Drug Development & Delivery

April 2011 Vol 11 No 3

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Strategies for Recovery

The science & business of drug development in specialty pharma, biotechnology, and drug delivery

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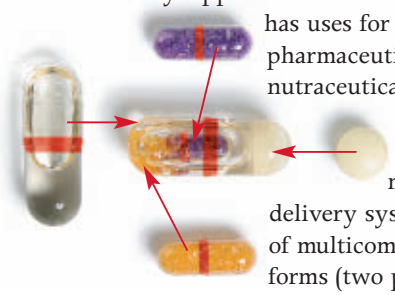
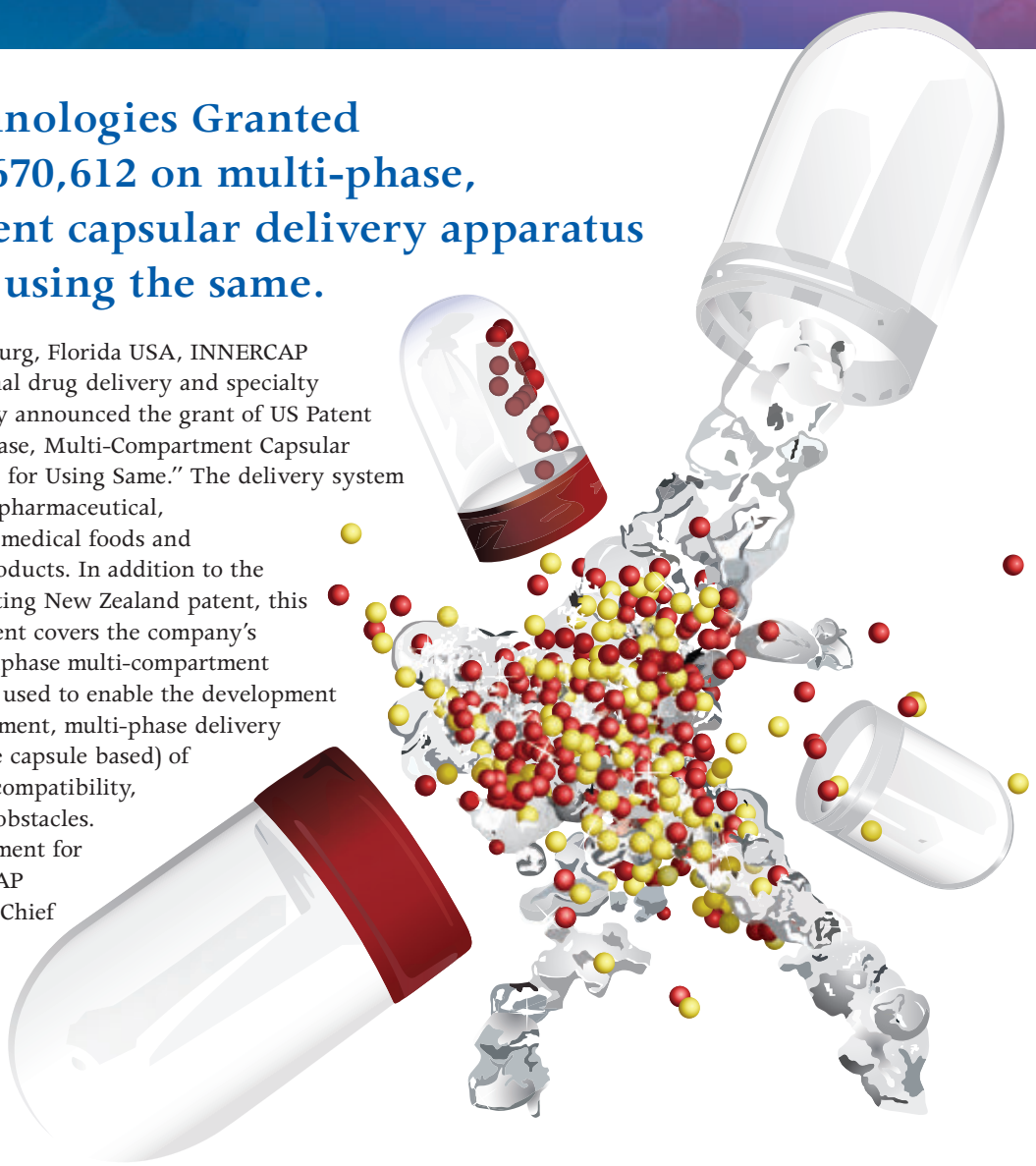
THE ADVANTAGES

OF MULTI-PHASE, MULTI-COMPARTMENT CAPSULES ARE CLEAR

INNERCAP® Technologies Granted US Patent No. 7,670,612 on multi-phase, multi-compartment capsular delivery apparatus and methods for using the same.





March 23, 2010, Saint Petersburg, Florida USA, INNERCAP Technologies, Inc., an international drug delivery and specialty pharmaceutical company, recently announced the grant of US Patent No. 7,670,612 entitled "Multi-Phase, Multi-Compartment Capsular Delivery Apparatus and Methods for Using Same." The delivery system has uses for bio-pharmaceutical, pharmaceutical, medical foods and nutraceutical products. In addition to the existing New Zealand patent, this patent covers the company's multiphase multi-compartment delivery system used to enable the development of multicompartment, multi-phase delivery forms (two piece capsule based) of combination products that have compatibility, formulation or targeted delivery obstacles.

"This is a significant development for INNERCAP Technologies NOVACAP technology," said Fred H. Miller, Chief Executive Officer at INNERCAP. "The continued growth of our patent portfolio establishes INNERCAP as one of the leading companies in this space."

The delivery system and combinations covered by the patent have the ability to deliver therapeutic entities that have never been combined previously and now can be administered together, via an oral, implanted, or suppository capsule, in the most advantageous pharmacokinetic profile, utilizing different physical phases. This technology can therefore be used to enable capsule administration of compounds that are not normally administered as a combination product. The efficacy, safety, and side-effect profiles of drugs can be substantially improved using this delivery technology. It will also provide very significant quality-of-life improvements for patients and substantial economic savings for hard-pressed healthcare systems.

"INNERCAP's multi-phase, multi-compartment technology has been commercially manufactured and validated in several products, demonstrating that INNERCAP's delivery system creates real value to consumers and branded manufacturers," added Mr. Miller.

INNERCAP was represented by Cliff Davidson, Esq. of the patent firm Davidson, Davidson & Kappel, LLC (www.ddkpatent.com) based in New York City.

For more information contact us at the telephone number and email address below:

INNERCAP® TECHNOLOGIES, INC

United States Patent No. 7,670,612
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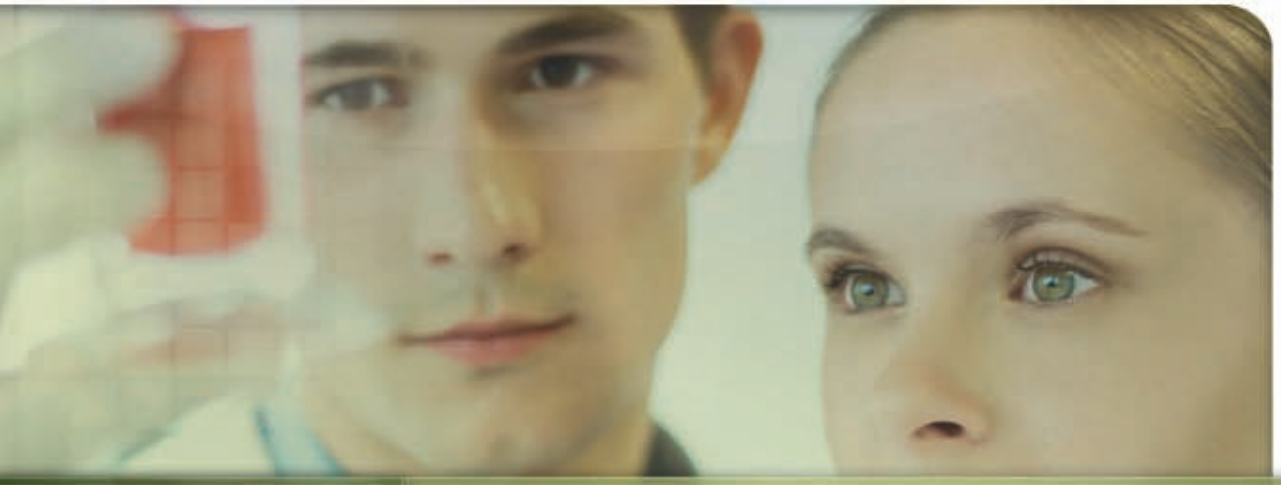

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"The availability of new technologies, such as virus-like particles, new viral or bacterial vectors, and DNA and RNA fragment technologies, opens the door for the development of prophylactic and therapeutic vaccines for diseases that until now have been out of reach, such as tumors, malaria, and cardiovascular conditions. Using the intranasal route will provide additional immunological benefits, in addition to it being a mature administration technology."

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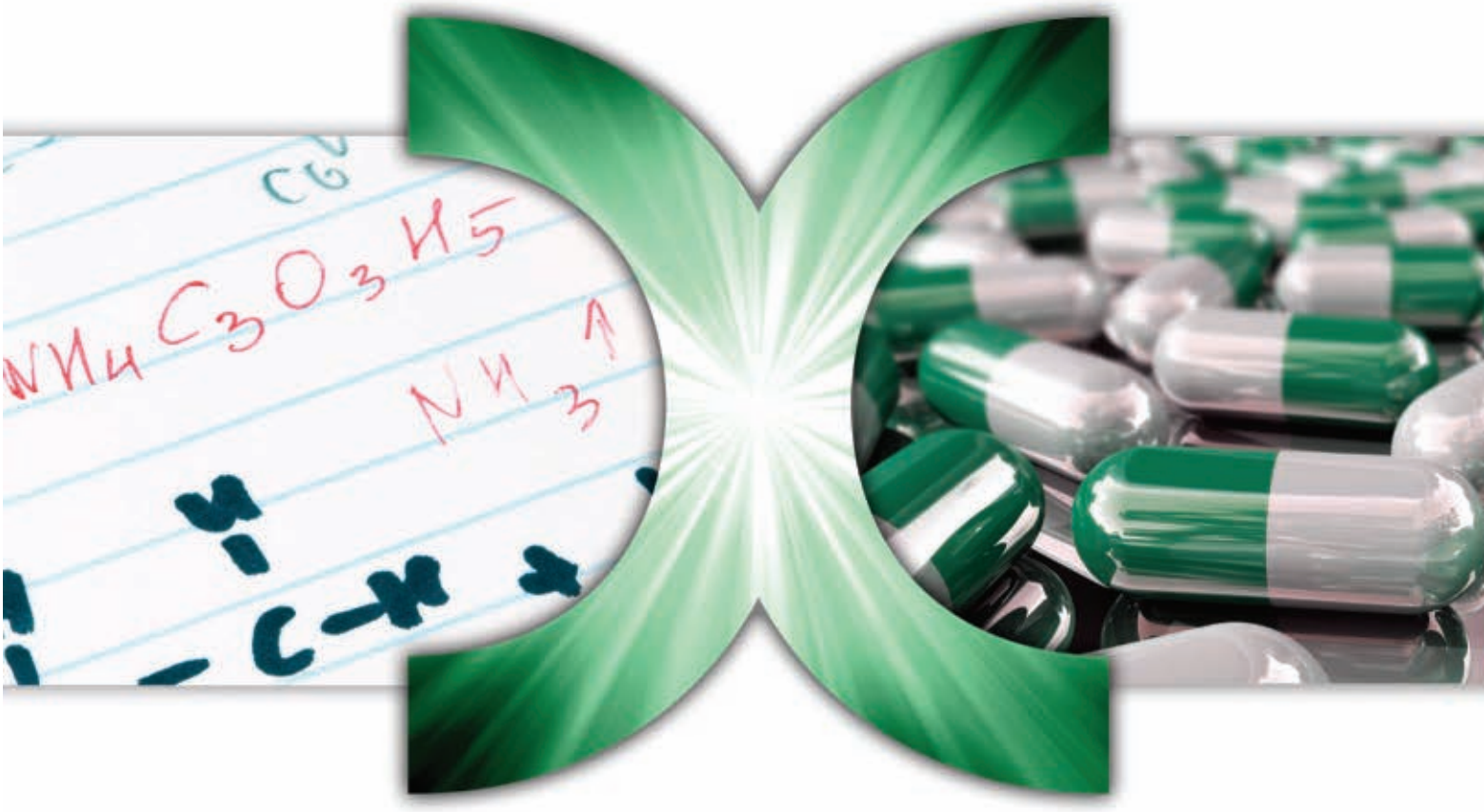

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MARKET NEWS AND TRENDS

Studies Show Triolex for Parkinson's Disease Crosses the BBB in Mice

Harbor BioSciences, Inc., which is investigating the use the company's proprietary compound Triolex as a treatment for Parkinson's disease (PD) with funding from The Michael J. Fox Foundation (MJFF), recently announced positive results in initial preclinical studies. These studies in orally treated mice demonstrate that Triolex crosses the blood-brain barrier in significant quantities.

Harbor BioSciences designed these studies to determine if Triolex, an anti-inflammatory, small-molecule compound believed to reduce inflammation in the brain, was capable of crossing the blood-brain barrier. The results described here show that more than 50% of levels of Triolex measured in plasma were found in mouse brains after oral administration. These positive findings support rodent efficacy studies, which are expected to be completed before the end of this year.

The terms of the collaboration call for MJFF to fund up to approximately $150,000 toward preclinical development of Triolex in rodents. If these studies are successful, additional funding may be awarded by MJFF to continue the clinical development of Triolex for the treatment of PD.

"Anti-inflammatory approaches to PD are of increasing interest to Parkinson's researchers, but even the most effective potential treatment must cross the blood-brain barrier in order to be translated into practical treatments for PD patients," said Todd Sherer, PhD, Chief Program Officer, The Michael J. Fox Foundation. "We are encouraged by the initial data showing that Triolex may accomplish both of these goals, and look forward to partnering with Harbor BioSciences to continue vetting this novel compound in the hope of speeding its path to the clinic."

Harbor BioSciences believes Triolex may decrease chronic inflammation in the brain, thereby protecting neurons whose loss would otherwise lead to the damage associated with PD. This belief is based on several factors: the company's previously reported data regarding the anti-inflammatory effects of Triolex in human clinical studies; attenuation of neuroinflammation in rodent models; and the extensive literature describing the benefits of related natural compounds in both humans and animal PD models. To date, Triolex has been well tolerated when administered to more than 180 people with type 2 diabetes and healthy volunteers.

"It is a privilege to be collaborating with MJFF to assess the potential benefits of Triolex in patients suffering from PD," commented James M. Frincke, Harbor BioSciences' Chief Executive Officer. "If Triolex demonstrates an effect on regulating key inflammatory cytokines in PD patients similar to that observed in animal models and in type 2 diabetes patients, it should provide an entirely new approach to the treatment of this debilitating disease. We are also hopeful that if Triolex is able to reduce neuro-inflammation and protect neurons in PD, it may also be useful in other inflammation-driven neurodegenerative disorders, such as Alzheimer's disease and multiple sclerosis."

Harbor BioSciences is a development-stage company with two product candidates that recently completed Phase I/IIa clinical trials: Apoptone (HE3235) in patients with late-stage prostate cancer, and Triolex (HE3286) in obese type 2 diabetes mellitus patients. Apoptone and Triolex represent two of the lead candidates from Harbor BioSciences' small molecule platform based on metabolites or synthetic analogs of endogenous human steroids.

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ICIG to Acquire Pharmaceutical Intermediates Business From Genzyme

International Chemical Investors Group (ICIG) recently announced it has entered into a purchase agreement under which an affiliate of ICIG will acquire the pharmaceutical intermediates business from Genzyme Corporation. Under the terms of the agreement, ICIG will purchase substantially all of the pharmaceutical intermediates business, excluding the drug delivery technologies portion of the business.

ICIG has agreed to offer employment to the unit's approximately 120 employees upon closing, and plans to maintain operations at its primary location, a manufacturing facility in Liestal, Switzerland. The acquired pharmaceutical intermediates business will be renamed Corden Pharma Switzerland LLC and will operate as part of ICIG's pharmaceutical business within the Corden Pharma platform. The companies' goal is to close the transaction during the first quarter of 2011. Financial terms are not disclosed.

As part of the agreement, ICIG will enter into a 5-year supply contract to provide Genzyme with materials needed for the production of eliglustat tartrate, an investigational treatment for Gaucher disease Type 1 that is currently in Phase III clinical trials. ICIG will also supply materials needed for the manufacture of other treatments in earlier stages of development, including neo-GAA, currently in preclinical development as a potential next-generation Pompe disease therapy.

Genzyme Pharmaceuticals develops and manufactures chemically synthesized pharmaceutical materials and technologies for the global pharmaceutical industry and focuses on lipids, peptides, carbohydrates, oligonucleotides, and custom small molecules.

ICIG is a privately owned industrial holding company focusing on mid-sized chemicals and pharmaceutical businesses. Since inception in 2004, ICIG has acquired 15 businesses, all of which have origins in major global chemical or pharmaceutical corporations and are independently managed. ICIG companies currently employ more than 3,000 people and operate 15 manufacturing facilities in Europe and the United States. Corden Pharma group companies offer contract development and contract manufacturing for advanced pharmaceutical intermediates, APIs, and drug product formulations with more than 1,500 individuals supporting their customers with specialized technologies in all international markets.

Ricerca Biosciences Announces Strategic Collaboration With Fulcrum

Ricerca Biosciences, an integrated preclinical CRO providing services to the biopharmaceutical industry, recently announced a strategic partnership with Fulcrum Pharma, an Aptiv Solutions company, a leading provider of drug development consulting and regulatory services essential to progress a development program from research to product approval. The collaboration provides unique, value-added capabilities to biopharmaceutical companies by creating a streamlined and efficient process to move a candidate from development to clinical evaluation.

"The Fulcrum Pharma collaboration is an attractive and exciting proposition for Ricerca and our clients," said Ian Lennox, CEO of Ricerca. "Fulcrum Pharma brings deep experience in IND authorship and regulatory approval, which fits well with Ricerca's preclinical services in discovery pharmacology, chemical development, and drug safety assessment. The relationship greatly expands our ability to serve our clients who are preparing for regulatory submission anywhere in the world."

"The collaboration offers our early stage clients a streamlined service to move efficiently through preclinical development to creation of high-quality IND submissions," added Patrick K. Donnelly, Aptiv Solutions' Chairman and CEO. "This approach, coupled with our expertise in adaptive clinical trial design, will provide our clients with the ability to accelerate the development of their products and stay ahead of the competition."

Ricerca Biosciences provides the full range of preclinical services from early discovery medicinal chemistry, compound screening, profiling, and lead optimization through full drug safety, metabolism, and efficacy development support, as well as clinical supply and commercial API production capability. Fulcrum Pharma is a global leader in the provision of strategic and operational regulatory support to assist clients in the authorship and approval of regulatory submissions. Aptiv Solutions is a global biopharmaceutical and medical device development services company focused on recognizing, understanding, and enabling clients to capitalize on rapid and fundamental changes facing companies developing products in the pharmaceutical, biotech, and medical device market.

Elan & PPD Announce Global Business Relationship in Drug Development

Elan, plc and PPD, Inc. recently announced they have formed a global business collaboration focused on the advancement, progression, and execution of Elan's development portfolio. The primary objective of this relationship is to deploy both companies' skills and expertise in a flexible, integrated manner to drive the execution of Elan's clinical programs in a parallel and expedited basis and on a global scale.

Under this business agreement, PPD will act as Elan's primary service provider for all development functions and activities. Elan will retain ownership of its assets and accountability for decision-making with regard to strategy and progression of the individual molecules as well as the overall portfolio. This business construct will enable Elan to leverage the significant and high-quality capabilities of PPD across project and data management, biostatistics, regulatory, clinical and medical monitoring, quality assurance, pharmacovigilance, and other areas. Elan and PPD intend to work closely to build upon this initial relationship and continuously explore opportunities to leverage both companies' respective competencies and maximize the full opportunity of moving Elan's science into clinical development and advancing toward patients.

"Establishing this strategic collaboration with PPD will enable Elan to accelerate the progression of our science into the clinical development setting in a rapid and global fashion," said Eliseo Salinas, Chief Medical Officer, Executive Vice President and Head of Development for Elan. "The ability to fluidly access additional expertise and execution capability on a global scale will complement our internal talent and may enable us to move multiple programs forward in a parallel manner."

