industry for a while; where do you see our industries heading in the next 3 to 5 years? What do people need to know to stay relevant at this time of shifting demands?

A: Well, that's an interesting question as we are here today in a position that we might never have anticipated a year ago. Clearly, the demonstrated potential for our peers to rally and respond to a healthcare crisis in record time is revealing more than we knew about ourselves and our capacity to invest and produce in record times. These events will forever be a part of our future, and we will learn and adapt and develop new ways of looking at these problems.

Still the movement toward personalized and regenerative cellular therapies will continue and will likely accelerate. Our ability to collect and analyze large amounts of data continues to develop. These advances will change our understanding of human physiology and disease.

The decentralized innovation model in industry has been persistent, and specialization creates hubs of expertise that are able to bridge disciplines and make advances quickly. The competition in these environments can sometimes be intense, but it drives innovation in a very natural way.

On the commercial side, the continued consolidation creates several options for industry in large, full-service CRO/CDMO partners. Yet, the rate of smaller, newly formed firms working to commercialize technologies is strong. In these environments, the gaps between firms and their specialties and expertise can seem wide. In those situations, the centralized CRO/CDMO outsourcing model can and will continue to provide attractive solutions for industry.

PLS strives to be an extremely flexible organization that can provide partners with the services, scientific knowledge, and attention needed to get drugs to patients efficiently. One way in which we achieve this is by staying up to date, if not ahead, of the science and technologies that will become the basis of the next round of therapies. Much like the big pharma players, the challenge is for us to invest in the right areas with the appropriate scientists, instruments, and facilities required. Working with several early, even virtual, organizations is one way we can stay abreast of the field and hopefully anticipate the needs such that our entire organization from development to cGMP testing is ready to usher in the newest therapeutics. ◆

ORAL MUCOSAL IMMUNOTHERAPY

Oral Mucosal Delivery of Allergenic Proteins for Inducing Tolerance in Food Allergic Individuals