"PPD will act as a strategic collaborator and deliver capabilities, resources, and expertise that will enable us to advance our business on a global scale while capturing efficiencies and flexibility from a business and operational point of view," added Elan's Executive Vice President and Head of Alliance Management, Doug Love.

"PPD and Elan have established an innovative relationship structure that enables Elan to continue to invest in and advance its world-leading work in biology and its broad application in neuroscience while leveraging PPD's expertise and execution capabilities across key program areas on a global scale. This relationship brings together the strongest resources of both companies, and we are pleased to have created this unique and strategic business arrangement with Elan," commented David Grange, Chief Executive Officer of PPD.

Elan Corporation, plc is a neuroscience-based biotechnology company committed to making a difference in the lives of patients and their families by dedicating itself to bringing innovations in science to fill significant unmet medical needs that continue to exist around the world. PPD is a leading global contract research organization providing drug discovery, development, and life-cycle management services.

Quintiles Supports Samsung's Entry Into Biopharmaceutical Market

Samsung Electronics Co., Ltd., the world's largest electronics company, and its affiliated companies (Samsung) recently announced they have entered into a strategic partnership with Quintiles, the world's leading pharmaceutical services company, to support Samsung's entry into the biopharmaceuticals market.

Under an agreement signed last week, Quintiles will make a minority 10% investment of approximately $30 million to start a new joint venture company with Samsung in the first half of 2011 to provide biopharmaceutical contract manufacturing services in South Korea. Samsung will own the remaining 90% of the joint venture company in the following proportions: Samsung Electronics Co., Ltd., 40%; Samsung Everland, Inc., 40%, and Samsung C&T Corporation, 10%.

The strategic partnership will support Samsung's entry into the biopharmaceutical market and reinforces Quintiles' role as an ally to help companies succeed in the New Health landscape.

"We are very pleased that Samsung has selected Quintiles as its ally to enter the biopharmaceuticals business through the manufacture of biosimilars," said Anand Tharmaratnam, Senior Vice President and Head of Asia Markets for Quintiles. "South Korea is an important part of our growth strategy in Asia. This strategic partnership illustrates how Quintiles can use its resources and expertise across the clinical, commercial, consulting, and capital spectrum to help companies achieve their strategic goals. We're also very pleased to drive innovation and advance the role of South Korea in the global biopharmaceutical industry."

"This partnership with Samsung demonstrates how at Quintiles we leverage not only our intellectual and human capital but also how we can invest to advance mutual interests and accomplish shared goals," added Paul Casey, Vice President and Head of Asia Corporate Development for Quintiles. "It shows we are committed to developing non-traditional alliances in order to help our customers navigate risks and seize opportunities in the complex industry landscape and ultimately help patients."

The joint venture company represents Samsung's first step into the biopharmaceutical business. Samsung also plans to commercialize biosimilars by 2016, and expand into innovative biologics in the future. Plans call for the joint venture company to construct a biopharmaceutical manufacturing plant in the Incheon Free Economic Zone in Songdo, Incheon, South Korea. Groundbreaking is expected in the first half of 2011 and full-scale operation is expected to commence in April 2013. The plant will be fully equipped with cutting-edge technologies and an 8,000-gallon (30,000 liters) mammalian cell culture bioreactor capacity capable of producing 1,300 pounds (600 kilograms) of biopharmaceutical products. The products are expected to be sold mostly on international markets.

Omthera Raises $33.9 Million to Fund Phase III Development

Omthera Pharmaceuticals, Inc., a privately held emerging specialty pharmaceuticals company, recently announced it has raised $33.9 million in a Series B funding round, led by new investor, New Enterprise Associates (NEA), one of the world's leading venture capital firms. Existing investor, Sofinnova Partners, also participated in the financing. The capital raised will be used to fund the Phase III clinical development of Omthera's novel Omega-3 fatty acid compound, Epanova, and for general corporate purposes. With this latest round, Omthera has raised approximately $40.4 million in venture capital since commencement of operations in 2009.

In a separate press release, Omthera also announced the company has initiated its pivotal Phase III EVOLVE (EpanoVa fOr Lowering Very high triglyceridEs) trial for Epanova, for the treatment of patients with very high triglycerides (greater than or equal to 500 mg/dL). In January 2011, the company announced data from its ECLIPSE (Epanova Compared to Lovaza In a Pharmacokinetic Single-dose Evaluation) trial, designed to evaluate the bioavailability of Epanova in comparison to Lovaza, the leading prescription Omega-3. Data from the trial showed that the bioavailability of Epanova was significantly superior to Lovaza.

"Omthera continues to generate very positive data, indicating Epanova has the potential to become the best-in-class therapy in the nearly $2 billion worldwide Rx Omega-3 market and an important treatment for the millions of patients suffering from high triglycerides," said Jerry Wisler, President, Chief Executive Officer, and Co-Founder of Omthera. "As such, we are delighted to gain the support of a venture capital firm of the stature of NEA to lead this substantial new funding."

New Enterprise Associates, Inc. is a leading venture capital and growth equity firm focused on helping entrepreneurs build transformational businesses across multiple stages, sectors, and geographies. With approximately $11 billion in committed capital, NEA invests in information technology, healthcare, and energy technology companies at all stages in a company's lifecycle, from seed stage through IPO.

Founded in 2008, Omthera Pharmaceuticals, Inc. is a privatelyheld, emerging specialty pharmaceuticals company focusing its efforts on the clinical development of new therapies for dyslipidemia. Led by a team of experts with exceptional experience in developing new therapies for lipid disorders, Omthera is dedicated to developing innovative therapies for the millions of patients who have elevated triglyceride levels and increased risk of cardiovascular disease.

Recovery Strategies

Lessons Learned From Genzyme, Johnson & Johnson & Baxter

Part 1 of a 6-part series on how not to blow the recovery.

By: Derek Hennecke, President & CEO Xcelience LLC

The recession is dead, long live the recovery! But not so fast. Just because your company made it through the recession, doesn't necessarily mean it will make it through the recovery. Yes, I'm dead serious. It is possible to screw this up.

Your company has just been through a savage economic battle. The pain of it is seared in your memory. But -hurray!- business is coming back. The risk now is that we will let our caution reign us in too much. After what we've been through, no one is actually turning away business, are they? But here's the rub: if we don't actively feed our companies as they grow - with quality hires and measured facilities growth - we risk major quality issues. In our industry, quality issues are death. At least, they should be.

Quality issues were a near-death experience for Genzyme. How does anyone get up in the morning with a whopping $175 million consent decree for a host of manufacturing shortcomings? The company closed one plant and ended fill/finish operations in another for products sold in the US. Fill/finish activities for Cerezyme® (imiglucerase for injection), Myozyme® (alglucosidase alfa), Fabrazyme® (agalsidase beta), and Thyrogen® (thyrotropin alfa for injection) for the US market now take place at Genzyme's Waterford, Ireland, plant and at an external contract manufacturer.

On top of the corporate humiliation resulting from these quality issues is the human suffering - some patients were left with little or no supplies in the middle of their treatment. Investors too were angered, including Carl Icahn, who waged a proxy fight and prompted a board probe into insider stock sales.

As a manufacturer myself, for days I cringed to read the latest Genzyme news. I'll confess I read of the warning letters and consent decrees with a measure of, "What were they thinking?" tempered by a dose of, "My God, could it ever happen to my company?" It's a blending of two emotions; schadenfruede - that secret, guilty delight we take in other's misfortunes - and plain old night terrors.

And yet, Genzyme lives on. Gone are the days when a consent decree rules out the possibility of growth or a merger. Genzyme soon found itself having to hold its nose and accept the advances of a suitor offering a price well below what it would've been worth without the quality issues. Even with this low bid, the acquisition remained uncertain. The suitor (Sanofi-Aventis) had to be sure it wasn't paying too much. How do you fashion a merger with a company like Genzyme in which so many costs and risks are unknown?

I will give the dealmakers their due. They crafted a deal of incredible creativity. Sanofi originally offered $69/share - an offer that sounds at first blush very generous given the fact that Genzyme was trading at $56/share before the merger rumors started. But insiders know the company was worth much more. Some say that Genzyme could easily have commanded a much higher price than the $74/share (a total of about $20 billion) they came out with, but Genzyme CEO Henri Termeer made the mistake of holding out in the hopes of creating a bidding war. Mr. Termeer was apparently a genius when it came to selling orphan drugs for awe-inspiring prices, but somewhat less adapt at selling a company.

But here's the creative part: the deal requires Sanofi-Aventis to cough up more money depending on various factors, such as fixing manufacturing problems and whether the Campath leukemia med is approved for multiple sclerosis (MS). These are called contingent value rights (CVRs), and payouts could be as much as $4/share per CVR later. Or there could be no payouts. Or there could be payouts but only years down the road.

CVRs are not new to deal-making, only to deals like this. You might see them with a big pharma/small start-up merger, but you don't see them in the merger of two large companies, like the Prizer-Wyeth deal or the Merck-SGP merger.

The implications of the structure of this deal go way beyond mere financial ingenuity. It means that regulatory actions - right up to the dreaded consent decree that was once considered a fatal blow to any company's growth or merger prospects - are now merely part of the cost of business in our industry. Consent decrees as a cost of doing business. This is stomach-turning. And it gets worse.

"Pharma M&As have officially entered the high-risk world of derivatives trading," writes Jim Prutow, a partner in the healthcare practice at the PRTM consulting firm.

"CVRs are basically options in which the investor is betting for or against whether certain milestones will be achieved, eg, FDA approval, etc. These types of trades are not regulated or even posted on any type of public exchange."

Welcome to our new industry. Quality issues are no longer a kiss of death, they're just a higher risk level you can price into a deal. Will it be worth it for Sanofi, acquiring a tainted company? In truth, quite possibly yes. For one thing, the structure of the deal makes it so that if the MS drug is approved, it's a win-win for both companies. More than that, Genzyme provides revenue streams and launches Sanofi into the rare disease business, where competition should be less. And there are fewer and fewer biologically focused biotechs out there, so it's not like Sanofi had lots to choose from.

Rare disease meds also command a lucrative pricing segment, though that may not be a long-term reliable assumption. As with any high-price segment, other potential competitors will be attracted and attempt to compete on price. There are other risks out there as well, such as the possibility that Sanofi won't integrate Genzyme well, particularly on the rare drug side, where the physician/patient/drug company dynamic can be quite different from other more mundane pharmaceutical products.

Most any acquisition leads to a shakedown amongst staff - both those who are laid off and those who choose to get out rather than face the coming uncertainty. Loss of key staff taxed with putting Genzyme back on track could further destabilize the situation.

I'm focussing on Genzyme here, but I could just as easily talk about the embarrassment of Baxter Healthcare, where CEO Bob Parkinson recently disclosed that he had received a warning letter from the FDA concerning problems at two plants in Puerto Rico. This comes just three years after the company was front and center in the contaminated Heparin scandal that led to deaths and a whole lot of intense FDA scrutiny.

Or I could have turned the lens to the hundreds of millions of products that have been recalled by the once venerable Johnson & Johnson. There was Tylenol, Rolaids, Benadryl, contacts lenses, and hip replacement devices. We've seen government probes, a factory closing, layoffs, bonus cuts, and then the story became about the company's bungled attempts to manage the fallout from the their actual bungling.

I'm not going to say that all these industry catastrophes were the result of trying to take on new business in the recovery without the necessary investment in people and facilities. Only that in this environment, with sales coming back while everyone is still battered and shell-shocked and in a cost-cutting state of mind, the risk of more quality failures is high.

We need to quit worrying. The recession is over. Take measured risks to expand to meet demand. We don't need anymore industry casualties. It should be us doing the hiring, not the FDA. We have a perfectly good recovery brewing, let's not blow it. ◆

BIOGRAPHY

Derek G. Hennecke, MBA
President & CEO
Xcelience

Derek G. Hennecke is a founding member, CEO and President of Xcelience. He has a long history of growing strong businesses around the world. Blending a scientific and business background, he has nearly 2 decades of international experience in the healthcare industry and a track record as a highly successful international turn-around manager in the global drug development community. Xcelience is the first company Mr. Hennecke has managed as an owner, having launched a management buy-out from MDS Pharma Services in 2006. The newly formed company immediately embarked on a robust pattern of strong growth. This growth was recognized in May 2008, when Mr. Hennecke was selected as a finalist for the coveted 2008 Ernst & Young Florida Entrepreneur of the Year award, a nomination based on the demonstration of extraordinary success in the areas of innovation, financial performance, personal commitment to community, and perpetual growth. Mr. Hennecke was also recognized as a finalist for the Ultimate CEO awards by the Tampa Business Journal and Small Business of the Year by the Greater Tampa Bay Chamber of Commerce, in 2008. Before founding Xcelience, Mr. Hennecke spent more than 10 years abroad working for the Dutch-based conglomerate DSM. In Montreal, he was GM of a 250-staff Biologics plant for more than 2 years. In Cairo, Egypt, as GM, he oversaw a radical turn-around in an anti-infectives plant that had been slated for closure. He spent 2 years in Holland developing new Pharma intermediates, and two years in Mexico as Commercial Director covering Central and South America. He also worked for Roche, both in Canada and Germany. Mr. Hennecke has a BSc in Microbiology from the University of Alberta and an MBA from the Erasmus University in The Netherlands.

MARKET BRIEF

Oral Administration Remains the Most Used & Preferred Drug Delivery Method for Neurological Diseases

By: Jennifer Brice, Industry Manager, Pharmaceuticals & Biotechnology, Frost & Sullivan

INTRODUCTION

Some of the most difficult diseases to manage are those that involve malfunctioning nerve or brain structures, such as dementias, seizures, vascular events, mental diseases, states of sleep and wakefulness, and pain. According to the World Health Organization (WHO), neurological disorders are estimated to account for more than 10% of the world's death and disability. Efforts to improve the treatment of these disorders are increasing, and biopharmaceutical and drug delivery companies are striving to improve treatments for these disorders.

A neurological disorder is a disorder of the body's nervous system consisting of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and the spinal cord, while the PNS includes 31 pairs of peripheral nerves and 12 pairs of cranial nerves.

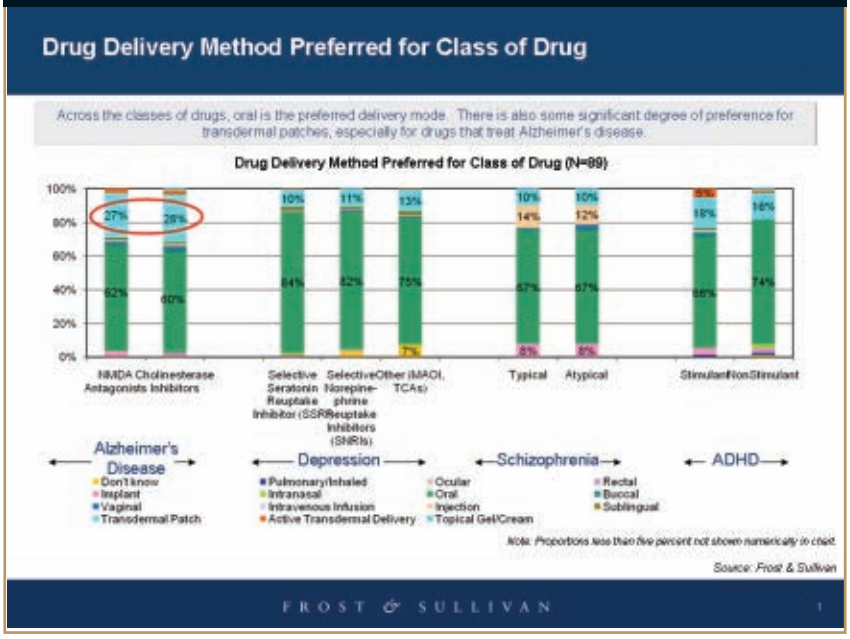
CURRENT TREATMENT METHODS FOR NEUROLOGIC DISEASES

In a recent survey, more than 100 physicians who treat neurological disorders were surveyed by Frost & Sullivan to determine drug delivery usage patterns, preferences, and opportunities in the US.[1] For this survey, neurological disorders were defined as depression, Alzheimer's disease, Parkinson's disease, attention deficit hyperactivity disorder (ADHD), and schizophrenia.

ALZHEIMER'S DISEASE

Cholinesterase inhibitor is the most commonly prescribed class of drugs for treating Alzheimer's disease, with 80% of neurologists prescribing this method of treatment. In Alzheimer's patients, research has shown there is a decrease in the level of acetylcholine, a chemical messenger that assists memory, thought, and judgment. Cholinesterase inhibitors improve the effectiveness of acetylcholine by increasing the levels in the brain or by strengthening the way nerve cells respond to the brain. This leads to an increase in communication between nerve cells that can temporarily improve or maintain

FIGURE 1



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symptoms of Alzheimer's disease. Cholinesterase inhibitors used to improve or stabilize symptoms for patients with Alzheimer's disease consist of donepezil (Aricept™ by Pfizer/Eisai), galantamine (Reminyl™ by Johnson & Johnson), and rivastigmine (Exelon™ by Novartis). All three treatments are administered orally. Exelon is also available as a transdermal patch. Tacrine (Cognex® by Shionogi USA, Inc.) was the first approved cholinesterase inhibitor; however, this drug is rarely prescribed today due to safety concerns.

N-methyl d-aspartate receptor (NMDAR) antagonists are the second most commonly prescribed class of drugs for treating Alzheimer's disease, with 18% of neurologists preferring this method of treatment. NMDA receptor antagonists are a class of anesthetics that inhibit the N-methyl d-aspartate receptor. Memantine (Namenda®) is a NMDAR antagonist used to treat Alzheimer's disease. It is believed that Namenda works by regulating glutamate, an important brain chemical. Memantine allows for Alzheimer's patients to continue with certain daily functions longer than if they were not on the drug.

Assuming any form of delivery is available for cholinesterase inhibitors and NMDAR antagonists, oral administration is the preferred delivery mode with approximately 60% to 62% of physicians preferring this delivery type for Alzheimer's patients. The second degree of preference is transdermal patch at approximately 27% to 28%.

Although the treatments in Table 1 have the potential to add therapeutic value for the treatment of Alzheimer's disease, the administration options do not seem to change versus what is currently on the market today.

TABLE 1

Drug	Company	Class	Status	Dose/Administration
Dimebon (in combination with donepezil)	Medivation, Inc	Inhibitor of cholinesterase and NMDA receptors	Phase III	5 or 20 mg orally 3 times daily
Bapineuzumab	Pfizer/J&J/Elan	Humanized monoclonal antibody	Phase III	10 mg s.c. once per week
Semagacestat/LY450139	Eli Lilly	y-Secretase Inhibitor	Phase III	60 mg orally once a day gradually escalated to 140 mg orally once a day
Immune Globulin	Baxter Healthcare Corporation	Immune Globulin	Phase III	200 or 400 mg/kg bodyweight every 2 weeks
Solanezumab/LY2062430	Eli Lilly	Humanized anti-A Beta peptide immunoglobulin G-1 (IgG1) monoclonal antibody	Phase III	400 mg i.v. every 4 weeks

Late-Stage Development Programs for the Treatment of Alzheimer's Disease

DEPRESSION

Selective Seratonin Reuptake Inhibitors (SSRIs) are the most commonly prescribed class of drugs for treating depression, with 79% of physicians preferring this method of treatment. SSRIs are believed to increase the extracellular level of the neurotransmitter serotonin, and are typically effective for the treatment of depression, anxiety disorders, personality disorders, and insomnia. Examples of SSRIs include citalopram (Celexa),

FIGURE 2

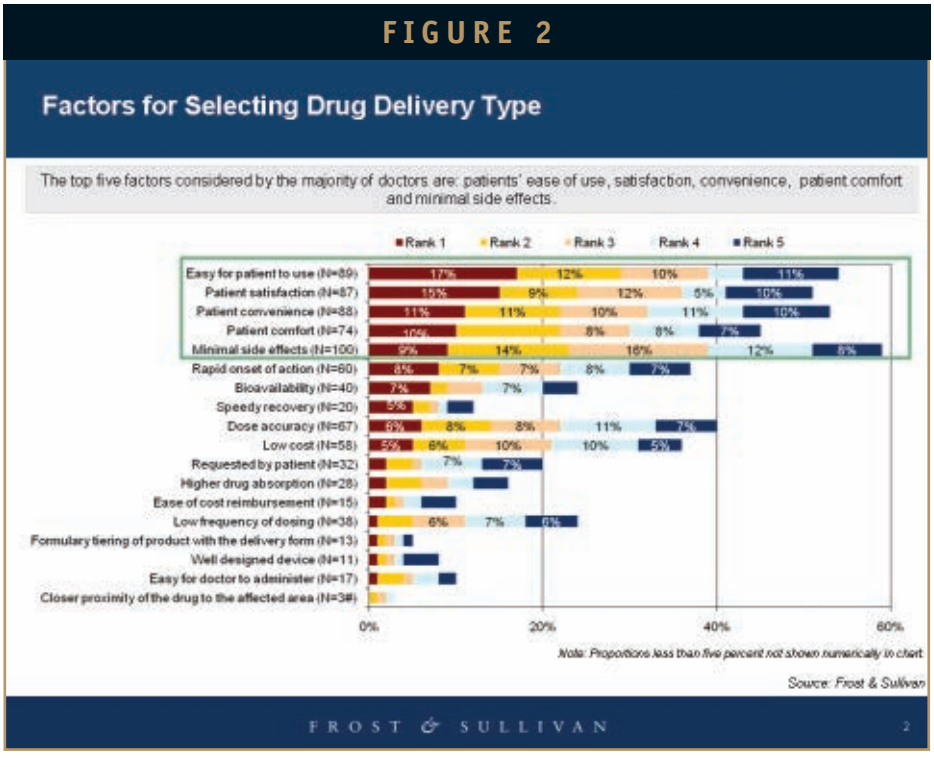
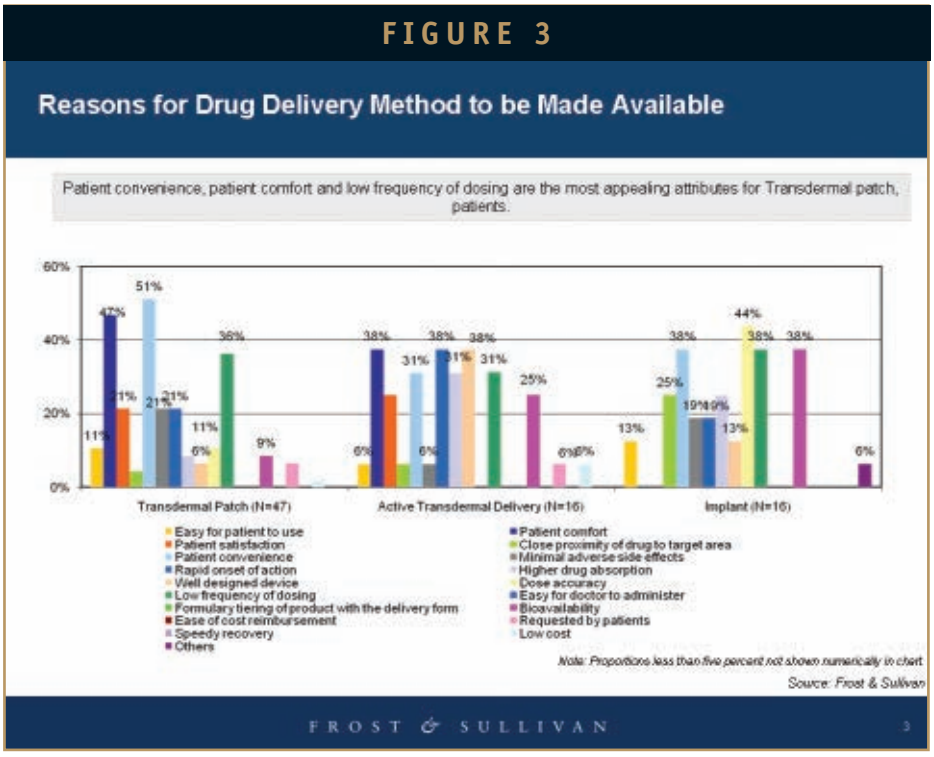


FIGURE 3



escitalopram (Lexapro), fluoxetine (Prozac, Prozac Weekly, Sarafem), paroxetine (Paxil, Paxil CR, Pexeva), sertraline (Zoloft), and fluoxetine combined with the atypical antipsychotic olanzapine (Symbyax). SSRIs typically come in an oral administrations.

Selective Norepinephrine Reuptake Inhibitors (SNRIs) are the second most commonly prescribed class of drugs for treating depression, with 16% of physicians preferring this method of treatment. SNRIs act upon two neurotransmitters in the brain, serotonin and norepinephrine. These neurotransmitters are known to be essential to mood regulation. Similar to SSRIs, SNRIs typically come in an oral administration. Examples of SNRIs include venlafaxine (Effexor), nefazodone (Serzone), milnacipran (Dalcipran/Ixel), desipramine (Norpramine/Pertofraneis), and duloxetine (Cymbalta).

Assuming any form of delivery is available for SSRIs and SNRIs, oral administration is the preferred delivery mode, with approximately 82% to 84% of physicians preferring this delivery type for Alzheimer's patients. The second degree of preference is transdermal patch at approximately 10% to 11%.

SCHIZOPHRENIA

Atypical (newer to the market than typical) antipsychotics are the most commonly prescribed drugs for treating schizophrenia, with 73% of physicians preferring this method of treatment. Atypical antipsychotics are a group of antipsychotic tranquilizing drugs used to treat psychiatric conditions. Atypical and typical antipsychotics both tend to block receptors in the brain's dopamine pathways; however, atypicals are less likely to cause extrapyramidal motor control disabilities in patients, including unsteady Parkinson's disease-type movements, body rigidity, and involuntary tremors.

The following list is atypical antipsychotic drugs marketed in various parts of the world:

- Amisulpride (Solian by Sanofi-Aventis)
- Aripiprazole (Abilify by Bristol-Myers Squibb)
- Asenapine (Saphris by Merck/Schering-Plough)
- Blonanserin (Lonasen by Dainippon Sumitomo Pharma in Japan and Korea)
- Clotiapine (Entumine by Teva Pharmaceuticals)
- Clozapine (Clozaril by Sandoz)
- Iloperidone (Fanapt by Novartis)
- Lurasidone (Latuda by Dainippon Sumitomo Pharma)
- Mosapramine (Cremin by Mitsubishi Tanabe in Japan)
- Olanzapine (Zyprexa by Eli Lilly)
- Paliperidone (Invega by Janssen Pharmaceutica)
- Perospirone (Lullan by Dainippon Sumitomo Pharma)
- Quepin (Specifar by Specifar ABEE in Greece)
- Quetiapine (Seroquel by AstraZeneca)
- Remoxipride (Roxiam by AstraZeneca)
- Risperidone (Risperdal by Janssen-Cilag)
- Sertindole (Serdolect by H. Lundbeck/Abbott Labs)

- Sulpiride (Sulpirid, Eglonyl by Unimed Pharmaceutical, Inc.) Ziprasidone (Geodon, Zeldox)
- Ziprasidone (Geodon, Zeldox by Pfizer)
- Zotepine (Nipolept by Aventis in Germany)

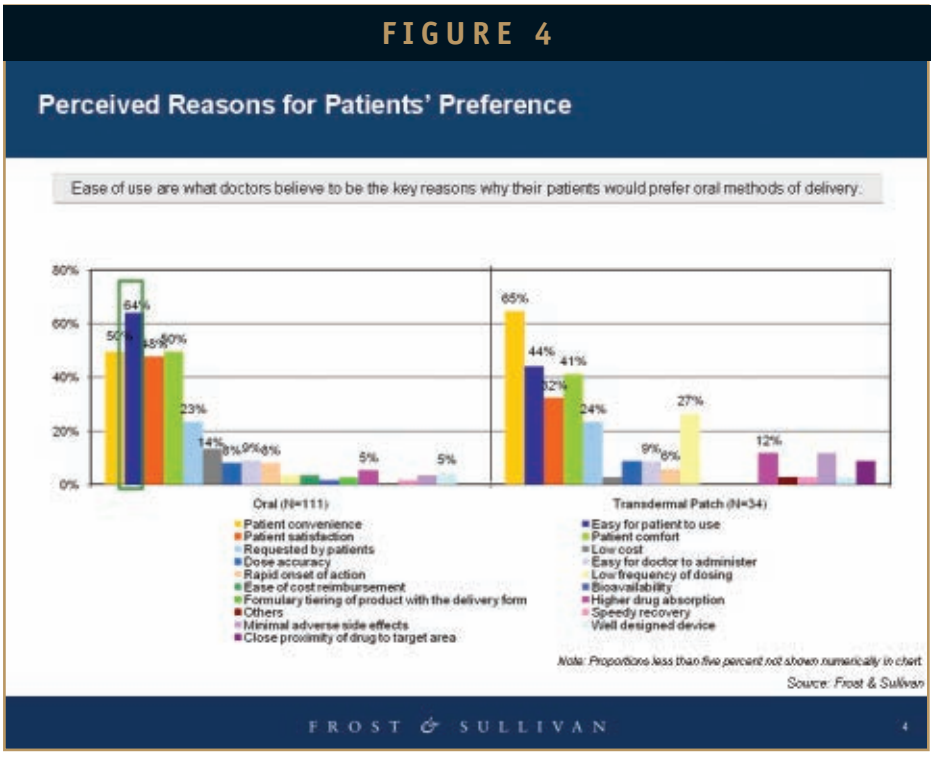
Atypical antipsychotics that have recently been evaluated for the treatment of schizophrenia include Bifeprunox (DU-127,090 by Solvay and Lundbeck), Pimavanserin (ACP-103 by Acadia Pharmaceuticals), and Vabicaserin (SCA-136 by Wyeth/Pfizer). In June 2009, Solvay and Lundbeck decided to terminate the development program for DU-127,090 due to insufficient efficacy data for non-acute patients with schizophrenia. Vabicaserin also does not appear to be in clinical development anymore. Pimavanserin, however, is currently in Phase II development for the treatment of schizophrenia.

Typical antipsychotics are the second most commonly prescribed drugs for treating schizophrenia, with 21% of physicians preferring this method of treatment. Typical antipsychotics are first-generation antipsychotics. Similar to atypical antipsychotics, typical antipsychotics block receptors in the brain's dopamine pathways. Typical antipsychotics are often classified by potency. Examples include the following:

Low Potency

- Chlorpromazine (Largactil, Thorazine by GlaxoSmithKline)
- Thioridazine (Mellaril by Novartis, Discontinued in 2005)

FIGURE 4



- Mesoridazine (Discontinued in 2004)

Medium Potency

- Loxapine (Loxapac, Loxitane by Watson Pharmaceuticals, Inc.)
- Molindone (Moban by Endo Pharmaceuticals, Discontinued in January 2010)
- Perphenazine (Trilifon by various generic manufacturers)
- Thiothixene (Navane by Pfizer)
- Trifluoperazine (Stelazine by GSK)

High Potency

- Haloperidol (Haldol, Serenace by J&J)
- Fluphenazine (Prolixin, by various generic manufacturers)
- Droperidol by J&J
- Zuclopenthixol (Clopixol by Lundbeck)
- Prochlorperazine by Teva

Most typical antipsychotics are available as an oral administration; however, some of the high-potency treatments are also available as an intramuscular injection. This is especially used when patient cooperation and compliance is required.

Assuming any form of delivery is available for atypical and typical antipsychotics, oral administration is the preferred delivery method, with 67% of physicians preferring this delivery type for schizophrenia patients. The second degree of preference is injection at approximately 12% to 14%.

MARKET BRIEF

ADHD

Stimulants are the most commonly prescribed class of drugs for treating ADHD, with 86% of physicians preferring this method of treatment. Stimulants induce temporary improvements in mental and/or physical function. Norepinephrine reuptake inhibitors (NRIs) and norepinephrine-dopamine reuptake inhibitors (NDRIs) are examples that provide a stimulant effect. Bupropion (Wellbutrin, Zyban) by GlaxoSmithKline is the most well-known NDRI.

Non-stimulants are the second most commonly prescribed drugs for treating ADHD, with 13% of physicians preferring this method of treatment. Atomexetine (Straterraby by Eli Lilly, an NRI) is the most well-known non-stimulant used to treat ADHD.

Oral administration is the most common delivery method for ADHD medication today. However, assuming any form of delivery is available for stimulants and non-stimulants, oral administration would still be the preferred delivery method, with approximately 66% to 74% of physicians preferring this delivery type for ADHD patients. The second degree of preference is via transdermal patch at approximately 16% to 18%.

SUMMARY

Oral administration is currently the most used and preferred delivery method for neurological conditions, including Alzheimer's disease, depression, schizophrenia, and ADHD. There is also a significant degree of preference for transdermal patches, especially for drugs that treat Alzheimer's disease (Figure 1).

Furthermore, the top five reasons physicians selected oral delivery for treating neurological disorders is due to minimal side effects, patient ease of use, patient convenience, patient satisfaction, and patient comfort (Figure 2).

Although there are a variety of different classes and drugs used to treat the different neurologic conditions, oral drug delivery is the most commonly used delivery type for all diseases in this space. Ease of use and convenience are the top attributes for oral delivery (Figure 3). Although oral administration is expected to continue to be the preferred form of drug delivery for neurological diseases, there seems to be increasing interest in transdermal delivery. In most cases, transdermal patch was selected as the second most preferred delivery type if available for the disease. Key considerations for transdermal patch include patient convenience, patient comfort, and low frequency of dosing (Figure 4).

For more information on the usage patterns, preferences, and opportunities in the US among physicians treating neurological disorders, please contact Jennifer Brice of Frost & Sullivan at (650) 475-4560 or jennifer.brice@frost.com.

REFERENCE

1. F&S 2008 U.S. Drug Delivery: Neurological Disorder- Physician Perspective.

BIOGRAPHY

Jennifer Brice currently serves as Industry Manager, Pharmaceuticals/Biotechnology at Frost & Sullivan in North America, Mountain View, where she devises strategies and leverage resources to deliver projects in an efficient manner from initial design to implementation. Ms. Brice has a strong ability to train, advise, and supervise analysts on project material and provide strategies for collecting primary and secondary information, as well as manages and executes quality control activities to ensure client deliverables meet top standards. Her industry expertise includes a strong network of key opinion leaders and senior executives within the pharmaceutical/biotech segments and an experience base covering a broad range of sectors within the life sciences space, including infectious diseases, biosimilars, rheumatology/inflammatory diseases, and ophthalmology. Previous experience includes both operational and project management roles in a consulting firm focused on the life sciences industry and Senior Analyst/Operations Manager at CIS Life Sciences/Business Research Group (now Prescient Life Sciences) in Mt. Olive, NJ. Ms. Brice earned her BSc from Ramapo College and her mini-MBA from Rutgers University.

DELIVERY REPORT

Drug Delivery: Products That Pop

By: Josef Bossart, PhD

In the process of putting together the material for the Delivery Report articles featured in this publication last year and more recently, our Drug Delivery Enabled/Enhanced Products report (DDEP2011), it was hard not to be impressed by a number of products that just seemed "different." But different in a good way. These products popped out and seemed to say "look at me, I'm special." The following discussion will take a look at these products and try to understand what makes them different and special.

WHAT DEFINES SPECIAL?

A good idea that doesn't make it to the market isn't special. A product that makes it to the market and enjoys mediocre therapeutic and commercial success isn't special. Commercial success is a necessary, but insufficient, criterion to be considered as special. There needs to be something else about a product that makes it pop out from among its peers.

To be considered commercially successful for this article, a DDEP (Drug Delivery Enabled/Enhanced Product[1]) needed to make it on the DDEP2011 Top 108 sales list. This list ranks DDEPs according to their US sales throughout the past decade, 2000 to 2009, primarily as presented in the SDI/Verispan Branded Drugs by Retail Dollars sales report.[2] The DDEP2011 Top 108 also includes some additional DDEPs that aren't captured in this sales audit. Why a Top 108? Well that's the number of DDEPs that made the annual SDI/Verispan Top 200 list at some point between 2000 and 2009. To make the SDI/Verispan Top 200 list, a product, whether or not a DDEP, had to have recorded more than $125 million in sales for a least 1 year in that period.

The DDEPs included in the DDEP2011 Top 108 sales list accounted for about 80% of all sales of DDEPs in that period, about $250 billion in total sales. By extension, we can estimate that total sales for all DDEPs were more than $300 billion, or about $30 billion per year, in the US in the years 2000 to 2009.

WHAT POPS?

The Top 108 DDEPs represent about a third of all DDEPs approved by the FDA in the period of 1990 through 2009. A DDEP that pops is not only commercially successful, therapeutically meaningful, and special, but in some fashion defines a new way of doing business. A once-a-day formulation of a successful antidepressant doesn't pop. Not because it isn't therapeutically valuable or commercially successful, but because it was anticipated and, dare we say, obvious. DDEPs that pop are different. How these DDEPs are different can point to new opportunities and new ways of understanding the potential of DDEPs. From the DDEP2011 Top 108 list, we identified a dozen products that popped (Table 1). We will look at six of these products a little closer to understand why they popped and what lessons they can teach us.

SUBOXONE – RECKITT BENCKISER

Suboxone has, perhaps surprisingly, captured more than $4 billion in sales worldwide since its launch. Suboxone, approved by the FDA in 2002 as a treatment for opioid addiction, combines buprenorphine, a partial agonist narcotic analgesic, and naloxone, a

narcotic antagonist, in a sublingual formulation. Two things make this product pop. Opioid addiction treatment is a niche indication that is not understood or pursued by many companies. This makes it an attractive indication because of limited competition and pricing flexibility. Reckitt Benckiser has leveraged this by pricing Suboxone at $5 to $15 per day, and capturing remarkable sales for such a niche market.

The second point that pops out is the sponsoring company. Reckitt Benckiser is better known for its OTC products and household supplies, such as Lysol and Calgon, than developing and selling pharmaceuticals. But the company has decades of experience with buprenorphine as an analgesic that they elegantly leveraged to create Suboxone. Good ideas and products depend more on seeing what is needed and what is possible than resources and critical mass. Sublingual delivery may not be cutting edge, but it can deliver.

TABLE 1

Product	Sponsoring Company	US Sales
Actiq	Anesta (Cephalon)	~$2 billion
Advair Diskus	GlaxoSmithKline	>$22 billion
Concerta	Alza (Johnson & Johnson)	>$7 billion
Maxalt MLT	Merck	>$1 billion
Niaspan	Kos (Abbott)	>$3 billion
OxyContin	Purdue Pharma	>$16 billion
Pegasys	Roche	>$3 billion
PegIntron	Schering-Plough (Merck)	>$2 billion
Pulmicort Respules	AstraZeneca	>$4 billion
Suboxone	Reckitt-Benckiser	>$2 billion
Tricor	Abbott	>$6 billion
Vyvanse	New River (Shire)	~$1 billion

Products That Pop (Estimated US Sales at AWP, 2000-2009)

OXYCONTIN – PURDUE PHARMA

Okay, what OxyContin are we talking about? That's a good question because OxyContin has become two different products sharing the same name. In a very subtle move, Purdue has migrated OxyContin commercialization from the original sustained-release formulation first approved in 1995 to a tamper-resistant sustained-release formulation approved in 2010. In the process, Purdue has changed the rules of the multibillion dollar business of extended-release oxycodone and created a very big, but remarkably quiet, pop.

Sustained-release oxycodone is big business in the US. Since being introduced in 1995, OxyContin has enjoyed relative exclusivity in the market by virtue of an elegant intellectual property strategy and Purdue's aggressive defense of these patents. Although there was a period in the mid-2000s when generics were introduced that cost Purdue billions in lost sales, this threat was nullified by Purdue's successful validation of its issued patents. If that was all Purdue did with OxyContin, it would have made the list. How many major pharmaceutical products have come back following the introduction of generics? How many DDEPs have been able to maintain market exclusivity with respect to both generics and functional equivalents for 15 years using an active that was introduced more than 50 years ago?

What really pops is Purdue's decision to introduce a tamper-resistant formulation and retire its original multibillion dollar formulation. The FDA Orange Book now lists only the non-tamper-resistant sustained-release formulation; the original formulation is listed as discontinued. So is this new OxyContin the comparator for future generics? What defines tamper resistant? What are the FDA rules on this point? Are there any rules? Will a non-tamper-resistant extended-release oxycodone be considered interchangeable with OxyContin even if it has the same bioavailability? The answers are: possibly; who knows; who knows; who knows; probably not; and very unlikely.