By: William R. Reisacher, MD

INTRODUCTION

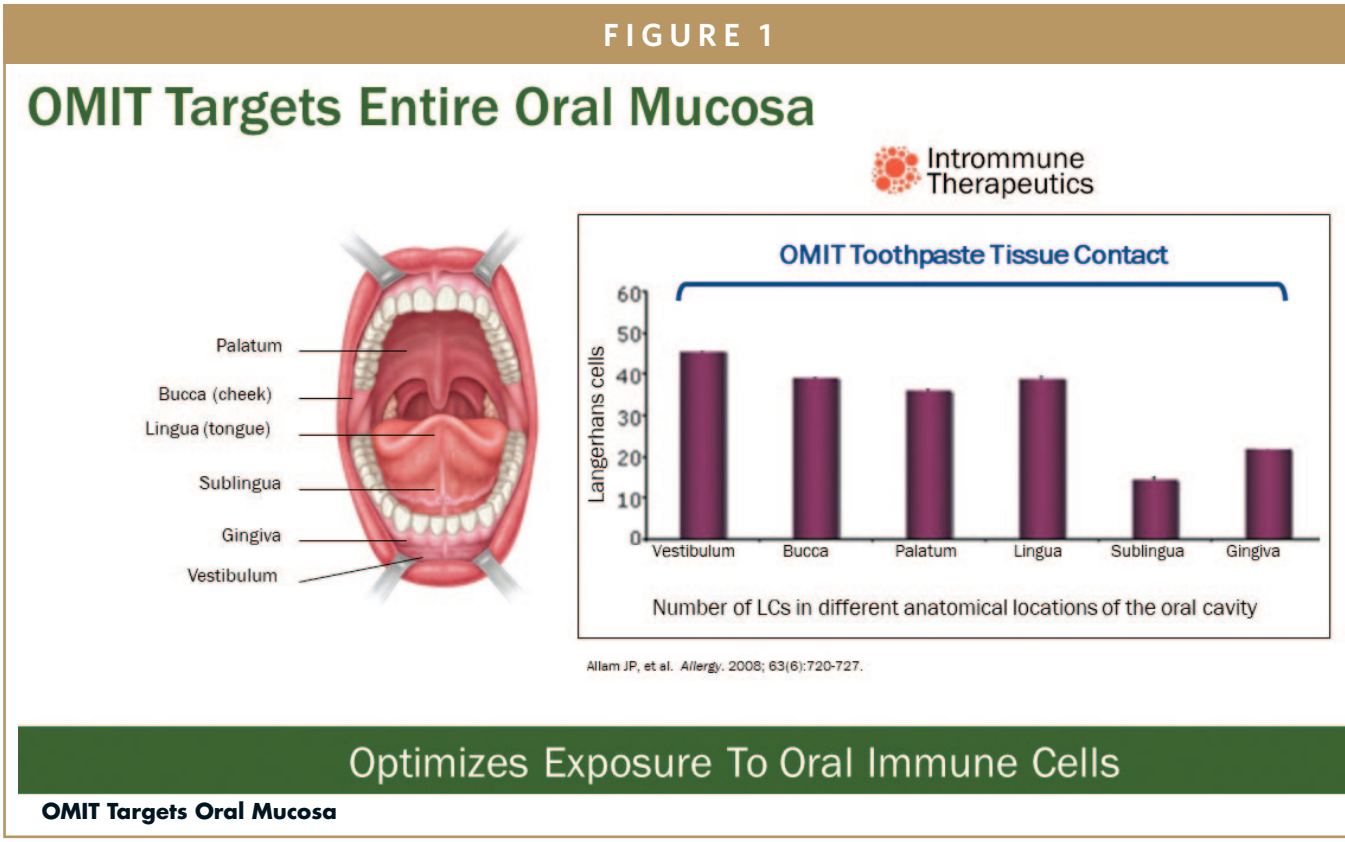
Currently, there is a tremendous amount of interest in the delivery of drugs through the oral mucosa for a vast array of medical conditions. The oral mucosa is more permeable than the skin by 1000-fold and lacks the enzymatic activity of the gastrointestinal tract.[1] One of the key benefits of this route, however, is the absence of first-pass metabolism. When a drug is delivered directly into the systemic circulation, rather than allowing it to absorb through the stomach and intestinal lining, it bypasses the hepatic venous system and avoids being metabolized by the liver. This can enhance the efficacy of a drug, theoretically lowering the therapeutic dose required. Other routes of drug delivery outside the gastrointestinal tract, such as injection and transdermal application, may be associated with increased discomfort, inconvenience, or undesirable side effects. Oral mucosal delivery is also very useful for children or adults who have difficulty swallowing tablets, or for people with gastrointestinal illnesses that are experiencing nausea, vomiting, or dysphagia.[2]

Pharmacokinetic data has been collected for the oral mucosal delivery of hundreds of small molecule drugs, commonly using the buccal or sublingual surfaces, of which the goal is for the drug to pass into the circulation. In contrast, the goal of oral mucosal delivery for allergy immunotherapy is to concentrate the large extract proteins in the mucosa itself, rather than promoting entry into the circulation, so that they can interact with the abundant levels of antigen presenting cells (oral Langerhans cells, or oLC) in this area, leading to immune tolerance.[3] The oral mucosal delivery of allergenic polypeptides for the purpose of allergy immunotherapy, or desensitization, has been in routine clinical use for several decades to treat respiratory allergies.

Oral mucosal delivery of allergenic proteins for respiratory allergies has been found to alleviate symptoms and reduce medication use, on a level comparable with subcutaneous immunotherapy (SCIT, or allergy shots); this method is also associated with improvements in quality of life, prevention of asthma, and a superior safety profile.[4,5] Oral mucosal immunotherapy (OMIT) for respiratory allergies has been commonly delivered through the sublingual mucosa, using either liquid extract drops or fast-dissolving tablets (also referred to as sublingual immunotherapy, or SLIT). More recently, a specialized toothpaste delivery vehicle (Allerdent®, Allovate, LLC) has been developed to stabilize the allergenic extract proteins and release them upon regular tooth brushing to a wider area of buccal, vestibular, lingual, and sublingual mucosal surfaces.[6] Further, INT301 (Intrommune Therapeutics), is an OMIT product being developed for the treatment of peanut allergies. INT301's unique formulation is designed to desensitize an individual with peanut allergy using a toothpaste delivery system, protecting them in the event of accidental peanut exposure. This innovative OMIT toothpaste delivery system has been shown to enhance adherence to therapy because it is linked to a common daily activity, and exhibits fewer gastrointestinal side effects because it is not swallowed.