By moving to a tamper-resistant, if not abuse-deterrent, formulation, Purdue has changed the game in the multibillion dollar market for sustained-release oxycodone. This doesn't mean that products like King's Remoxy won't be approved and be competitive. But Purdue's biggest fear must be the introduction of generics, not me-too products that are not substitutable. Pop the champagne corks on this one; Purdue will be making billions and improving patient therapy for years to come by tenaciously beating back generic companies in the courtroom and at the same time developing a product that made its own billion-dollar product obsolete.

PEGASYS – ROCHE, PEGINTRON – SCHERING-PLOUGH

These two products are often pointed to as models of a successful DDEP lifecycle strategy. Both of these products are built on the success of the original non-conjugated proteins for the treatment of hepatitis C (HCV). While the native proteins in combination with ribavirin defined the sate-of-the-art of therapy for HCV, the dosing regimen, three injections per week for 26 to 52 weeks, was demanding, and long-term remission rates were in the 20% range. Reconceived and reformulated as PEG-conjugates, both of these products immediately improved dosing to once per week.

But what made these products pop is their almost doubling of long-term remission rates. Hindsight is 20/20, but it is likely that even Roche and Schering-Plough were counting on improved compliance to drive sales, and were surprised by the significant improvement in efficacy. This improvement in efficacy results from a much longer residence time for the PEGylated protein that provides less opportunity for the virus to rebound. And by making dosing less onerous, with only one injection per week, compliance was without a doubt improved, fewer doses were missed, and there was again less chance for virus rebound. This is a textbook example of how improved dosing convenience translates into better compliance and better efficacy, without the need for a new and improved active.

There were two commercial pops with these DDEPs. The first was seen in improved treatment levels that translated into greater

sales. It was easy for patients, physicians, and payers to rationalize treating HCV with PegIntron and Pegasys; less doses and better outcomes. Exact cumulative sales for both products are hard to determine because they are packaged with ribavirin, but each has cumulative sales approaching $10 billion worldwide since launch.

The second pop came with a reset of the exclusivity clock for both company's interferon franchises. While the native molecules were once on the verge of patent expiration worldwide, these new PEGylated proteins, with new composition of matter patents, started a new clock. Who was going to use the unPEGylated proteins in the clinical setting given the more demanding dosing regimen and the poorer outcomes? How much cheaper would an unPEGylated interferon alpha need to be to make it a bargain? The FDA still doesn't have a pathway for the approval of generic biotechnology products, such as interferon alpha. But even when it arrives, these billion-dollar products will still have a long and profitable future ahead of them.

MAXALT MLT – MERCK

This is perhaps the most surprising selection of the group. Maxalt MLT, an orally disintegrating tablet (ODT) formulation of the migraine treatment rizatriptan, was approved and launched in 1998 in tandem with the immediate-release tablet. What pops is the consumer-oriented decision to give patients and physicians a choice in dosing: with or without water. It's hard to think of another product that introduced immediate-release tablet and ODT formulations at the same time, rather than one following the other by a couple of years. Yes, there was additional cost to Merck to license the Zydis technology used in Maxalt MLT, but these costs have been more than paid for by the increased sales of the Maxalt brand.

As the third triptan to the market, and first offering an ODT formulation, even without a nasal or injectable form, the Maxalt brand has racked up total sales of more than $3 billion in the US and $5 billion worldwide. Of this total, Maxalt MLT has contributed to more than half of the sales, a nice pop for a late-to-market product that distinguished itself by improving convenience and the treatment experience.

NIASPAN - KOS

An Oral SR formulation of niacin, a B Vitamin, Niaspan provided a big pop to the sales and prospects of Kos. By formulating niacin into a once-daily oral dosage form, Kos leveraged the well-known ability of niacin to lower LDL and triglycerides and provide clinicians with an enhanced therapeutic option synergistic with HMGCoA reductase inhibitors, such as Lipitor. Approved in 1997, Niaspan has racked up sales of more than $4 billion through the end of 2010 despite, or perhaps as a result of, a settlement and license agreement with Barr. The commercial success of Niaspan led Abbott to acquire the company late in 2006 for $3.7 billion. A validated molecule, a relatively generic drug delivery technology, and a large medical need all provided the means to develop a high-value DDEP. US sales of Niaspan in 2010 popped up again to a little bit less than $1 billion. These are remarkable results for a reformulated vitamin that once again reinforce the value of vision and execution.

REFLECTIONS

All 12 of these DDEPs (Table 1), and many others, are excellent examples of what is possible when creativity aligns with market needs. With the exception of Maxalt MLT, all of these DDEPs were based on previously approved actives; often generic actives. If there is one common feature of these products that has contributed to their commercial success, it has been their ability to retain a reasonably long extended period of market exclusivity. While the FDA provides only 3 years of exclusivity for a new formulation of a previously approved active, these products have in many cases been able to extend their exclusivity to 10 years and beyond.

The epitaph for Jack King, a gambler from Alfred Henry Lewis' 1897 book Wolfville, reads "life ain't in holding a good hand, but in playing a pore hand well." That sentiment relates well to these DDEPs. While these companies certainly weren't dealt a poor hand, they were able to create something of real durable value from molecules and technologies that others ignored. ◆

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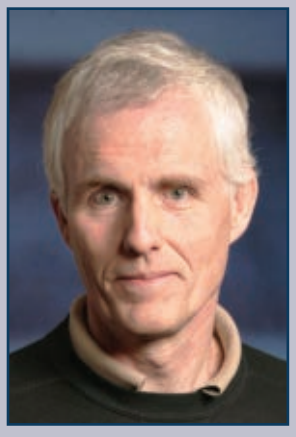
BIOGRAPHY



Dr. Josef Bossart is Managing Director of The Pharmanumbers Group, a boutique research and consulting group providing the biopharmaceutical industry with analysis and insights that improves business outcomes. In addition to issuing industry reports, such as DDEP2011 - Drug Delivery Product Success Rates, Development Times, Costs and Marketing Exclusivity under its Bionumbers label, Pharmanumbers provides strategy consulting and forecasting support for emerging and commercial-stage drug delivery companies. Dr. Bossart has more than 3 decades of experience in the biopharmaceutical sector, including senior sales, marketing, business development, and management positions with Enzon Pharmaceuticals, GeneMedicine, US Ethicals, and Rhône-Poulenc Rorer. Dr. Bossart earned his PhD in Medicinal Chemistry from The Ohio State University, College of Pharmacy.

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PHARMACEUTICAL DEVELOPMENT

Defining & Addressing Solid-State Risks After the Proof-of-Concept Stage of Pharmaceutical Development

By: Joanna Bis, PhD, David Igo, PhD

INTRODUCTION

Selection of an appropriate solid-state form (non-solvated or hydrated/solvated parent, salt, or cocrystal) is one of the most important decisions in the development of a small-molecule API due to the inherent link between the physico-chemical properties of the solid-state form and factors important to the success of its commercialization.[1,2] Although the significance of this decision is generally well appreciated across the pharmaceutical industry, strategies can vary widely between different drug development programs as companies attempt to balance the tension between quality, speed, cost, and risks.[3] In the earlier phases of development, attrition rates can be as high as 90% due to the unknown safety profile of the drug candidate, human exposure, and clinical efficacy.[4] This failure rate has redirected development activities away from rigid workflows and processes that focused on optimizing the solid form based on the intended commercial profile and toward defining intermediate preclinical and clinical objectives and activities necessary to enable these objectives to be achieved.[5] Examples of these fit-for-purpose objectives targeted at early stages of development may include identifying a suitable solid form of the API that 1) will exhibit adequate bioavailability and/or stability, 2) can be formulated to support evaluation of the toxicity of the API in preclinical species, 3) enables isolation and purification of the API, and 4) is compatible with the simple dosage form required for the first human trials (eg, powder-in-bottle or powder-filled capsule). Because the scope of early phase studies is becoming more focused and constrained, APIs are more likely to enter the later phases of development with gaps in knowledge and understanding. This means that when a molecule demonstrates its clinical efficacy, or proof-of-concept (PoC), focus will need to quickly shift toward defining and understanding the solid-form space, surfacing and mitigating risks associated with these forms, and ensuring that manufacturing operations are sufficiently robust in delivering the desired materials. For more advanced drug programs, a comprehensive knowledge of solid forms and their attributes can also afford a practical means of prolonging the life cycle of an asset after its commercialization and maximize the return on the discovery and development investments.

CRYSTAL-FORM SPACE

Because the crystalline form can impact attributes important to the performance of a medicine (eg, shelf-life and bioavailability/efficacy), an understanding of the solid-form space is essential in defining the quality attributes associated with the manufacturing and formulation processes.[6,7] Undiscovered stable crystal forms pose a direct risk to commercial supplies, particularly for compounds exhibiting solubility-limited bioavailability, because both properties typically decrease with higher stability.[8,9] Therefore, shortly after achieving PoC, extensive crystal-form screening studies are employed in order to maximize the opportunity for discovering important solid-state forms. These comprehensive studies typically involve 1000 to 2000 experiments and utilize an array of solvents that allows the effects of solvent polarity, hydrogen-bonding, and geometric attributes on crystal-form space to be thoroughly exploited. This typically involves a large number of solvent classes (eg, alkanes, alcohols, ketones, aldehydes). Diversity is also obtained through the use of binary and ternary organic mixtures, which can extend the range of solubility achievable using neat solvents while providing a chemically diverse solvent system. Incorporating water is often of critical importance because hydration by vapor or liquid water can have serious consequences on formulation methodology and ultimately on the stability of the active ingredient and formulated product. Such risks are assessed via the use of water and aqueous solvent mixtures in the solvent set to facilitate the discovery of hydrates and, in the case of salts, probe

their disproportionation.

The diverse solvent arrays are used in a variety of crystallization modes (eg, slurry ripening and evaporative, cooling, and solvent/antisolvent crystallizations) to explore the impact of temperature, degree of supersaturation, and solvent composition on the ability to nucleate and grow important crystal forms. Solvent-less screening studies are also employed and may include exposure of the solid API to a variety of conditions: elevated humidity, solvent vapors, elevated pressures, milling, melting/cooling, etc. The products of the comprehensive screen are subjected to analyses to establish the nature of the unique forms and assess their thermodynamic stability and relationships to each other. These analyses may include thermal analysis, solid-state stability studies, solubility determinations, and competitive-ripening studies conducted at various temperatures.

CRYSTALLIZATION PROCESS SUPPORT

When an API exhibits polymorphism or can solvate with a process solvent and/or water, more detailed experiments are required to understand the thermodynamic and kinetic behavior of the system. In these cases, a diagram of the thermodynamic stability of the different phases with respect to temperature and solvent composition needs to be generated to assist in understanding the risks posed to the crystallization process. Once the phase-diagram is known, the kinetics of the form inter-conversions (eg, relative rates of crystallization, nucleation, and crystal-growth) can be evaluated to ensure the avoidance of any undesired form in the isolated product.

As the scale of the manufacturing process increases, mixing and heating become significantly more non-homogeneous compared to small-scale laboratory studies. The resulting concentration and thermal gradients can promote the appearance of new crystalline forms and highlight the importance of monitoring the product throughout crystallization and/or salt-formation process.[10] Monitoring can be achieved by in situ monitoring (eg, by spectroscopy or reflectance) or by collecting grab-samples and analyzing them off-line (eg, by PXRD or vibrational spectroscopy). Solid-state forms can be stable when in contact with the crystallization mother liquor, yet physically unstable when isolated, resulting in conversion to the desired, or sometimes, undesired crystal form. Therefore, the grab-samples should be evaluated as a wet and freshly isolated cake and throughout the drying process.

API FORMULATION & DRUG DELIVERY SUPPORT

Secondary processing operations can lead to the production of new crystal forms or result in a loss of crystallinity and corresponding increase in amorphous content.[11,12] Hence, the solid-state characteristics of the API should be monitored prior to and following each unit process wherever possible. Laboratory-based experiments (eg, compression and milling) can often be used to predict the likelihood of processing-induced changes. However, while crystalline transformations are possible with these unit processes, some processes may also induce disorder, in which case it is important to establish the fate of the disordered phase, the kinetics of change, and the influence that it has on drug product performance and stability.

If an API can exist as a hydrate, aqueous-based manufacturing processes of anhydrated form, such as wet granulation followed by tabletting are at risk. Properly designed stability studies can be employed to determine the critical water activity below which the anhydrate is thermodynamically stable. Such studies may enable the use of aqueous organic solvent system with appropriately low water content to destabilize the hydrate and to granulate the anhydrate form. Similar studies should be considered for programs involving, for instance, aqueous suspension formulations.

For salts, in particular those containing weak counterions, disproportionation risk should be assessed in formulation medium and under mechanical processing conditions. Specifically, micronizing or compacting can induce formation of amorphous phase, which under appropriate humidity conditions, may absorb moisture and disproportionate.

SOLID-STATE FORM STUDIES TO SUPPORT LIFE CYCLE MANAGEMENT

The commercial opportunities of a pharmaceutical asset can be expanded via a new indication, combination therapy, route of administration, or a formulation. These new targets, or product line extensions (PLEs), are identified as clinical data from Phases IIa onward become available and highlight specific physico-chemical properties of the molecule required to achieve a more desirable or new clinical response. PLE can be enabled through the introduction of a new solid form of the same API that exhibits the desired pharmaceutical performance. Alternative salts are frequently considered for PLE, and recent research has indicated that pharmaceutical cocrystals may exhibit desirable attributes.[13,14]

Discovery of new solid-state forms may enable an extension of the original patent term if the novelty, inventiveness, and utility can be demonstrated.[15] For example, undesired side-effects in the gastrointestinal track posed by an oral formulation of a marketed API salt can prompt the search to identify, patent, and develop a topical formulation of another salt.[16]

Extensive and thorough solid form screens are conducted in order to identify and evaluate the potential solid form candidates for a variety of PLE strategies. These screens may involve hundreds to thousands of experiments and utilize a broad range of counterions (CIs) and cocrystal formers (CCFs) to maximize the probability of producing new solid form with a desired property. The discovery of the novel multicomponent solid-state forms raises the need to consider compatibility of the CI and/or CCF attributes with those exhibited by the API and those that are desired in the final product. In addition to the toxicity and physical property profiles of the coformers (eg, hydrophilicity), which will likely affect the property of the product (eg, solubility), a

comprehensive-screen design should also address crystal engineering aspects.[17,18] These aspects affect the likelihood of producing a stable crystalline material and may include: propensity of components to interact via strong ionic (eg, pKa considerations) and/or weaker neutral supramolecular synthons, proton donor/acceptor balance, geometrical fit (size and shape), conformational flexibility, stereochemistry, etc. The successful application of crystal engineering approaches to achieving enhanced dissolution characteristics has been demonstrated for new salts and cocrystals of several marketed drugs, indicating its usefulness in supporting life-cycle management strategies.[19-21]

CONCLUDING REMARKS

Solid forms can impact the API and drug product attributes that are important to shelf-life, robustness of the manufacturing operations, and reliability and efficacy of the medicine. Once a compound achieves a successful PoC, efforts intensify in selecting the proper solid-state form for development, optimizing manufacturing operations, and identifying and mitigating risks that may be posed by other solid-state forms. The knowledge gained from these activities increase confidence in the ability to deliver the commercial product reliably and can play a key role in successful life cycle management. In addition, discovering commercially viable crystalline forms and protecting these forms with patents has become a common practice in both innovator and generic pharmaceutical sectors to develop and commercialize FDA-approved APIs.

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BIOGRAPHIES

Dr. David Igo earned his PhD from the University of Cincinnati, where he studied the structural behavior of inorganic/polymeric electrochemical sensors using extended X-ray absorption fine-structure (EXAF) spectro-electrochemistry under the direction of William R. Heineman and Richard C. Elder. Dr. Igo began his industrial career with Glaxo Inc. in 1991 (now GlaxoSmithKline; GSK), where he supported various aspects of drug development, including preformulation, product development, chemical development, materials characterization, and technology development. In his nearly 2-decade tenure at GSK, he co-invented a variety of high-throughput technologies utilized in solid-state screening along with a range of unique crystalline salts and solid-state forms of GSK compounds. He is currently Director of OptiformTM Technologies at Catalent Pharma Solutions. Optiform Technologies combines novel automation tools and solid-state workflows to support the discovery and evaluation of crystalline forms, salts, and cocrystals.

Dr. Joanna Bis earned her MSc in Analytical Chemistry from Jagiellonian University, Kraków, Poland, in 2002. In 2006, she earned her PhD from the University of South Florida, where under the supervision of Dr. Michael Zaworotko, she studied crystal engineering of organic compounds. While in the PhD program, she worked as a Research Assistant for TransForm Pharmaceuticals Inc. and applied the fundamental crystal engineering strategies to the design and preparation of novel pharmaceutical cocrystals and salts. In 2006, she assumed a scientific position at GlaxoSmithKline in the Solid Form Sciences department, where she was responsible for supporting solid-state form screening and evaluation activities for late-stage drug development projects and addressing solid-state issues encountered during the development of crystallization and formulation processes. Currently, Dr. Bis continues to support solid-state aspects of early, middle-, and late-stage pharmaceutical development as a Principal Scientist of OptiformTM Technologies at Catalent Pharma Solutions.

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Analysis of New FDA Transdermal Draft Guidances: Insights on Study Design for Bioequivalence Assessment of Transdermal Systems

By: Paul A. Lehman, MSc

INTRODUCTION

Since 2007, the US FDA has issued 12 bioequivalence draft guidances for generic transdermal patch designs. While these guidances provide insight into current regulatory views on study designs for individual transdermal patch bioequivalence assessments, differences among the guidances make it challenging for product developers to glean essential principles for study design. This review summarizes the recommendations to identify commonalities and differences important to the design of successful studies.

OVERVIEW: FDA TRANSDERMAL GUIDANCES 2007-2010

Designing studies for bioequivalence assessment of transdermal patch systems had involved considerable speculation until the FDA issued a series of draft guidances, beginning with the transdermal patch for lidocaine. The FDA website on individual product bioequivalence recommendations maintains the most current draft guidances.[1] In this rapidly changing environment, it is recommended that product developers review the FDA website frequently for new and updated guidances. Table 1 provides the most current list of those guidances related to transdermal patch delivery systems.

COMMON ELEMENTS: FOUR TYPES OF STUDIES

Collectively, the draft transdermal bioequivalence guidances recommend four distinct types of studies. The first is the standard requirement for evaluation of pharmacokinetic (PK) bioequivalence. The other three are to assess product irritation, sensitization, and adhesion performance.

The draft guidances also address dissolution testing and analytical issues related to PK studies. However, these topics, as well as the new draft guidance on the evaluation of residual drugs in transdermals, are beyond the scope of this article, and product developers are advised to consult the FDA guidances for details on those specific topics.[2]

Pharmacokinetic Bioequivalence Study Requirements

Although there are drug-specific requirements for PK study designs, the common elements across the draft guidances for PK bioequivalence studies include:

- Design (single-dose, two-treatment, two-period cross-over)
- Number of subjects (36 to 48 subjects recommended)
- Dose duration as indicated for the Reference Listed Drug (RLD)
- Analysis (appropriate validated analytical method)
- Acceptance criteria (bioequivalence based on 90% confidence interval)

Dose duration is indicated by the RLD with pharmacokinetics being followed throughout that dose period. For example, Methylphenidate is indicated for a 9-hour dose

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duration, Oxybutynin is indicated to be worn for a 96-hour dose duration, etc. Dose strength is the dose provided in the full-size patch. In most cases, the highest strength is recommended. Lower strengths may be considered for a waiver under certain circumstances. The appropriate frequency and timing of sample collection is a function of the patch design and is unique to each product, but if not already known, clinical research organizations (CROs) can often provide recommendations on these parameters.

Irritation & Sensitization (I/S) Study Requirements

To evaluate skin irritation and sensitization, the common elements across the draft guidances for combined irritation and sensitization studies include:

- Induction-irritation phase (21 days of exposure)
- Challenge phase (a single, 48-hour dose duration)
- Scoring scale (Standardized Visual Assessment)
- Number of subjects (completion with 200 evaluable subjects)
- Study sites (multiple clinical sites with different climate conditions)
- Control treatments (optional vehicle patch and negative control)
- Acceptance criteria (non-inferiority of the test product compared to the RLD)

The initial induction-irritation phase consists of 21 days of exposure to both the test patch and the RLD. The frequency of application, application duration, and dosage strength vary depending on the patch design. Before each next sequential patch application, irritation is measured using two standardized scoring scales: one to score Dermal Response and one to score Other Effects. Following the induction-irritation phase, there is a 14-day rest phase, during which no patch is applied. After the rest period, a challenge phase is conducted to determine whether the subject has developed a sensitization response to the products. This consists of a single 48-hour dose duration with monitoring for skin reactions during the 72 hours following removal.