In fact, OMIT is safe enough to be performed as a home therapy. This has led to new strategies for desensitization against food allergies, a treatment proven to be too dangerous for clinical use when delivered by subcutaneous injection.[7] Kim and colleagues demonstrated long-term clinical and immunologic toler-

FIGURE 1



OMIT Targets Oral Mucosa

ance, along with a low rate of adverse events, when children were treated with liquid peanut extract applied to the sublingual space over a period of 3 to 5 years.[8] Oral immunotherapy (OIT), using swallowed peanut protein flour, has also been found to produce a robust degree of desensitization, but is associated with a higher degree of serious adverse events, including anaphylaxis.[9] This paper will highlight some of the differences between food allergy immunotherapy via the oral mucosal route versus exposure through the stomach and intestines.

THE PHARMACOKINETICS OF ORAL MUCOSAL DELIVERY

Saturation Solubility

The journey for allergenic proteins entering the oral cavity and encountering the mucosa begins with the saturation solubility, or how much of the protein gets dissolved in the liquid/slurry from the protein application modality plus the roughly 1 ml of saliva that is present in the oral cavity.[10] If the protein level is above saturation, the absorption into the mucosa requires a longer exposure, because some would have to first be absorbed into the mucosa, allowing the excess protein in the mouth to then dissolve in the saliva. In one study using SLIT, 26% to 30% of the allergenic protein was expelled in the saliva, presumably because the small surface area of the sublingual space kept saturation levels high.[11] The interaction of the protein molecule with the mucosal membrane is also facilitated by ionic forces, whereby the slightly positive charged protein molecules binds to the slightly negatively charged mucosal cells. In the case of OMIT using the toothpaste delivery vehicle, it is believed that brushing across the mucosal surface also removes some of the epithelial debris and keratin, exposing more of the area available for absorption.

Distribution Equilibrium

The second part of the process is the distribution equilibrium, which directs the actual absorption of the allergenic extract proteins into the mucosa. This is a rapid process, with an estimated mean absorption half-life of 52 seconds.[10] Rapid clearance of the protein from the oral cavity, however, does not imply rapid systemic exposure. The mucosa acts as a reservoir, reaching an equilibrium with the protein in the saliva/foam in the mouth. This is independent of the dose of protein in the mouth. In other words, once the mucosa has fulfilled its quota in the region, more drug will stay in the mouth and be swallowed or expelled, as previously noted. This raises concern that concentrating the proteins in liquid sublingual drops could lead to a wasting of excess protein due to oversaturation in one area. Thus, distributing the protein to a wider area would be potentially very beneficial in terms of utilizing all available mucosal surfaces. Even

TABLE 1

Oral Immunotherapy (OIT)	Oral Mucosal Immunotherapy (OMIT)
Requires ingestion of allergen, which may trigger eosinophilic esophagitis (EoE)	Toothpaste delivery platform is not ingested
Requires metabolism of food allergens in the stomach and intestines to reach the lymph system	Antigen presenting cells escort non-metabolized food allergen to regional lymph nodes
Requires mixing a powder into pudding or applesauce for each dose	Brush teeth at home once daily to receive each dose

Oral Immunotherapy (OIT) & Oral Mucosal Immunotherapy (OMIT)

after extensive mouth rinsing, 2% of the radiolabeled allergen remains in the oral cavity mucosa 20 hours after administration, suggesting that processing of the allergen was still occurring within the mucosal reservoir.[10]

Bioavailability

The bioavailability, or how much of a drug or protein reaches the systemic circulation, is largely independent of the dose delivered into the oral cavity because of the partitioning phenomenon of the oral mucosa. Drinking water 2 minutes after sublingual administration of Asenapine, an anti-psychotic drug, does not affect the speed of bioavailability.[10] This would be analogous to a car, which can run at the same speed whether or not it has a half-filled tank or a full tank. Whether or not the drug will reach the circulation is based more on the characteristics of the molecule itself, such as size, molecular weight, configuration, how extensively it is processed, pH, affinity for blood vessels, ability to penetrate blood vessel endothelium, and interactions with other cells in the mucosa. Peanut proteins, which are well-known to cause anaphylaxis, are large molecules that are unable to cross the oral mucosal membrane without the assistance of oLCs. Ara h 2, one of the major peanut allergen proteins with a molecular weight of 17,000 g/mol, whether avidly bound to oLC or not, does not have a realistic pathway to reach the systemic circulation under normal conditions, particularly if blood is actively flowing out of a small vessel. Nonetheless, temporarily suspending OMIT treatment when open wounds, ulcers, lesions, or significant gum bleeding are present is a prudent recommendation.

In general, intact proteins are not transported directly into the bloodstream. The exceptions to this rule are the placenta, where immunoglobulins can be transferred directly to the fetal blood supply, and the human small intestine in the very first few weeks of life, where a "leaky" mucosa helps facilitate absorption of immunoglobulins present in breast milk.[12] Normally, the cells of the GI tract absorb digested proteins and then transport the fragments, which are rarely more than four amino acids long, into the bloodstream. In contrast, Ara h 2 has over 100 amino acids and a complex 3D structure. The oLC do not process food proteins to a point when they are small enough to gain access to the circulation, and therefore preferentially transports them to locoregional lymph nodes for processing. It has also been suggested that ongoing gut leakiness, either through disease or medications, such as proton pump inhibitors, promotes an ongoing cycle of allergic inflammation that increases the risk of developing food allergies.[13]

MOUSE STUDIES

Immunoglobulin E (IgE) from C3H/HeJ mice can recognize the same peanut allergen isoforms and epitopes as IgE from human peanut-allergic patients, making it a good model for anaphylaxis.[14] Mouse studies have also shown that dendritic cells from the oral mucosa acquire allergenic proteins in the buccal epithelium and transport them toward the draining lymph nodes, where they are processed and presented to MHC class II-restricted T cells.[15] Specific IgE and IgG responses were actually decreased when dosing newborn Brown Norway rats in the oral cavity with ovalbumin, as opposed to the allergenic priming effect seen when the exposure was through intragastric intubation. This supports a distinct pathway of antigen presentation in the oral mucosa, as well as a robust protective effect from early exposure of allergens to these tissues.[16] The distribution of a sublingually applied solution of Timothy grass allergen in sensitized mice has been investigated using 1% Evans Blue solution in phosphate-buffered saline (PBS) with 1.2% carboxymethylcellulose (CMC, an ingredient commonly seen in toothpaste, including Allerdent®, and other cosmetic products).[17] Thirty minutes after sublingual administration, the dye was detectable in the sublingual tissue, but not in the esophagus or in

the stomach. Without using CMC, the dye was also found in the stomach, demonstrating the positive impact of using viscous materials to support longer retention of proteins delivered to the oral cavity mucosa.