Recommendations call for sensitization studies to be completed with 200 evaluable subjects, which may require 240 to 300 subjects for initial enrollment. Subject compliance and retention is affected by the propensity of drug-related adverse events that may occur, study duration (6 weeks), frequency of return visits, time of year, competency of the CRO, and other factors.

The guidances indicate that irritation and adhesive properties of transdermal patch systems may be sensitive to climate, and recommend that studies be conducted at a minimum of two sites with different climate conditions. However, it is not specified what the different climate conditions should be that are relevant to a North American population. When placing these studies, it is advisable to utilize a CRO that has clinic sites at different locations within North America with average temperature differences of 10°F to 15°F, and with average relative humidity differences of 10% to 15% between sites.

Scoring systems for assessing dermal irritation have evolved over the years. The FDA has provided a standardized scoring system for dermal irritation assessment for consistency across all of the guidances. The system uses two scales: the Dermal Response scale for erythema and edema, which assigns numeric values from zero (no evidence of irritation) to 7 (strong reaction spreading beyond the test application site), and the Other Effects scale, which measures physical changes to the skin (e.g., glazing, peeling, cracking, fissures). The Other Effects scale assigns letter grades with numeric equivalents, from A (0) (slightly glazed appearance) to H (3) (small petechial erosions and/or scabs). The Dermal Response score and Other Effects score are combined to determine the final I/S scores. One can refer to any of the draft transdermal guidances for the specific score definitions.

The key to a successful I/S study is ensuring the CRO has an intensive irritation-score training program with validation of scoring competency. Ideally, the same grader (scorer) should be used throughout the study. Because this is not always possible, particularly when two or more clinic sites are being used, it is important that all graders are cross-trained to achieve the same degree of accuracy and reproducibility in their scoring assessments.

TABLE 1

Active Ingredient	Date Issued
Clonidine	11/2009
Estradiol (7-day patch)	11/2010
Estradiol (3.5-day patch)	11/2010
Ethinyl Estradiol: Norelgestromin	5/2009
Fentanyl	2/2010 (Revised)
Lidocaine	5/2007
Methylphenidate	7/2010
Nitroglycerin	12/2009
Oxybutynin	3/2009
Rivastigmine	11/2010 (Revised)
Scopolamine	12/2009
Selegiline	8/2009

Draft Guidances Available from the FDA as of January 2011

Adhesion Performance Study Requirements

The need for adhesion performance assessment arose in parallel with advances in patch adhesive matrix designs, in which patch detachment from the skin may be a concern. Adhesion performance is to be assessed on both the test and reference transdermal patches. The common elements for adhesion performance assessment in the draft guidances include:

- Dose type (single full-size, full-strength patch application)
- Dose duration (matched to that of the RLD)
- Scoring scale (Standardized Visual Assessment)
- Study sites (multiple clinical sites with different climate conditions)
- Acceptance criteria (non-inferiority of the test product compared to the RLD)

Adhesion performance assessment is conducted on a single dose application with a full-size patch for the dose duration dictated by the RLD. As with I/S studies, adhesion studies are recommended to be conducted in at least two sites with different climate conditions.

An issue for adhesion studies is the uncertainty regarding the number of subjects required to sufficiently power the study for demonstration of non-inferiority. In addition, the draft guidances do not offer clear recommendations on frequency of adhesion scoring during the patch application period. An experienced CRO can often recommend appropriate parameters for conducting adhesion studies, such as the number of subjects needed for a particular product, the frequency of assessment, or the most appropriate statistical evaluation of the data.

The FDA has provided a standardized visual scoring scale for adhesion assessment. The system ranges from a score of zero (≥ 90% attachment to the skin) to 4 (completely detached). One can refer to any of the draft transdermal guidances for the specific score definitions.

Assessment is based on the perceived percentage of detachment from the skin. This can be challenging, depending on the size of the patch and the type of detachment. Detachment along the edges is easy to see and score. However, with larger patches, detachment in the inner central areas may also occur, which is more difficult to assess visually. Again, a comprehensive, competency-based training program is essential for study personnel assessing adhesion, and should be expected of the CRO conducting the study.

Adhesion Assessment: Issues When Combined With PK Studies

Several of the draft guidances recommend combining the adhesion performance assessment with the PK study. However, the guidances do not offer clear recommendations for situations when poor adhesion is encountered. It is advisable that the study protocol addresses conditional outcomes, to anticipate whether a subject should be discontinued when significant detachment is observed, or how much detachment constitutes an inadequate dose exposure. Enrollment in combined PK/Adhesion studies should take into consideration the potential for poor adhesion, and ensure there are a sufficient number of subjects to evaluate non-inferiority for adhesion performance.

Adhesion Assessment: Issues When Combined With I/S Studies

When adhesion performance assessment is combined with the I/S study, adhesion is typically conducted on the first patch application only. Following that, tape re-enforcement of the patches may be used, if needed, to ensure full contact exposure for the remainder of the 21-day induction phase. In the protocol, it is important to differentiate the adhesion performance data (for non-inferiority assessment) from adhesion confirmation data (ensurance of continuous exposure during the remaining period of the study), which is not used for the non-inferiority comparison analysis.

Only in those I/S studies in which a full-size patch is indicated can adhesion performance be evaluated. When the I/S study is indicated with a reduced sized patch (e.g., 1/2 cut patch), adhesion performance cannot be evaluated.

GUIDANCES BY GROUP: INSIGHT INTO DESIGN APPROACHES

Patterns can be observed in the 12 existing draft guidances that yield insight into regulatory expectations for assessment of transdermal products that have not yet been addressed by an FDA guidance. As product developers venture into transdermal products where a guidance is not available, comparing the test product to the most similar drug among existing guidances may

help in developing study designs.

There are four distinct groups of draft guidances for transdermals that distinguish whether adhesion may be evaluated in parallel with a PK or I/S assessment:

1. Adhesion performance is conducted with PK
2. Adhesion performance is not conducted with PK
3. Adhesion performance is conducted with I/S
4. Adhesion performance is not conducted with I/S

Group 1: Adhesion Performance is Conducted With PK

The guidances recommend that PK evaluation be combined with adhesion performance for the following seven transdermal systems:

- Estradiol
- Ethinyl Estradiol: Norelgestromin
- Fentanyl
- Nitroglycerin
- Oxybutynin
- Rivastigmine
- Scopolamine

The common design is the single-dose cross-over study with a full-size patch. Tape reinforcement is not allowed.

Group 2: Adhesion Performance is Not Conducted With PK

The draft guidances recommend that adhesion performance is not conducted in the PK study for the following four transdermal systems:

- Methylphenidate
- Clonidine
- Lidocaine
- Selegiline

There are specific distinctions within the recommendations for this drug group. First, fasting for PK is indicated for methylphenidate and lidocaine, but not the others. Second, the use of an overlay is indicated exclusively for clonidine. In addition, one noteworthy observation is applicable to the lidocaine transdermal system. A specific body site for patch application is not indicated. Given the size of the lidocaine patch, the upper back is a logical choice.

Group 3: Adhesion Performance is Conducted With I/S

Adhesion performance assessment is indicated to be included with the I/S study for the following four transdermal systems:

- Methylphenidate
- Clonidine
- Nitroglycerin
- Selegiline

This group presumes a low risk for drug-related adverse events during the 21 days of exposure in which full-size patches are used. However, one should still anticipate that even at the lowest recommended dose, adverse events are likely to affect a small number of subjects. Adhesion performance is assessed on the first dose application only; tape reinforcement may be used thereafter if needed. Patch dose durations during the 21-day induction phase are a function of the indicated RLD patch application period.

Group 4: Adhesion Performance is Not Conducted With I/S

Adhesion performance cannot be combined with the I/S study for the following six transdermal systems:

- Ethinyl Estradiol: Norelgestromin
- Lidocaine
- Oxybutynin
- Rivastigmine
- Fentanyl
- Scopolamine

The reason adhesion performance cannot be combined with the I/S studies is due to the risk of drug-related adverse events from the double dose delivered if two full-strength patches were to be used. To minimize drug-related adverse events, half-size or quarter-size patches are indicated. As adhesion performance must be evaluated on full-size patches, adhesion performance cannot be included in these I/S studies. Tape reinforcement can be used, and may even be required to ensure continuous patch exposure to the skin during the induction phase.

Unique to fentanyl and scopolamine, the irritation and sensitivity tests actually evaluate only a vehicle patch - there is no drug involved. Irritation comparison is made to the positive control, which is nominally indicated as a 0.1% sodium laurel sulfate in

water solution, dosed under an occlusive chamber or dressing.

CONCLUDING OBSERVATIONS

Value of Pilot Studies

In the course of a transdermal product's development, particularly when different backing materials, adhesives or excipients, or manufacturing processes are used that differ from the RLD, there may be uncertainty in the PK, adhesion performance, and irritation properties for the patch. For patches in development, less-expensive pilot studies can provide early warning of unanticipated issues before initiation of pivotal studies.

Lifestyle Guidelines in the Protocol

It is important to include subject lifestyle guidelines in study protocols. In the course of a 21-day irritation study in which the subjects are outpatients, activities, such as tanning, sunburn, hot tubs, sweat-producing sports, and exercise, can have a major impact on patch adhesion, irritation, and compliance. Defining the lifestyle guidelines in the protocol and informed consent, and providing clear instructions to the subjects, improves compliance, retention in the study, and data quality.

Volume of Data

Regulatory agencies are requesting significantly greater amounts of data, particularly from I/S and adhesion studies. Product developers should be prepared to manage extensive data tables, listings, and information; and the CRO conducting these specialized studies should be experienced, with processes and templates already in place, proven, and validated, to ensure that all the required data is appropriately captured in a timely and efficient manner.

Protocol Submission

Because these guidances are still in draft form, and are considered as recommendations, if there is any uncertainty regarding interpretation of the guidance, or if there is any planned study design variance to the guidance, it is recommended that the protocol be submitted to the FDA for review and comment prior to initiating a study.

Keys to Success

One is encouraged to study the guidances carefully to ensure proper study design, partner with an experienced CRO, and monitor the FDA website for new or revised guidances.

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BIOGRAPHY

Paul Lehman is the Director of the Clinical and Preclinical Dermatology at Cetero Research. He has conducted internationally recognized research in the field of topical pharmacokinetics and topical bioequivalence for 30 years and has been a Principal Investigator or Sub-Investigator on more than 500 clinical and preclinical dermatology studies for numerous pharmaceutical and skin care companies. He earned his BA in Biology and Bachelor of Business Administration from Incarnate Word College in San Antonio, Texas. Mr. Lehman later earned his MS in Pharmaceutics at the University of Washington in Seattle. His prior appointment was Executive Vice President of Clinical and Preclinical Dermatology at DermTech International in San Diego. Mr. Lehman has also held faculty appointments at both the University of Arkansas for Medical Sciences and at the University of Washington. He has also worked for 2 years at the National Center for Toxicological Research (FDA) in Jefferson, Arkansas, and is currently an Adjunct Professor at North Dakota State University in Fargo, North Dakota. Mr. Lehman has been an integral partner with Dr. Thomas Franz in the conduct of *in vitro* and *in vivo* topical pharmacokinetics and the development and validation of dermatopharmacokinetic bioequivalence methods for topical formulations. His credentials include several manuscript and book chapter publications, as well as numerous poster and lecture presentations.

Ultrasound & Microbubbles for In Vitro Gene Delivery

By: J.M. Escoffre, PhD; A. Novell, MSc; A. Zeghimi, MSc; A. Bouakaz, PhD

INTRODUCTION

Gene therapy is a potent strategy for the treatment of a wide variety of inherited and acquired diseases for which the current treatments are inefficient or non-existent.[1] Therapeutic genes can be transferred using viral or non-viral vectors. In clinical trials, viral vectors are preferentially used due to their high gene delivery efficiency and their ability to induce high-level and long-lasting gene expression in a wide range of tissues.[2] Their effectiveness lies in the infectious properties of viruses controlled by viral proteins. However, these proteins can induce specific immune responses that would limit the ability to re-administer the viral vector and inhibit the efficiency of gene delivery.[3] Moreover, some of the viral vectors can induce insertional mutations during their integration into the host genome.[4] In addition, previous investigations reported recombination events that can lead to the formation of replication competent viruses.[5]

In contrast, plasmid DNA (pDNA) is straightforward covalent closed circles of naked double-stranded DNA. pDNA is simple to mass produce, and easier to store compared to viral vectors.[6] These molecules could stimulate the immune system due to the recognition of immuno-stimulatory sequences associated with unmethylated Cytosine-phosphate-Guanine (CpG) motifs present in bacterial-generated DNA. However, the use of mini-circle DNA, which does not harbor these sequences, does not induce an immune response and can be re-administered.[7] Low immunogenicity and lack of integration of pDNA make it a highly attractive molecule for gene therapy provided that an efficient, safe, and targeted delivery method can be achieved.[6]

The use of ultrasound waves with gas microbubbles as a safe tool to deliver pDNA to tissues and organs has been rapidly developing throughout the past decade. The method increased transiently the native permeability of cell membranes when submitted to ultrasound waves in the presence of gas microbubbles. This process is commonly known as sonoporation or microbubble-assisted ultrasound permeabilization. This review focuses on the mechanisms of membrane permeabilization with ultrasound and microbubbles and its use for in vitro gene delivery.

BIOPHYSICAL MECHANISMS OF MEMBRANE PERMEABILIZATION WITH SONOPORATION

To understand the principle of microbubble-assisted ultrasound for gene delivery, it is important to describe the physics behind the interactions between microbubbles and ultrasound.

Microbubble-Ultrasound Interactions

Ultrasound is a longitudinal pressure wave with a frequency higher than 20 kHz. An acoustic pressure wave alternatively compresses (compression phase) and expands (rarefaction phase) the medium through which it travels. Therefore, when ultrasound waves pass through a medium, the molecules that comprise this medium can be physically and locally moved. These molecules are compressed at high pressures and expanded at low pressures.[8]

Microbubble response to an ultrasound excitation depends on the applied acoustic pressure. For small acoustic pressures, the microbubble oscillates and hence its radius shrinks and expands following the respective effects of the high- and low-pressure phases of the ultrasound wave. For higher acoustic pressures, the microbubble oscillates strongly, giving rise to nonlinear components at multiples of the transmitted frequency, the so-called harmonic components. This acoustic regime is the basis of modern ultrasound contrast imaging methods. Such strong oscillations induce intense liquid flows around the microbubbles, termed acoustic microstreaming.[9] At much higher

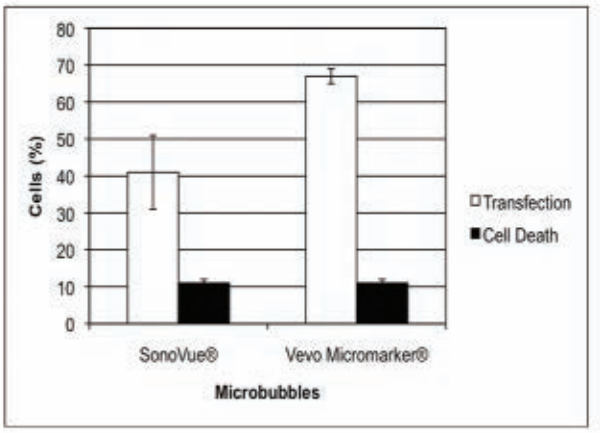
ultrasound amplitudes, the microbubble grows rapidly during the rarefaction phase, and then collapses during the compression phase. These strong oscillations result in the disruption of the microbubble, which is accompanied by high-amplitude nonlinear components. During the collapse of microbubbles, various acoustic phenomena might be generated, including shock waves and acoustic microjets.[10] In the case of asymmetrical collapse, jet formation can occur when a collapsing microbubble is located nearby to a surface, such as a cell membrane.[11]

Membrane Permeabilization

In spite of the increasing applications of microbubble-assisted ultrasound for drug and gene delivery, the mechanism(s) involved in the membrane permeabilization remain to be elucidated. Although no consensus is available, three different scenarios are usually hypothesized: (1) membrane poration, (2) endocytosis, and (3) the formation of large cell membrane wounds.

The generation of transient pores has been ascribed to the increased uptake or release of marker compounds.[12,13] Indeed, Mehier-Humbert et al demonstrated that dextran molecules with a diameter between 11.6 nm and 37.0 nm were able to enter into the cell via pores, and no differences were found between molecule sizes.[12] These data are in contrast with the recent study of Meijering et al, who showed that the contribution of transient pores is less important for the delivery of macromolecules with a molecular weight larger than 155 kDa.[13] The formation of transient pores has also been shown by measuring changes in ionic conductivity.[14] Indeed, using voltage clamp techniques, Deng et al demonstrated that the application of ultrasound in the presence of Optison® microbubbles on Xenopus oocyte increased the transmembrane current, as a direct result of decreased membrane resistance due to pore formation.[14] These different technical approaches suggested that the intracellular uptake is governed by passive diffusion through membrane pores with a size range from 30 nm to 100 nm. Moreover, indirect estimates of the membrane recovery time showed to range from a few seconds to a maximum of a few hours, with different kinetics depending on the molecular size.[12-16] Recent investigations suggested that membrane resealing is an energy- and Ca^{2+}-dependent process that involves the aggregation and fusion of lipid vesicles trafficked to the pore site.[17,18]

FIGURE 1



Transfection Rate & Cell Death of U-87 MG Cells at 48 hrs After Insonation Using Sonovue® & Vevo Micromarker® Microbubbles
Before insonation, microbubbles (ie, SonoVue and Vevo Micromarker) and pEGFP were added in the sample to a concentration of 5 microbubbles per cell and 5 micrograms/mL, respectively. Cells were insonated at 1 MHz, with 40% duty cycle during 30 secs at an applied acoustic pressure of 0.6 MPa. Transfection efficiency and cell death were determined by flow cytometer after propidium iodide staining.
West's ConfiDose® Auto-Injector System

In addition to triggering transient pore formation, recent electrophysiological studies showed that microbubble-assisted ultrasound induces a cascade of events characterized by the activation of BKCa channels, a subsequent local hyperpolarization of the cell membrane followed by an increase of the intracellular Ca^{2+} concentration.[19] The local hyperpolarization of the cell membrane facilitates uptake of macromolecules through endocytosis and macropinocytosis. In the recent investigation from Meijering et al, primary endothelial cells were subjected to microbubble-assisted ultrasound (1 MHz, 0.22 MPa, 6.2% DC 30 sec, SonoVue®) in the presence of fluorescent dextrans (4.4 kDa to 500 kDa).[13] Fluorescence microscopy showed homogeneous distribution of 4.4 kDa and 70 kDa dextrans through the cytosol and localization of 155 kDa and 500 kDa dextrans in distinct vesicles. The ATP depletion reduced the uptake of 4.4 kDa dextrans but no uptake of 500 kDa dextrans. Moreover, the independent inhibition of clathrin and caveolae-mediated endocytosis and macropinocytosis significantly decreased the intracellular delivery of 4.4 kDa and 500 kDa dextrans. Thus, the vesicles of 500 kDa dextrans colocalized with caveolin-1 and clathrin. This study showed that the contribution of endocytosis and micropinocytosis processes are dependent on macromolecule size.[13]

The last mechanism involves molecular uptake through membrane wounds.[20,21] Schlicher et al showed that microbubble-assisted ultrasound facilitated the intracellular incorporation of macromolecules up to 28 nm in radius

TABLE 1

Microbubbles	Mean Diameter (µm)	Shell Composition	Surface Charge	Gas	References
Albunex®	4.5	HSA	Negative	Air	27,37
BR14®	2.6	Phospholipid	Neutral	C_4F_{10}	26
Definity®	1.5	DPPC/DPPA/MPEG 5000-DPPE	Negative	C_3F_8	49
Levovist®	2-3	Galactose/ PA	Negative	Air	27,50
Optison®	3-5	HSA	Negative	C_3F_8	27,38,51
PESDA	4.7	HSA/dextrose	Negative	C_4F_{10}	33,51
Sonidel MB101®	2.7	Stabilized lipid	Neutral	C_4F_{10}	52
SonoVue®	2.5	DSPC/DPPG/PA	Negative	SF_6	52-54
Targeson®	2.5	Surface-modified lipid	Variable	C_4F_{10}	55

HSA, human serum albumin; PA, palmitic acid; DSPC, distearoyl-phosphatidylcholine ; DPPC, dipalmitoyl-phosphatidylcholine; DPPA, dipalmitoyl-phosphoric acid; MPEG 5000-DPPE, polyethyleneglycol 5000-dipalmitoyl-phosphatidylethanolamine; C_4F_{10}, perfluorocarbon; C_3F_8, octafluoropropane; SF_6, sulfur-hexafluoride.