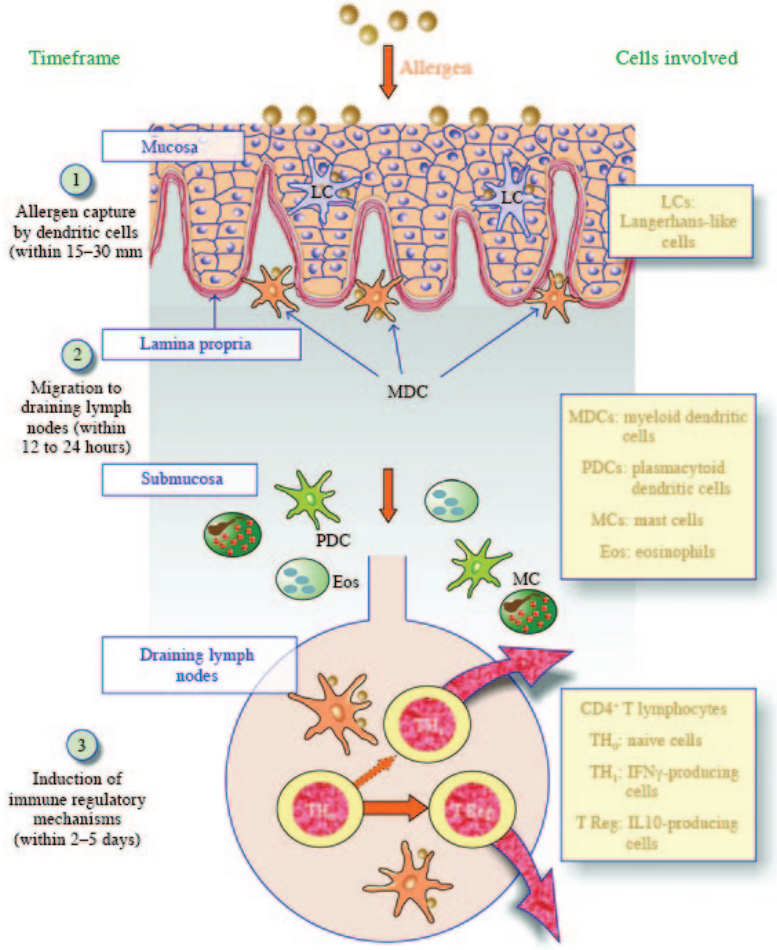
After Japanese Cedar pollen was painted on the sublingual and buccal mucosal surfaces in BALB/c mice, there was a rise in the oLC population at 2 hours, which peaked by 10 hours, after which the activated oLC population decreased and was gone by 24 hours in both groups.[18] The density of oLC was found to be lower in the sublingual mucosa than in the buccal mucosa, consistent with the human studies performed by Allam and colleagues.[3] With repeated paintings, equal percentages of resident and newly recruited oLC in the regional lymph nodes suggested that there was a consistent supply of new oLC at local buccal mucosa sites. In contrast, the supply of new oLC to the sublingual mucosa was very limited, demonstrating a marked decrease of oLC recruitment with repeated paintings and supporting the benefit of allergenic protein application to regions outside the sublingual space.

HUMAN STUDIES

In healthy, non-allergic individuals given radiolabelled allergen by the sublingual route, no significant deiodination or degradation of the protein was noted, even after the allergen was kept under the tongue for 20 minutes.[19] Serum detection of radioactive iodine alone and radioactive iodine-labeled small peptides increased only after swallowing, providing further evidence that large, intact peptides cannot gain access to the circulation via the oral mucosa under normal circumstances. In another study, plasma levels were achieved in sublingual-swallow patients only after swallowing and digestion within the stomach, with peak levels seen at 2 hours.[11]

Evidence suggests that even small molecules will have difficulty accessing the circulation through the oral cavity unless they are destined to do so by virtue of their inherent properties, such as 3D structure, pH, ionic charge, or biologic activity. Teeguarden and colleagues determined that oral exposure of bisphenol A, a

FIGURE 2



Immunology & Biology of OMIT

monomer with a molecular weight of 228 g/mol, equivalent to the 95% upper percentile of total daily exposure, would produce less than 1% absorption through the mucosa.[20] By using computer modeling of the oral cavity, compounds with a low rate of diffusion are predicted to have negligible systemic absorption, and that regions with a thicker mucosa, such as the buccal mucosa with 40-50 layers of non-keratinizing cells, would have a slower diffusion when compared with the relatively thin mucosa of the sublingual space.[21]