A survey of the different microbubble types used for in vitro gene delivery.

through repairable micro-scale wounds in the plasma membrane with lifetimes > 1 min.[21] Like the pore resealing, previous studies showed that cells resealed these membrane wounds by an active process involving trafficking of intracellular vesicles (eg, lysosomes) to the site of membrane injury. Then, vesicle fusion resealed the membrane on a timescale of minutes.[20,21]

In conclusion, these data demonstrated that pore formation, endocytosis stimulation, and membrane wounds are likely to be key mechanisms of molecular delivery by microbubble-assisted ultrasound. The contribution of these mechanisms is dependent on molecular size.

IN VITRO GENE DELIVERY

Microbubble-assisted ultrasound as a safe and efficient method to deliver plasmid DNA to target cells has rapidly evolved throughout the past decade. Several cell lines, including cancer and primary, have been successfully transfected. This method is efficient even in endothelial and smooth muscle cells that are known to be resistant to conventional transfection methods. The transfection level and efficiency are comparable to or even higher than the results obtained by lipofection and electroporation.[22,23]

The efficiency of microbubble-assisted ultrasound lies in a narrow combination between microbubbles (type, acoustic properties, and concentration), acoustic parameters (excitation frequency, acoustic pressure, insonation time), and the physiological state of the cells.

The Microbubbles

The microbubbles are echo-contrast agents used in ultrasound imaging to improve the scattering of ultrasound waves and to enhance the contrast.[24] The microbubbles are gas-filled cores surrounded by a stabilizing shell. The shell prevents gas leakages and enhances the stability of the microbubbles and their circulation time in blood.[24,25] Examples of currently available microbubbles are shown in Table 1, and this list is indicative of the heterogeneity of these reagents in terms of shell composition, surface charge, and gas core.

In most cases, gene delivery with high-frequency ultrasound (1 MHz to 10 MHz) requires the use of microbubbles. Indeed, Sakakima et al showed that BR14® microbubble-assisted ultrasound induced a six-fold increase of transfection level of SK-Hep1 cells compared to ultrasound alone.[26] Moreover, the type of microbubbles has been demonstrated to be a major parameter for gene delivery. However, few investigations allow drawing up a comparative table of microbubbles in terms of their transfection efficiency and safety. Li et al led a comparative study of Albunex®, Levovist®, and Optison® microbubbles for gene delivery. The authors showed that the use of Optison® microbubbles induced a six-fold increase of transfection level compared to Albunex® and Levovist® microbubbles.[27] These results can be explained by an enhanced acoustic activity with Optison® microbubbles. The concentration of microbubbles is a second key parameter for optimal gene transfer. Indeed, a linear relation between the concentration of microbubbles and the percentage of transfected cells for Albunex® and Optison® microbubbles has been reported.[28,29] However, above a microbubble concentration threshold, the transfection level is stabilized and thus does not exceed its maximal rate. These results can be explained by a strong attenuation of ultrasound waves due to the high concentration of the microbubbles. At higher concentrations of the microbubbles, the transfection level remained constant, but the cell viability decreased.[28,29] Recently, we performed a comparative study using two types of microbubbles, ie, SonoVue® and Vevo Micromarker® microbubbles currently used in contrast imaging. The results showed that the transfection level achieved with Vevo

Micromarker® microbubbles is higher than SonoVue® microbubbles with a comparable cell viability. The transfection rate obtained with Vevo Micromarker® microbubbles reached approximately 70% (Figure 1).[30]

Although the studies were often successful in increasing pDNA delivery, it would be even more beneficial to bind the pDNA to the microbubbles.[31] The pDNA uptake can be achieved by mixing cationic microbubbles and pDNA, by pDNA incorporation in the microbubble shell, or pDNA enclosed within the microbubble shell.[32-35] There are different attractive advantages to load the pDNA to the microbubbles: (1) pDNA-carrying microbubbles can locally release their content and instantaneously enhance the pDNA uptake into the cells, thus reducing the putative side effects of pDNA. (2) pDNA release during the collapse of the microbubbles would result in a very high pDNA local concentration near to the target cells. (3) Closer contact between microbubbles and pDNA would enhance the likelihood of pDNA uptake through microstreaming and be easily pushed through the permeabilized membrane.[31] Frenkel et al, reported that upon attaching pDNA to albumin microbubbles during bubble synthesis, an enhanced gene expression was achieved.[34] Using 293-cell line, the pDNA-loaded microbubbles demonstrated a five-fold increase in luciferase reporter expression over that of unloaded microbubbles. In the same way, the transfection efficiency was better for pDNA-loaded microbubbles than unloaded microbubbles (41% ± 3% vs 9% ± 3%).[34]

Current developments in the field of microbubbles are a generation of targeted microbubbles to increase specificity of gene delivery.[36,37] The basic strategy to target microbubbles is to couple covalent or non-covalent targeting ligands to the shell. Specific ligands, such as monoclonal antibodies, receptors, glycoproteins, carbohydrates, and peptides, have been used. Indeed, Negishi et al have used the properties of AG73 peptide derived from the laminin alpha-1 chain is a ligand for syndecan-2 highly expressed in some cancer cells, to develop tumor-targeted gene delivery.[36] The authors have developed a new strategy including the combination of AG73-PEG liposomes, microbubbles, and ultrasound exposure to enhance transfection efficiency by promoting the escape of the liposomes from the endosome to the cytosol. This approach induced a sixty-fold increase of transfection efficiency compared to the combination using ultrasound and microbubbles alone.[36]

TABLE 2

Cell	pDNA	Microbubble	Microbubble Amount	Frequency	Acoustic Pressure	Insonation Time	Transfection Level	Transfection Efficiency	Cell Viability	Reference
CHO	Luciferase	Albunex®	10%	2.25 MHz	0.35 MPa	1 min	ND	0.15[b]	40%	39
	EGFP	Albunex® Levovist® Optison®	10% 10 mg/mL 2%	1 MHz	0.5-1 W/cm^2	20 secs	4% 5% 36%	ND ND ND	60% 61% 62%	27
BAEC	Luciferase	SonoVue®	1.25% v/v	1 MHz	0.1 W/cm^2	30 secs	ND	1300[d]	ND	53
HUVEC	EGFP	SonoVue®	2%	1.9 MHz	80 mW/cm^2	5 mins	20%	ND	90%	54
PC-3	Luciferase	Levovist® YM454 MRX-815H	0.2 mg/mL 3 µL/mL 1 µL/mL	1 MHz	0.2 MPa	1 min	ND ND ND	0.25[c] 50[c] 7.5[c]	15% 70% 65%	50
MATB-III	EGFP	Experimental Microbubbles	25-30[a]	2.25 MHz	0.57 MPa	10 secs	20%	ND	90%	22
VSMC	Luciferase	Optison® PESDA	25% v/v 25% v/v	1 MHz	0.41 MI[¶]	30 secs	ND ND	0.040[c] 0.072[c]	61% 59%	51
HepG-2	EGFP	Experimental Microbubbles		0.8 MHz	1 W/cm^2	2x30 secs with 5 min interval	45%	ND	84%	43
HEK-293	beta-Gal	loaded PESDA unloaded PESDA	1600[a]	1.3 MHz	1.6 MI	120 secs	40% 10%	ND ND	90% 85%	33
	EGFP	Targestar-P Cationic MB	4.10^7 MB/mL	1 MHz	2 W/cm^2	3 mins	70%	ND	70%	55
BHK-21	Luciferase EGFP	Optison®	10%	1 MHz	2 W/cm^2	30 mins	ND 44%	0.19[c] 75000[e]	80% 80%	40

CHO, Chinese hamster ovarian cells; BAEC, bovine aorte endothelial cells; HUVEC, human umbilical vein endothelial cells; PC3, human prostate carcinoma cells; MATB-III, rat mammary carcinoma cells, VSMC, vascular smooth muscle cells; HepG2, human hepatocellular liver carcinoma cell; HEK-293, human embryonic kidney cells; BHK-21, Baby Hamster Kidney; MB, Microbubbles; EGFP, Enhanced green fluorescent protein; β-Gal, beta-Galactosidase; [a]microbubbles/cell; [b]ng/10^6 cellules; [c]10^6 RLU/mg protein; [d]Luciferase activity (%); [e]Arbitray unit; [¶]Continuous wave.

In vitro gene delivery by microbubble-assisted ultrasound.

Acoustic Parameters

A range of acoustic parameters of the applied ultrasound waves has been investigated in order to increase the efficiency of in vitro gene delivery (Table 2). The ultrasound frequencies used for gene transfer range from 0.5 MHz to 4 MHz. The choice of the transmitted frequency is dependent on the size of the microbubbles and thus their resonance frequency. Indeed, the resonance frequency of the microbubbles decreases when their size increases.[39] In most reported studies, an optimal transfection level has been observed at 1 MHz frequency for all types of microbubbles.

The generated acoustic pressures during the insonation are variable and are usually expressed in different units depending on the authors (eg, Pa, W/cm^2, MI), making it impossible to provide a direct comparison between the different studies (Table 2). Nevertheless, numerous data showed that the transfection level increased with the acoustic pressure. However, above an acoustic pressure threshold, the transfection level declined with a concomitant decrease of cell viability. Indeed, Bao et al showed that the increase of the acoustic pressure from 0.28 MPa to 0.8 MPa induced a ten-fold increase of the transfection efficiency of CHO cells

and a 60% decrease of the cell viability.[40]

The total insonation time also plays a major role in gene delivery using ultrasound and microbubbles (Table 2). In the majority of published protocols, the insonation time ranges from 1 sec to 30 mins, and the extension of the insonation time induced an increased transfection.[41] Indeed, the increase of the insonation time from 10 mins to 30 mins (2 W/cm^2, 1 MHz, Optison 10% v/v) led to a three-fold increase of transfection efficiency of BHK cells and 10% decrease of cell viability. However, the authors report that 30 secs was the optimal insonation time for gene delivery into BHK using the same acoustic conditions. Hence, an insonation time of 40 mins caused respectively a three- and seven-fold decrease of transfection efficiency and decrease of cell viability compared to the 30 mins insonation time.[41] These results can be explained by the fact that the intracellular trafficking of pDNA would depend on the insonation time. As shown in the same study, the increase of insonation time from 10 mins to 30 mins induced a two-and-a-half- and ten-fold increase of BHK cells containing the pDNA into the cytoplasm and the nucleus, respectively.[41]

Physiological State of Cells

In addition to acoustic parameters, cellular factors may influence the degree of transfection efficiency and cell viability after insonation. Thus, the membrane fluidity has been proved to be a key factor for transfection efficiency. Using the optimal conditions (500 kHz, 20 J/cm^2, [11] Optison® microbubbles/cell), Zarnitsyn et al showed that changing the temperature from 21°C to 37°C induced a two-fold increase of the transfection efficiency without viability loss.[42] In addition, Nosaki et al demonstrated that the lidocaine (1 mM) and the temperature (42°C to 44°C) significantly increased luciferase expression approximately eighteen-fold and nineteen-fold higher than the microbubble-assisted ultrasound alone.[43] These investigations showed that the increase of membrane fluidity might facilitate the permeabilization of cell membrane. Moreover, the increase of temperature may provide the necessary conditions for the cell to reseal and survive its membrane permeabilization.

Thus, the cellular architecture is another key factor for gene delivery. To date, most cell lines that have been successfully transfected were adherent cells,[34,44] whereas only few attempts to transfect cells in suspension have been reported.[45-47] Thus, Kinoshita et al demonstrated using the same acoustic conditions (1.7 MHz, 1.6 W/cm^2, 15 secs, Optison® 2%), that the permeabilization level was similar in both cell suspension and adherent cells set-ups (around 20%). However, the viability of adherent cells was two-fold higher than that of cell suspension (80% vs 30%).[48] The development of more appropriate set-ups and the systematic optimization of the applied acoustic parameters would allow a high transfection level with an optimal cell viability for cell suspension.[45,49]

CONCLUSIONS

Microbubble-assisted ultrasound is a new and elegant delivery method with low toxicity, easy implementation, and adaptability to different cell types. The take-home message of all theses studies is that the efficiency of in vitro gene delivery depends on acoustic parameters, type of microbubbles, and physiological state of cells. Moreover, biophysical mechanism(s) of membrane permeabilization should be elucidated to improve molecule delivery. We sincerely believe that this method has a promising future for gene delivery within stem and primary cells, which are used in an exponential way in repair medicine and in basic research. Microbubble-assisted ultrasound is a promising strategy for gene delivery in order to treat (eg, gene therapy) and to prevent (eg, genetic vaccination) diseases in the human clinic. We believe that the clinical application of this approach will depend on the identification of optimal disease targets to develop targeted microbubbles, further refinements to minimize the dose of microbubbles and pDNA required while increasing efficiency, and further optimization of ultrasound parameters. The combination of this approach with ultrasound imaging would allow for targeted, efficient, and safe delivery.

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BIOGRAPHIES

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2011 DDP Awards Indicate Traditional Drug Delivery Model Isn't Dead

By: Marc Dresner, Contributor

So much has changed in the drug delivery sector since the Institute for International Research (IIR) inaugurated its 1st annual Drug Delivery Partnerships (DDP) event in the mid-90s. At that time, the "blockbuster" model was still in its heyday, although by then, patent cliffs and generic encroachment were among many factors keeping pharmaceutical executives awake at night. Faced with increasingly parched pipelines, many such executives turned to drug delivery technologists to extend lucrative patent lives, and drug delivery thus became a lifeline. Indeed, drug delivery innovation became something of a surrogate for genuine molecular innovation.

Fifteen years later, industry analysts, venture investors, regulatory watchers, and drug delivery specialists - many of whom attended IIR's 15th annual DDP event this past January in Miami - spent a great deal of time wrestling with the question of whether or not the "traditional" drug delivery model was dead. The most obvious example would seem to be companies' migration from pure drug delivery technologists to specialty pharmaceuticals manufacturers who are in fact developing their own medicines in tandem with the delivery technologies required to optimize them and who are building the highly focused, lean marketing infrastructure required to move their products.

In addition, many Big Pharmas - while not altogether abandoning the concept and/or option of partnering with delivery technologists - have bolstered their internal capabilities because they recognize that delivery innovation will be part and parcel of R&D in a world where biotechnology will play a critical role in health science progress. Clearly, the line demarcating the traditional pharmaceutical and drug delivery technology industries today has blurred, and it will likely continue to do so. But is the traditional drug delivery model dead? Absolutely not.

While much of the low-hanging fruit have been plucked, the need for specialist expertise in drug delivery has never been greater, especially as Big Pharma and biotechs look to develop solutions to myriad lower-incidence disease states and medical conditions that in the past were perhaps not considered worth pursuing from an investment standpoint. Many of these solutions, invariably, will require novel delivery platforms that account for outcomes, compliance, and healthcare economics beyond anything we've seen before.

Happily, drug delivery providers demonstrated they are up to the challenge at IIR's second annual Drug Delivery Product Showcase Awards on January 28. This year's competition featured 11 nominees, including even one Big Pharma (GlaxoSmithKline), across four categories: Industry Achievement, Technology/Company Innovation, On-The-Rise Company, and Pipeline Value Creation. The winners and

FIGURE 1

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What do you *really* know about end-users of drug delivery technologies?

Drug delivery technologies are an important part of the changing Pharma & Biotech industry. Feedback from patients and physicians, in terms of factors such as perception, desired attributes, compliance, and drivers of adoption/non-adoption for different drug delivery types, is therefore vital to developers. Is your company positioned to understand and take advantage of these opportunities for growth?

Frost & Sullivan's Pharmaceutical & Biotechnology group can provide your organization with the research and support it needs to fully understand end-users of Drug Delivery Technologies, and to identify and take advantage of the best opportunities for growth in this market.

Our expert Healthcare analysts:

- Provide objective, 3rd party analysis
- Identify a range of growth options
- Evaluate which options will produce the best Return on Investment
- Work with clients to develop effective implementation strategies

For more information on growth opportunities in the Drug Delivery market, please contact Britni Myers at britni.myers@frost.com or 210.477.8481.

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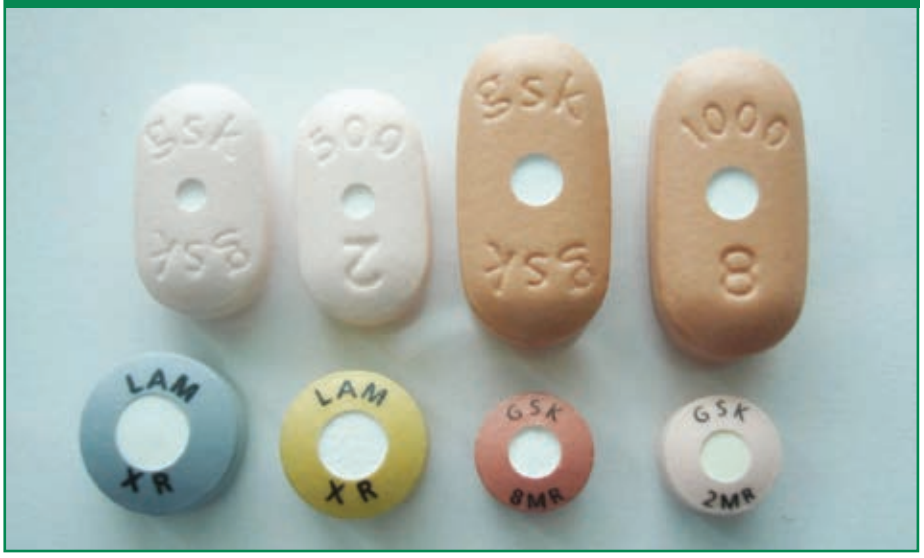
finalists per category are summarized further. It can be fairly said that each entrant took ingenuity and progressive science in drug delivery to an entirely new level.

INDUSTRY ACHIEVEMENT

WINNER: ZOGENIX - TAKING THE HEADACHE OUT OF INJECTABLES

Since 2006, Zogenix has pursued a needle-free injection technology that is commercially viable, patient-preferred, single-use, disposable, and prefilled. This pursuit culminated in the first FDA-approved needle-free subcutaneous drug delivery system for self-administration of prefilled, single doses of liquid drugs. The first Zogenix product (Figure 1), SUMAVEL®DosePro™ (sumatriptan injection), is a fast-acting therapy for the acute treatment of migraines. The patient simply snaps off the tip, flips the lever, and presses the device to the thigh or abdomen, which delivers the medicine under the skin without a needle. It was developed to address the major reasons for the under-utilization of current injectable therapies: fear of needles, lack of confidence to use a needle injector, and concerns with storage and safe disposal. The achievement embodied by the introduction of SUMAVEL DosePro breaks the barrier to self-administered subcutaneous injection for life-improving therapies. With over 1 million commercial units produced within the first year of production, the industry now has a proven, reliable, single-use, disposable, prefilled needle-free technology for use in other important therapeutic applications.

FIGURE 2



FINALIST: BIOCHEMICS - BECAUSE PATCHES ONLY GO SO DEEP

BioChemics has developed a novel, transdermal drug delivery system called VALE® (Vaso-active Lipid Encapsulated) as well as a suite of other transdermal and intradermal technologies. The lead technology, VALE, is a major breakthrough in transdermal science and, for the first time, may allow almost any drug to be efficiently delivered through the skin. VALE is a patchless cream- or gel-based technology enabling targeted or systemic delivery of drugs. All other transdermal technologies combined can only deliver about 12 to 15 drugs (eg, nicotine, testosterone, estrogen, fentanyl, etc, which have a rare molecular structure). Only VALE technology has demonstrated it can work with the majority of the pharmacopoeia. With the launch of VALE products, BioChemics hopes to provide a significant technological advance in the field of transdermal drug delivery.