In the case of food allergy, both OMIT, applied to the sublingual space and/or other regions of the oral cavity, and OIT, whereby swallowed food proteins in powder reach the lymph tissue of the intestines, have been shown to be effective in promoting tolerance.[8] One of the key differences between these methods is that OIT uses a much larger amount of allergenic food protein, inducing a robust clinical effect but also producing a higher risk of adverse allergic events compared to the oral mucosal route. Recent studies have also determined that the mechanism of action is different for these two routes of delivery. Each route stimulates a different subpopulation of dendritic cells, which are antigen-presenting cells that

are essential for the development of tolerance, leading to different patterns of cytokine modulation.[22] These findings also suggest that these two methods of food allergen delivery might be combined to function in a complementary fashion.[23]

SUMMARY

The accelerated interest in drug delivery via the oral mucosa, coupled with the significant increase in severe food allergies throughout the world, has shined a spotlight on oral mucosal immunotherapy. The World Allergy Organization (WAO) 2013 Position Paper on sublingual immunotherapy stated that "compared to the sublingual region, targeting the vestibule with allergen vaccine has the potential to induce enhanced immune deviation or tolerance, possibly with a lower potential for mast cell-related local side effects."[24] This position reflects the understanding that immunologically active, tolerance-producing cell populations are present in a high degree in regions beyond the sublingual space, a target traditionally used because of easy access and the pull of gravity. With more advanced delivery techniques, such as a buccal patch or a toothpaste vehicle, however, food allergy proteins can now reach these areas and gain access to the locoregional lymph nodes through an elegant escort system orchestrated by oral Langerhans cells. It is important to remember that one of the main functions of the oral cavity mucosa is to teach the immune system what is safe to consume.

With the recent FDA approval of the first oral immunotherapy platform for peanut allergy, improved platforms using oral mucosal immunotherapy are sure to follow for other food allergies, along with multi-food products. ◆

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BIOGRAPHY

William Reisacher, MD, FACS, FAAOA, is a board-certified otolaryngologist, allergist, inventor, and Associate Professor at Weill Cornell Medicine in New York City. He has published and presented numerous chapters and research papers and served on the Board of Directors of the American Academy of Otolaryngic Allergy. Dr. Reisacher has founded companies in the biopharmaceutical, biotechnology, and digital healthtech spaces and lives in Manhattan with his wife and three children. Dr. Reisacher is an advisor and shareholder for both Allovate and Intrommune Therapeutics.

PRODUCT DEVELOPMENT STRATEGY

ESCP, Estimating Product Performance Part 1 - Playground Physics

By: Josef Bossart, PhD

INTRODUCTION

After more than 4 decades in the industry, I continue to be surprised by companies chasing new product opportunities that carry not only the usual development risks, but also a surprisingly high risk of commercial failure.

While risk is a given when it comes to pharmaceutical product development, formulation-enhanced products using previously approved actives take on more risk than they should. The real risk for these products, beyond a technology failure, lies in commercial acceptance. If you make it, they will only prescribe it if there is a perceived benefit.

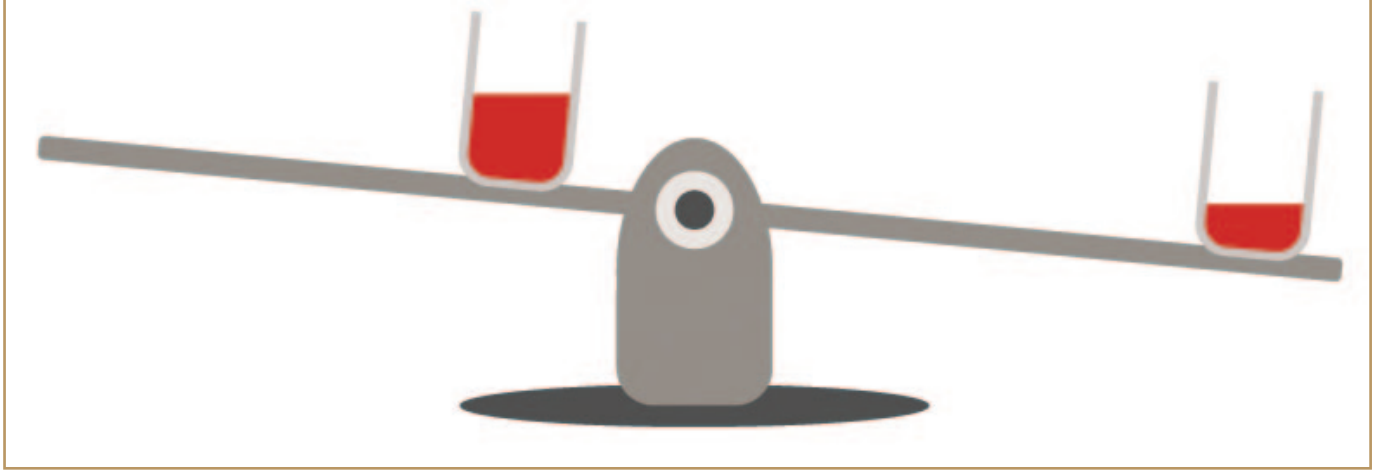
In a series of short articles, I will introduce a qualitative model to help understand and visualize the potential of a product with prescribers, patients, and payors. This simple model can help weed out product ideas that may at first glance seem attractive but offer little potential in the real world. Any number of sophisticated models are available to forecast product opportunity. Too often, these models are unreasonably complex, hard to "feed" with the necessary information, and don't provide an easily grasped sense of why a product does, or doesn't, represent a worthy opportunity. More importantly, these quantitative models don't provide guidance of how to improve a product's potential and turn good into better.

A classic physics model using playground equipment provides a useful visual framework.

ESCP

Efficacy (E), Safety (S), Convenience (C), and Price (P), or ESCP will be familiar concepts. These are the parameters that determine the performance and acceptance of a product. The relative importance or "weight" of these parameters follows the order E>S>C>P.

FIGURE 1



In the real world of prescription pharmaceuticals, you will not secure regulatory approval for any product that does not demonstrate some degree of efficacy. What qualifies as efficacious in the eyes of a regulator depends on the condition it serves. A product that can demonstrate a statistically significant improvement of cognitive or functional performance in any number of neurological diseases, even if relatively minor, is likely to be approved given the significant need. In the case of indications such as hyperlipidemia or hypertension, the standard for efficacy may be very high. The net/net is that without suitably validated efficacy, a product is unlikely to gain regulatory approval.

Once efficacy is demonstrated, the next parameter of importance is safety and tolerability. As with efficacy, there is a variable scale. A treatment for cancer that is very effective but relatively toxic might still be approved if the risk/benefit ratio is deemed acceptable for the indication. A product that demonstrates comparable efficacy, but has more safety or tolerability issues, may be approved but probably will not compete effectively against a comparably efficacious, but safer, product.

A new product that offers comparable efficacy and safety benefits to an approved product but is more convenient as evidenced by increased patient adherence is likely to gain significant market acceptance. A product with greater convenience at the expense of reduced efficacy or safety is unlikely to receive significant market acceptance in most cases.

In the case of two products with comparable E, S, and C, things will be tipped by price. Price is actually a rather weak parameter. Most everyone is willing to pay more to achieve a better clinical outcome.