FINALIST: MANKIND - BREATHING NEW LIFE INTO PULMONARY DELIVERY SOLUTIONS

Technosphere® technology represents a versatile drug delivery platform allowing for the pulmonary administration of therapeutics currently requiring administration by injection. Technosphere technology offers several competitive advantages over other pulmonary drug delivery systems. Most

FIGURE 3

notably, the pharmacokinetic profiles of drugs inhaled as Technosphere formulations are characterized by very rapid systemic absorption into the arterial circulation. In addition to rapid arterial delivery, drugs administered as Technosphere formulations avoid both hepatic first-pass metabolism and degradation in peripheral circulation. To facilitate the delivery of Technosphere-formulated drugs to the deep lung, Mannkind has developed a series of delivery systems:

- Mannkind's first-generation inhaler, MedTone®, is light and easy to use, and fits in the palm of the patient's hand. It utilizes single-use, disposable plastic cartridges containing drug-loaded powder. The inhaler is breath-powered, which means patients do not need to coordinate a breath with any manipulation of the device, such as priming or pumping.

- Based on feedback from clinical trial participants, Mannkind began Project Dreamboat™ - technology that provides a re-useable, miniature, breath-powered inhaler in combination with single-use cartridges containing pre-metered doses. The sleek inhaler design fits within the palm of the hand and is ready to use with a quick and intuitive cartridge load mechanism. These attributes result in an easy-to-use, elegant delivery system providing optimal discretion during use.

Author's note: *Mannkind's insulin inhaler for diabetics, Afreeza, was not considered in the competition after the FDA rejected its NDA. Mannkind continues to pursue approval for this potential breakthrough delivery system.*

FIGURE 4

TECHNOLOGY/COMPANY INNOVATION

WINNER: GLAXOSMITHKLINE - ADDING NEW LEVEL OF CONTROL TO RELEASE

GSK has pioneered a new generation of controlled-release tablets through its DiffCORE technology (Figure 2). It's hard to ensure patients take their medications as often as they're supposed to. One way the pharmaceutical industry is helping tackle the problem of compliance is by improving options for the delivery of medications. The GSK-developed DiffCORE technology involves creating holes of different size and number into coated tablets, allowing the active ingredient to be released in a much more controlled way. When the tablet is swallowed, gastrointestinal fluids enter the tablet hole in the coat and penetrate the core, releasing the drug. The rate of release also depends on the make-up and composition of the internal matrix. GSK is using DiffCORE technology in an increasing number of products for treatments, including epilepsy and metabolic disorders.

FINALIST: STEVANATO GROUP - FILLING GLASS CONTAINERS JUST GOT EZ-ER

Stevanato has developed a standardized packaging concept for ready-to-be-filled pharmaceutical use glass containers as an alternative to syringes: EZ-fill™ (Figure 3) for vials and cartridges. Today, only syringes are available on the market in a kind of arrangement that ensures particle content below limits and sterility. Thanks to the implementation of the innovative concept, OMPI will put on the market an extended range of clean, sterile, non-pyrogenic glass containers, ready to be filled, with the following peculiarities: 1) WFI - washed and rinsed glass vials or cartridges, using a validated washing and drying cycles; 2) glass containers arranged in innovative trays or nests

to prevent glass-to-glass friction; 3) packaging operations in Grade A/Class 100 environment; 4) packaging units subjected to a validated ETO sterilization process (3-log endotoxin reduction). Pharmaceutical companies can outsource the first part of the manufacturing process, thereby reducing their costs and concentrating their resources on their own aseptic fills. The EZ-fill for vials and cartridges can be easily integrated into existing pharmaceutical manufacturing capabilities, both automatically and semi-automatically. An extended range of available containers for direct filling operations will result in more flexibility in pharma R&D activities for new formulations. Very specific production, like clinical trials or orphan products, will be possible without investing huge capital for the washing and sterilization of these containers, resulting in a consistent time-to-market reduction.

Finalist: Pierre Fabre Medicament - Tastful, High-Performance, Green Coating Innovation

Pierre Fabre Medicament has extensive expertise in SuperCritical fluids and has developed completely new and very innovative tools. Three currently patented processes are high performance: the results are better than those obtained by conventional processes and, moreover, these processes are totally environmentally safe. The processes called FOMULPLEX (complexation with cyclodextrins), FORMULDISP (stable solid dispersion), and FORMULCOAT (taste-masking) performed with supercritical CO2 are mild and green processes that can be applied even to OTC drugs. The results are so efficient that they permit not only increased solubility and bioavailability of an API, but also the possibility to develop drug candidates that are currently too poorly soluble. Pierre Fabre's coating process is not a "one more" coating process; it provides high-performance taste-masking even for very small and sensitive particles, without organic solvent use, at room temperature with a high productivity (30 Kg/h) not ever proposed by preformulators.

ON-THE-RISE COMPANY

Winner: MedinCell Biopolymer - Reducing Life-Cycle Management Risk

MedinCell™ (Figure 4) offers game-changing technology for delivery of peptides, small molecules, and biologics. The company's biodegradable depot chemistry requires no API modification, and can target subcutaneous durations from 4 days through 6 months. Product feasibility studies can be completed within 6 months to 1 year, making this an economical, low-risk strategy for life cycle management.

Finalist: Oval Medical - Injecting Innovation into a Staid Market

Oval Medical is revolutionizing the autoinjector market. Since its February 2009 launch, Oval has achieved tremendous commercial traction - securing two pharmaceutical company deals with another 30 in the pipeline, closing two oversubscribed funding rounds, and establishing manufacturing partners - all within in its first full year of operation. Oval's technology differs from category competitors because its devices focus both on patient ease of use and the pharmaceutical industry's need to contain fragile drugs. Oval has taken a fresh approach by designing from the outside in. The device is able to handle extremely viscous drugs and volumes of 0.1 ml to 3.0 ml, which is reportedly not possible for other autoinjectors currently on the market, many of which have a history of unreliability and market recalls. Oval's design is robust; it's made of cyclic olefin plastic instead of glass (Figure 5). This has enabled its autoinjector to be produced at half the size of any other autoinjector on the market, making it the preferred choice of users - and much easier to use. Not only does it offer advantages to the patient and the drug, but it offers significant potential commercial advantage to pharmaceutical companies; it's highly cost effective compared with other autoinjectors, and in the long- term, Oval claims its autoinjector will increase a pharmaceutical client's market share.

Finalist: Neos Therapeutics - A Profile in Customized Controlled Release & Flexibility

Neos Therapeutics has developed proprietary drug delivery technologies that

FIGURE 5

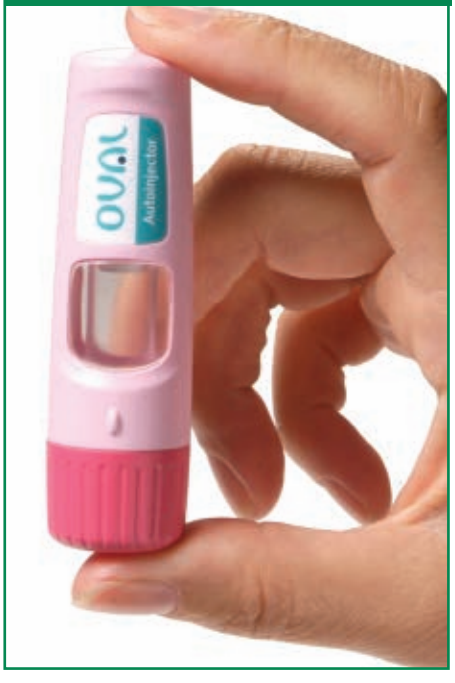


FIGURE 6

enable the creation of stable controlled release (CR) products; CR liquids and CR oral disintegrating tablets (ODTs), with suitable flavors and mouth feel. Neos' technology can provide a customized release profile and accommodate the need for a variety of release profiles, including the combination of IR and CR profiles for a single active ingredient or a combination of active compounds. The Neos technology platform leverages a drug/resin complex to create controlled release Rx or OTC products in convenient oral liquid and ODT dosage forms to better serve patients who experience difficulty swallowing oral solid forms, benefit from titration flexibility, or prefer the portability of ODTs.

PIPELINE VALUE CREATION

Winner: Elan Drug Technologies - Epitomizing Delivery Portfolio Perfection

In the past decade alone, Elan Drug Technologies' (EDT) solutions have been applied to more than $17 billion of client in-market product sales. Since their founding in 1969, Elan's technologies have been employed in over 35 products, which have been commercialized in more than 100 countries. Central to EDT's success over the past decade has been the creation of a broad and unique portfolio of drug delivery assets that include technology solutions for oral controlled release, delayed release, pulsatile release, and poorly water-soluble compounds (Figure 6), coupled with the know-how and expertise to bring such products successfully through development. Presently, Elan has 14 compounds in clinical development for clients. Examples of value-creating products developed by EDT include:

- The Cardizem® SR and CD products in the late 80s, which resulted in the successful building of one of the first blockbuster franchises in the US.
- TriCor® 145, launched with Abbott in 2005, which has consistently achieved over $1billion in sales in the past 5 years.
- NCEs such as Acorda's AMPYRA®, which was approved last year and has demonstrated strong performance in the initial stages of its launch with gross sales by the end of Q3 of approx. $85 million, and Emend® with Merck (consistent annual sales of more than $300 million).
- Other successes include the methylphenidate franchise - Ritalin® LA and Focalin XR® with Novartis, the long-acting injectable INVEGA® SUSTENNA® with Janssen, Rapamune® with Pfizer, once-daily morphine Avinza® with King, and liquid megesterol acetate, Megace® ES with Par Pharmaceuticals.

Since 2001, 12 products using EDT's technologies have been launched for their clients, making them the most successful drug delivery company in the past decade in terms of product launches.

Finalist: Halozyme - Subcutaneous Made Powerful & Painless

Enhanze™ technology (Figure 7), a proprietary drug delivery platform using Halozyme's first approved enzyme, rHuPH20, is a broad technology opportunity that can potentially lead to additional partnerships with other pharmaceutical companies. When formulated with other injectable drugs, Enhanze technology can facilitate the subcutaneous dispersion and absorption of these drugs. Generally, MAbs require a higher dosage (100 to 1000 mg/dose) than typical protein therapeutics; however, it is commonly accepted that subcutaneous (SC) injections over 1 mL cause skin distortion and pain. As a result, most biotech companies spend much effort concentrating MAbs to 100 mg/mL or more and then try to stabilize these formulations to avoid aggregates and particulates. Halozyme's Enhanze technology permits subcutaneous dosing much greater than 1 mL per injection, which enables bypassing the formulation challenges associated with achieving high concentrations. ◆

For more information or to submit a nomination for the 2012 Drug Delivery Product Showcase Awards, please visit www.iirusa.com/ddp or contact SSlobodskoy@IIRUSA.com.

INTRANASAL VACCINES

Do We Need New Devices for Intranasal Vaccination?

By: Degenhard Marx, PhD, Matthias Leitz, Christophe Fagot

INTRODUCTION

After years of getting very little attention, vaccines are back in the spotlight of big pharma. According to the WHO, the vaccine industry is experiencing a new, more dynamic period. The global vaccine market reached over $22.1 billion in revenue in 2009, making vaccines one of the fastest growing sectors of the pharmaceutical industry with a predicted 9.7% CAGR for the next 5 years. In the past, the highest share came from routine vaccination programs for children, proposed by WHO and adopted by local authorities and health insurance systems. An increasingly important market is preparation for pandemic outbreaks of highly infectious, fast-spreading diseases, such as H1N1 or swine flu, bird flu, and severe acute respiratory syndrome (SARS), which may have a severe global social and economic impact. When they can afford to, countries invest in new vaccine technologies or create stockpiles for vaccination campaigns to maintain essential services during pandemics. Following the 9/11 terrorist attacks and the mailing of anthrax-contaminated envelopes, the fear of biological warfare was revived, and substantial support for the development of vaccines for anthrax and smallpox was made available. Although less recognized, prevention of travel-related diseases, such as hepatitis A and diarrhea, is another attractive market. One result of globalization is the enormous increase in people traveling for pleasure or business to areas where there is high risk of infection. Preventive measures, such as vaccination and anti-malaria medication, are normally paid for by travelers out of their own pockets.

In the past, intramuscular and oral administration of prophylactic vaccines were considered to be the ultimate vaccination methods. Fine hypodermic needles and prefilled syringes make vaccine administration safe and less painful, but injections are still linked with pain and fear of an anaphylactic response. Moreover, because of the appearance of HIV, needlestick injuries, and disease transmission have become important threats, these obvious disadvantages of injections led to the search for more gentle alternative administration routes. Intranasal vaccination provides a promising non-invasive alternative. Used successfully in veterinary medicine for years, this route should transfer across to use in humans and may gain a reasonable share of the market in the near future.

WHY CHOOSE THE INTRANASAL DELIVERY ROUTE?

Using a Natural Immune-Competent Site

For most microbes, the nasal mucosa is the first barrier that must be conquered. It is then no surprise that this mucosa is extremely immune-competent. Intramuscular vaccination primarily induces systemic immune response, mainly via formation of vaccine-strain-specific circulating antibodies. Intranasal vaccination elicits broader protection. It induces mucosal (protection at the site of infection) and systemic immunity, which includes antibody formation as well as activation of circulating immune cells. It has also been reported that the nasal route induces cross-protection against variant strains, an observation that may contribute to the development of so-called "universal vaccines." There is also evidence this administration route may enable the development of therapeutic vaccines for chronic, hard-to-treat diseases, such as hepatitis B.[1] Although there are many publications supporting this view, this route may not work for all antigens or vaccines.

Patient Comfort

One unquestionable advantage of intranasal vaccination is the nasal cavity is easily accessible to liquids and even dry powders. Intranasal spray

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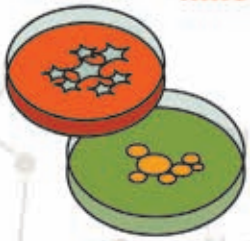

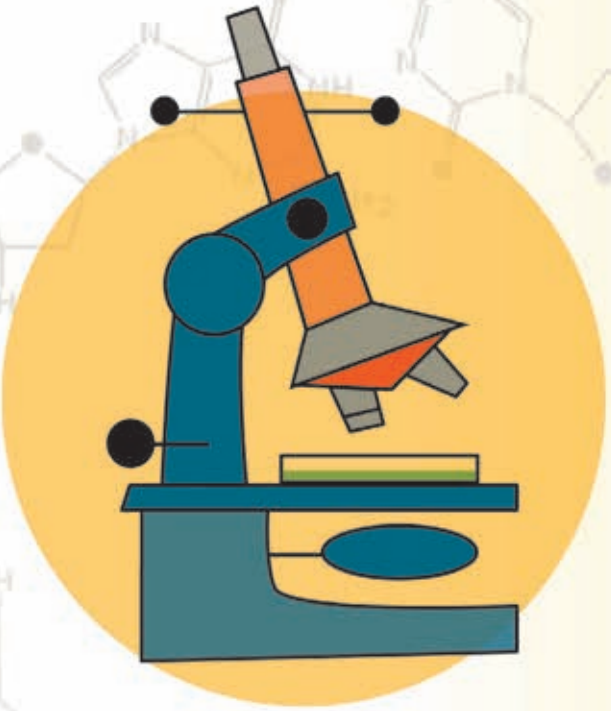

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administration is not invasive and causes little discomfort to patients. This is important, because many people fear injections, associating them with pain, the risk of transmission of diseases, such as HIV and hepatitis B, and the possibility of an anaphylactic response. Fear of injection can become a serious medical condition known as needle phobia. It is estimated that at least 10% of American adults are needle phobic (this is sometimes also known as trypanophobia). It is likely the actual number is larger, as the most severe cases are never documented due to the tendency of the sufferer to simply avoid all medical treatment, which of course includes vaccinations.[2]

Ease of Manufacturing

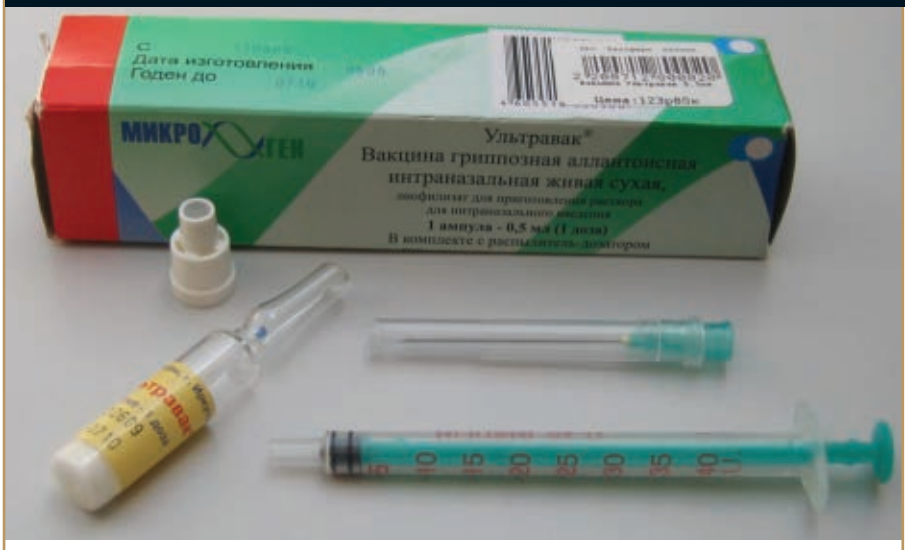
Another advantage arises from the constraints on the pharmaceutical formulation. Injection requires a sterile, particle-free liquid and a sterile syringe, while for intranasal administration the device does not need to be sterile.

Limitations & Potential Solutions

In spite of these potential advantages, there are three general concerns linked with intranasal vaccination:

1. Antigens may be unable to penetrate nasal mucosa in sufficient amounts to elicit an immune response.
2. The vaccine's nasal residence time may be too short to get a reliable response.
3. The activity of the vaccine may be hampered due to swirling, pressure, and shear forces when generating the spray.

FIGURE 1



MicroGen's Ultravac® for the Russian market. The live attenuated influenza virus is delivered lyophilized in the glass ampoule and is reconstituted using boiled and cooled down water. The vaccine (0.5 ml) is taken up into the syringe using the needle, which is now replaced by the sprayer. Then 250 microliters is sprayed into each nostril.

These issues need careful evaluation and must be addressed during the development of the vaccine. The use of appropriate adjuvants or vector systems (eg, adenoviruses) will generally help to solve the first two problems. Good device design will avoid the third potential issue. In addition, to gain wide acceptance, intranasal vaccines must not cause discomfort following administration due to unpleasant odors, itching, or nosebleed.

CAN INTRANASAL VACCINATION HELP TO SAVE COSTS?

There is great pressure to keep costs down in the healthcare sector. Even though vaccines are considered to be very cost-effective, the price of new vaccination programs must be justified. The price for a vaccine itself, its primary packaging, and the delivery device is not equal to the cost of vaccination. So cost reduction may come from different areas.

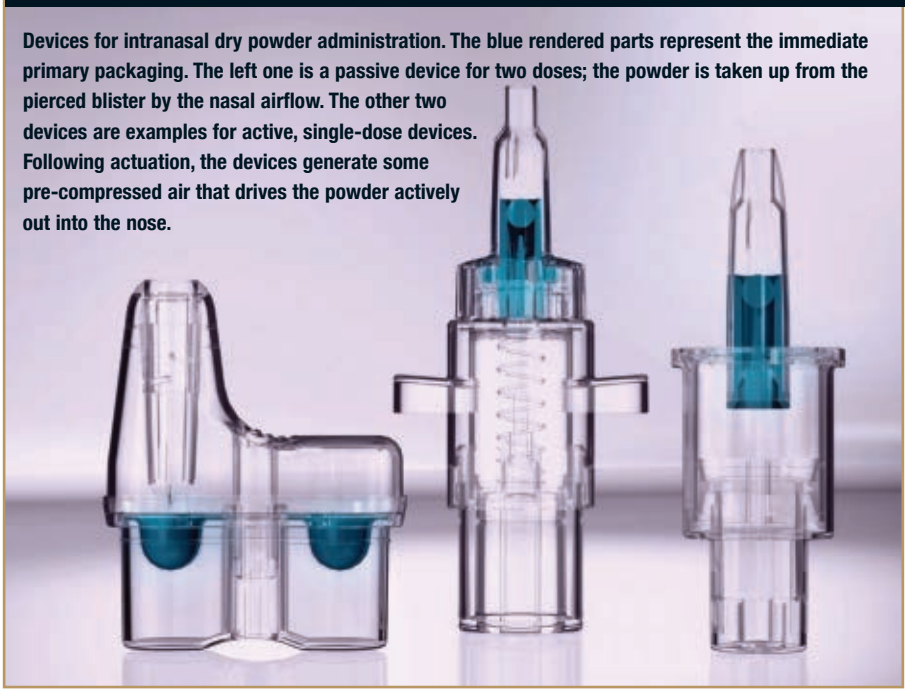
Cost Reduction of Antigen Per Shot

The amount of antigen per shot necessary to elicit protective immune response is a major cost-driving factor. This is obvious for antigens, which are hard to manufacture and expensive. To keep antigen costs down, new adjuvant systems have been developed to increase the effectiveness of the delivered antigen by a factor of two to four. The adjuvants for injected vaccines are not welcomed by all authorities (eg, US FDA) and may be seriously challenged as happened in Europe during the 2008/2009 swine flu

FIGURE 2

Needle-free transfer device and sprayer used for Serum Institute of India's intranasal flu-vaccine, Wolfe Tory's mucosal atomization device (MAD), and the sprayer used by Microgene (from left to right).