PLAYGROUND EQUIPMENT, THE SEESAW

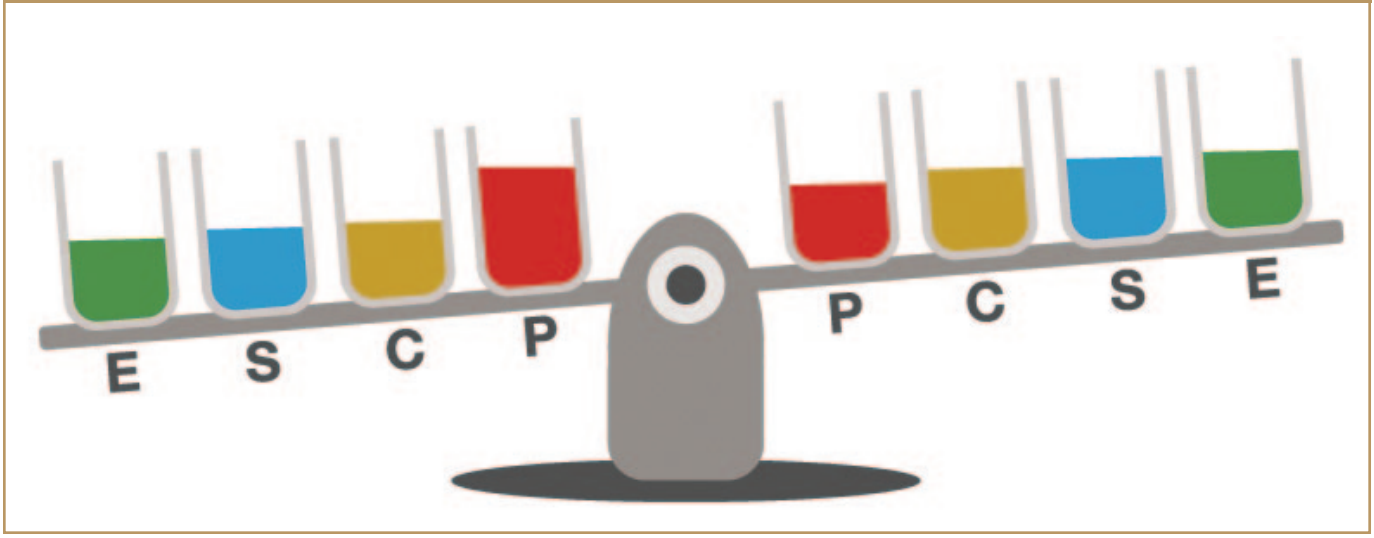
Think back to being on the playground as a child. The seesaw (Figure 1), or teeter totter, introduced you to one of the earliest real-world demonstrations of physics. Being able to lift the person on the other side of the seesaw depended on your weight and where you were positioned on the seesaw. By properly managing weight and location, you could seize the advantage. In this way, a seesaw is quite different than a simple double beam scale; a seesaw responds to both weight and relative position.

Another important difference between a seesaw and a scale is the pivot point. On a scale, the pivot point is designed to provide as little friction as possible. In the case of a seesaw, the pivot point, depending on how well it is maintained, can have a big impact on how easy it is to gain leverage when differences in weight and position are reasonably small. Put an elephant on one side of a seesaw and a mouse on the other and no amount of friction at the pivot is likely to prevent the obvious outcome. But, put two different sized mice on either side of the seesaw and a rusted pivot will disguise any weight difference or relative position on the seesaw.

BALANCING PRODUCTS ON A SEESAW

It may be pretty obvious where this is headed. Product features and benefits using the ESCP parameters can be visualized using a seesaw-type device. How a product concept stacks up against a competitor, or competitors, can be imagined based on a few simple concepts:

FIGURE 2



Concept 1 - the seesaw is a fair device, meaning that both sides are the same length and the same weight.

Concept 2 - the weight of products on both sides is modeled using buckets that are of infinite size and are weightless, filled with the same "stuff." A greater benefit or weight of a bucket is achieved by adding in more "stuff."

Concept 3 - the order of the buckets from furthest to closest to the pivot is Efficacy, Safety, Convenience, and Price. This is consistent with their inherent clinical and market "weight."

Concept 4 - the pivot point can be manipulated by managing its friction.

AN EXAMPLE

Perhaps the simplest example to explain the process is to compare two products that are identical except for price; one is a generic (left), and the other is a full price branded product (right). As shown in Figure 2, it is pretty clear the generic product tips the balance. That extra "weight," or greater value, in the Price bucket makes the difference.

Things get more interesting when we compare two branded products and all four parameters come into play. Does a bit more weight in the Efficacy bucket overcome a little bit less weight in the Safety bucket? Is it possible, or desirable, to "grease" a rusted pivot hinge? What are the options to gain leverage? What is the best bucket in which to "invest" resources?

In future articles, I will examine these questions in the context of real products and how they have been accepted as evidenced by commercial success. Product success and failure should never come as a surprise. ◆

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