FIGURE 3



Devices for intranasal dry powder administration. The blue rendered parts represent the immediate primary packaging. The left one is a passive device for two doses; the powder is taken up from the pierced blister by the nasal airflow. The other two devices are examples for active, single-dose devices. Following actuation, the devices generate some pre-compressed air that drives the powder actively out into the nose.

pandemic. Depending on the antigen, intranasal vaccines may outperform here. For example, the influenza virus is still widely grown on eggs and as a rule of thumb, the antigen yield from one egg is used for one injection. The same amount would be sufficient for up to four adjuvanted intramuscular doses (inactivated) or for 20 to 100 intranasal shots of live- attenuated viruses.

Cost of Safe Administration

Another factor affecting cost is the level of skill necessary for safe administration of the vaccine. Egg protein remaining in the vaccine can cause an anaphylactic response in susceptible people, and the presence of a physician to administer first aid can save lives. Even in people with allergies, intranasal administration of antigens causes a much milder, non-life-threatening response, so it can be performed by nurses or pharmacists. Intranasal vaccines may also be suitable for self-administration, an option for travel-related diseases or pandemic situations.

Waste Disposal

Following successful administration of the vaccine, the used device and the secondary packaging must be safely disposed of in an environmentally friendly manner. The amount of potentially harmful medical waste is steadily increasing and is also a cost factor, which devices without needles and blood contamination make it much easier to deal with. The use of material that can be incinerated completely at low temperatures without producing toxic fumes will be highly appreciated in developing countries.

Dry Powder Vaccines to Avoid Need for Cold-Chain Storage

Most vaccines are temperature sensitive, and a cold chain is therefore mandatory. Transportation and storage under cold-chain conditions can cause substantial costs (estimated at 20% of vaccination costs) and require an appropriate infrastructure. With the introduction of new vaccines and the inclusion of additional target groups for certain vaccines, the volume within the cold chain is increasing. Although many guidelines are available, quite a high percentage of vaccines have to be discarded due to failure in the cold chain or its documentation (so-called wastage). Any technology that dispenses with the cold chain, such as dry powder preparations, would help save costs and increase the availability of vaccines. So developing dry powder vaccines for intranasal administration or inhalation would be a straightforward approach to reduce costs for vaccination programs, particularly in countries with poor infrastructure.[3]

SELECTING AN APPROPRIATE DEVICE FOR INTRANASAL VACCINATION

Existing Market References

Although there are some intranasal vaccines on the market for pets and farm animals, only three intranasal influenza vaccines are on the market for human use. All are intended for the prevention of seasonal or pandemic influenza and use live-attenuated viruses. Although the seasonal influenza market is quite large at an estimated $3 billion for 350 million doses per year, intranasal products are still not widely used. The first on the market was Microgen's Ultravac® seasonal vaccine, which is available for about $3 in Russia only (Figure 1). The live-attenuated virus form of this particular vaccine is under development by BioDiem/Nobilon for developed countries, and for developing countries, the project is supported by WHO. Very recently, it became available as Nasovac® in India to prevent pandemic flu. For both vaccines, the administration device is very cheap and simple but inconvenient: a single use syringe with an attached

sprayer (Figures 1 and 2). The third device on the market and probably the best known is MedImmune's Flumist®, which uses a prefilled syringe fitted with a sprayer and a removable clip to separate the two actuations (one per nostril). The moderate commercial success of these intranasal vaccines is not due to low effectiveness but linked to inconvenient handling and storage conditions and the risk of flu-like symptoms after immunization.

User-Friendly Nasal Vaccine Sprayers

There is certainly no device that can accommodate all intranasal vaccines in development, but there are some common considerations. For nasal administration, the applied volume is comparably low. For liquids, 100 microliters per nostril is optimum in adults, but should be reduced for children to avoid nasal dripping, which produces discomfort and reduces effectiveness. When using live-attenuated viruses, a simple dropper should be sufficient because the virus will behave like a wild-type infection, which normally does not need a sophisticated device to infect people. The simple blow-fill-seal container approach for vaccines is used by the Swedish company Eurocine. The disadvantage here is that to administer nasal drops properly, the recipient has to lie down or carry out some head movement to ensure proper distribution and to prevent immediate drip off. A more user friendly spray device would allow more convenient and much faster vaccination in an upright position, which would be an advantage in pandemic situations and for mass vaccinations.

For virus-like particles or purified antigens, a more elaborate device with good spray performance will certainly reduce the amount of antigen needed to elicit reliable protection. In this case, the cost of the saved antigen should balance out the likely higher price for the device. Another aspect to consider is whether the vaccine should be administered in one or both nostrils. The latter option seems to give patients more confidence and will increase acceptance for the intranasal route.

Prefilled Nasal Delivery Devices

Prefilled syringes are increasingly replacing multi-dose bottles for vaccines because they are easy to handle. So it is safe to predict a bright future for prefilled intranasal devices. The packaging of the vaccine (dry powder or liquid) must avoid loss of antigen during storage and transportation (e.g., adhesion). Although well established, glass vials in combination with rubber stoppers are not as cheap as one might believe. For effective filling and optional Water for Injection (WFI) washing, the vials need to be nested to fit into existing washing and filling lines. The filling volume for nasal administration is also much smaller than the 0.5 to 1.0 ml for prefilled syringes. To avoid investment in new expensive filling equipment, device manufacturers are working intensively on a solution to adapt the existing filling technology for Ready-to-fill syringes to the technology for nasal sprays. The future will see alternative approaches to keep costs down and speed up filling. It may include the use of primary packaging molded from new plastic materials developed for prefilled syringes, such as Cyclic Olefin Copolymer (COC) or Daikyo's Crystal Zenith® to replace glass vials, or pouches made from laminated foils.

Dry Powder Devices

In the near future, the development of intranasal vaccines will probably focus on dry powder vaccines to take advantage of improved storage conditions. It may be a challenging task

FIGURE 4

For use with a multi-dose pump, the vaccine is freeze-dried or filled as liquid into a standard multi-dose bottle and sealed with a simple tear-off closure. Only the filled bottle has to be transported within the cold chain if necessary. Just before the vaccination should start, the closure is removed (Step 1) and the content reconstituted if necessary and the pump is snapped on by hand (Step 2). After priming the pump with 5 to 7 actuations, the system is ready to use. For each patient, a disposable cap is used to prevent disease transmission. No re-priming is required between patients.

to generate a powder with the right particle size without harming the vaccine, but the filling of blisters and capsules is well established. For dry powders, electrostatic charge and moisture ingress must be considered. Devices that actively drive out the powder, using compressed air generated by a pump-like mechanism, seem to be better accepted than passive devices in which the powder is taken up by the nasal air flow (Figure 3).

Multi-Dose Spray Pumps for Mass Vaccinations

Multi-dose spray pumps are a very cost-effective option for liquid vaccines. The major challenge is to prevent microbial contamination of the bottle content when a single device is used by many people. The principle of how this could work is shown in Figure 4. Some of the so-called preservative-free pump systems, and in particular, systems with the means to prevent drain-back of liquid into the nasal actuator (also called a tip-seal), can fulfill this requirement. Transmission of diseases from patient to patient (eg, common cold or rhinoviruses) can be effectively prevented using disposable sleeves or protection caps. This is a very cost-effective approach not only for mass vaccinations in developing countries but also for the stockpiling of vaccines for pandemics or to fight bioterrorism. In this case, one would be well prepared without spending too much money on vaccines and devices, which hopefully would never have to be used.

CONCLUSIONS & PERSPECTIVES

The availability of new technologies, such as virus-like particles, new viral or bacterial vectors, and DNA and RNA fragment technologies, opens the door for the development of prophylactic and therapeutic vaccines for diseases that until now have been out of reach, such as tumors, malaria, and cardiovascular conditions. Using the intranasal route will provide additional immunological benefits, in addition to it being a mature administration technology. Examples of the wide use of nasal sprays include treatment of allergic rhinitis and nasal congestion, and providing fast pain relief. This means that easy-to-use and price-competitive devices for intranasal administration are already available. The near future will see the development of cheap sophisticated devices, optimized for fast filling and safe disposal.

Intranasal vaccination is set to become an attractive segment of the vaccine market in the near future. Non-invasive administration and the potential to use dry powder formulations may further assist its wider use. The intranasal route is also likely to gain support from patients who fear injections.

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BIOGRAPHIES

Dr. Degenhard Marx, following the study of veterinary medicine and the successful completion of his thesis at the University of Leipzig, joined the Arzneimittelwerke Dresden/Asta Medica co-operate research in 1992. In 2001, he took over a Senior Research position at Altana Pharma/Nycomed in Constance, Germany. During this time in the pharmaceutical industry, he collected ample experiences in the drug development of anti-inflammatory and cardiovascular drugs. Since 2008, he is Business Development Manager at Ing. E. Pfeiffer, Pharma Division, which became Aptar Pharma in 2010. Now he is Director Scientific Affairs within the Consumer Healthcare division.

Matthias Leitz, following training as a paramedic, he successfully graduated in Business Engineering at the University of Cooperative Education Stuttgart - Horb. In 2004, Mr. Leitz joined the Aptar Group, initially working on ophthalmic drug delivery technologies. After gaining first experiences in this field, he started focusing on single-dose devices administered via the nasal and oral route, which target vaccination, pain management and the central nervous system. He currently works as Product Manager in Aptar Pharma's Prescription Division.

Christophe Fagot is Associate Director, Business Development Manager for Pain & Vaccines at Aptar Pharma, where he worked for 13 years. He graduated in Mechanical Engineering from Supmeca (Paris) in 1994 and is currently part of the Executive Part-Time MBA programme of the Ecole Supérieure de Gestion (Paris). From 1998 to 2009, he worked in different technical positions in the Engineering Department and then in the R&D Department as a Design Engineer, Project Manager, and Director of DPI development. In 2010, he decided to join the Business Development team. He can be reached at christophe.fagot@aptar.com.

DRUG DEVELOPMENT Executive

Covaris flow systems

COVARIS: ENABLING NEW DRUGS & DELIVERY SYSTEMS USING ADAPTIVE FOCUSED ACOUSTICS™

Carl Beckett

Covaris Flow Division General Manager

Covaris, Inc

Covaris Incorporated leverages Adaptive Focused Acoustics™ (AFA™) technology to provide premier sample processing instruments and solutions to the analytical and life sciences industry. Founded in 1999, Covaris is a privately held technology company headquartered in Woburn, MA, and was built upon its team's in-depth knowledge in fields ranging from acoustic physics and mechanical engineering to biophysics and molecular biology. The Covaris technological foundation is based on it's proprietary and patented AFA technology. AFA enables a vast array of non-contact, isothermal processes to be developed for use in a broad range of applications in formulation, drug delivery systems, genomics, proteomics, cell biology, and drug discovery research. In many cases, the ability to impart precision, kinetic control of application processes could not be obtained prior to the development of the isothermal, non-contact AFA technology. These important characteristics provide Covaris with a truly sustainable competitive advantage. With over 1500 systems installed world-wide for processing of bench top volumes, Covaris was constantly receiving requests to process larger sample volumes. In 2010, the Covaris Flow Division™ was launched to deliver upon the pent-up demand for processing of higher volume, continual processes. With all the same attributes as the core technology and the ability to scale to higher volumes, it is now possible to address new and exciting applications with Covaris AFA. Indeed, AFA enables performance previously unobtainable with currently available technologies. Drug Development and Delivery recently sat down with Carl Beckett, Covaris Flow Division General Manager, to discuss how AFA is enabling new drug delivery systems.

"The scientist is able to use the same process technology from 0.1 ml through pilot-scale volumes. This is significant because it enables methods and processes to carry through the entire discovery/development cycle, eliminating the need to re-engineer and re-design formulations when a higher volume of material is needed for preclinical and clinical dosing. This results in a significant reduction of the overall development timeline for a new molecule."

Q: Can you please tell our readers what makes your technology unique?

A: Acoustic energy, at both sonic and ultrasonic frequencies, has been utilized for many years for a variety of diagnostic, therapeutic, and research purposes. Some uses of acoustic energy in materials processing include sonication. This is an unrefined process of mechanical disruption, typically involving the direct immersion of an unfocused sonic source into a fluid suspension of the material being treated. The sonic energy often does not reach the target in an effective dose because the energy is scattered, absorbed, and/or not properly aligned with the target. One result of this unrefined, unfocused energy is undesired heat generation that can negatively impact sample quality (causing degradation, for example). Another issue is recovery, as the unrefined process can cause sample loss due to lack of experimental control. In addition, this approach is prone to cross-contamination and finally, sonication processes cannot effectively scale from small (< 1 ml) through

Pilot Scale volumes (> 100 L) as the energy is unfocused (ie, due to the fundamental long wavelength of the sonicators, typically 10 to 15 cm). While there are also specific clinical examples of the utilization of therapeutic and diagnostic ultrasound (eg, fetal imaging), until AFA, ultrasonics have not been controlled to provide an automated, broad range, precise materials processing or reaction control mechanism for both bioanalytical and bioprocess sciences.

Covaris systems are uniquely capable of providing controlled delivery of acoustic energy to closed vessels. Using AFA with sample sets can improve both the quality and efficiency of the drug development process, with unparalleled reproducibility, precision, and recovery for a variety of sample types with a broad range of sample output sizes.

Q: How does AFA accelerate the drug development process?

A: AFA can be used for a variety of process applications, but the common theme is that it is a highly scalable process. The scientist is able to use the same process technology from 0.1 ml through pilot-scale volumes. This is significant because it enables methods and processes to carry through the entire discovery/development cycle, eliminating the need to re-engineer and re-design formulations when a higher volume of material is needed for preclinical and clinical dosing. This results in a significant reduction of the overall development timeline for a new molecule. The use of high mechanical energy at the molecular level, such as the controlled acoustic dosing available with Covaris, often enables a process or reaction with fewer catalysts or solvents. By reducing or eliminating certain solvents/surfactants, the results of a particular study are less error prone by eliminating possible false negatives that could be due to the solvent and not the active ingredient itself. Complete temperature control during the process eliminates the molecular degradation that is common for many of today's methods. Less degradation means more of the active ingredient is maintained intact and available for biological uptake. It also creates flexibility in the dosing, formulation choices, and ultimately end efficacy of the drug. More choice and design freedom during the discovery/development cycle results in more drugs entering the market more quickly. Overcoming even a small design hurdle can make a difference that saves weeks or months of effort.

Q: What particular application areas has AFA been able to have a significant impact?

A: We have demonstrated some tremendous benefits of applying AFA in the area of preclinical formulation of poorly soluble media in which we were able to reduce certain processing times from 2 days to less than 3 minutes. At the same time, we were able to reduce material degradation by 1500%. This means more than 2.5 times more of the API was delivered. Furthermore, using a repeatable, automated system allows standard procedures for formulation to transfer across organizational boundaries. The formulation scientist can develop a method that directly translates to the PK group, or even the CRO performing a study. Problems and issues with a study are not uncommon, and when they occur, there is always an investigation into why. Common questions include: were the doses homogenous, was the formulation properly prepared, were procedures properly followed, what were the subjective grading elements, and were these calibrated to those specified? AFA has proven to be extremely helpful in that it reduces the source for many of these types of errors by providing a precise, repeatable, automated process. Obviously, this results in a better animal study, saving time and money, but most importantly, improves the quality of the study itself by eliminating unknowns.

Q: Are there other application areas in which AFA makes a significant impact?

A: Perhaps even more exciting is the relative ease and benefits by which AFA can form liposomes. In a matter of seconds, AFA is able to produce 2-ml samples of liposomes, while maintaining a process temperature of 4°C. There are no other technologies available to

produce liposomes of the quality, and with the ease and convenience, of the Covaris system. Also, the AFA process is completely self-contained, meaning all the wetted surfaces are disposable and any risk of contamination is eliminated. This is a significant benefit over some of the current processes that heat or contaminate the material. Again, volumes from as low as 0.1 ml up through pilot scale are possible. Delivery of sensitive biologicals, such as RNA/DNA-based active components, is enabled as long as the strand is below a certain size range. We have multiple research projects under way validating the benefits of the process, including one with Dr. Jean-Bosco Tagne of the Boston University School of Medicine, to evaluate the potential as a delivery system for miRNA molecules in respiratory disease research.

Q: What about drugs with extremely low solubility levels?

A: The formation of nanosuspensions is another exciting area for Covaris and AFA. More and more of today's molecules are problematic in terms of solubility, which creates a problem in effective formulation. We see an increase in applications needing to micronize to a particle size below 200 nm, after which the material can be passed through a sterile filter before dosing. The current methods almost always contaminate or degrade the material, or result in significant material loss. Not only are we able to produce nanoparticles as small as 13 nm, we can do so in just a few minutes, while controlling heat and contamination, as well as scaling this same process from 1 ml through 1000 ml. As an example, a 200-ml batch of material is reduced to ~60 nm mean particle size in 30 minutes. The distribution was very monodisperse with a PDI ~0.2, resulting in a stable formulation validated 1 month later. Looking ahead, we envision producing even larger volumes of material (multiple liter scale), as the AFA process is completely computer controlled, repeatable, and a hands-off operation.

Q: Where does AFA end and formulation begin?

A: The two are deeply interrelated, and each molecule and application is a unique design challenge. Although we have formulation expertise in-house, oftentimes a customer is working with a highly confidential compound that must remain secret. We understand these needs, and our best results are achieved when the customer formulation scientists work closely with our formulation and acoustical process scientists to optimize a result. The type of formulation does impact the optimum processing conditions; therefore, by working together, the results are almost always better (and certainly more efficient) than when a customer purchases our equipment without understanding the underlying unique molecular interactions of the AFA technology.

One of the salient features of this technology is when the optimization is complete; the process is highly repeatable, and can then be scaled to whatever volume of material is needed. In summary, the time and effort invested in a discovery phase can be carried through to larger studies, such as cell culture, animal, and even clinical trials.

Q: There are a growing number of applications using biological materials. Can AFA be applied for these?

A: Covaris was founded on the basis that the initial preparation of biological samples will become an increasingly critical step for analytical sciences. This is evident in applications in which best-in-class processing of biological materials is required for advanced instrumentation systems (eg, next-generation DNA sequencing). Lysis, separation, disruption, or extraction of a target molecule is at the core of our expertise and capability. With the ability to scale these processes, we believe there is a significant opportunity in the area of biological production that includes vaccine development. Transfection, sonophoresis, and extraction/lysis are also key capabilities having tremendous potential. Because we are able to precisely control the energy level, we are able to achieve a very high process efficiency rate, without damaging the target molecule. Often, a currently required detergent or intermediary step can be significantly reduced or even eliminated entirely by using AFA, resulting in higher overall yields. For example, a small increase in the yield of a vaccine production of 10% could have a considerable commercial impact. ◆

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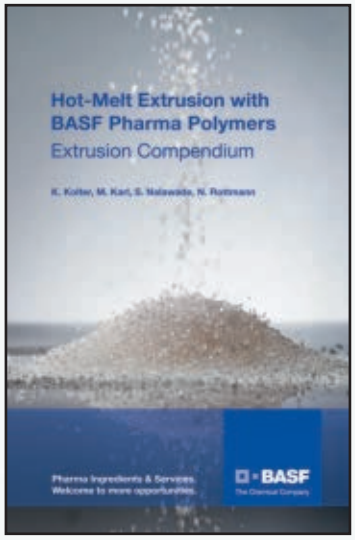
Hot-Melt Extrusion



The first edition of the Hot-Melt Extrusion (HME) Compendium is now available. In this compendium, scientists at BASF present a range of polymers with both low and high glass transition temperatures for pharmaceutical technology. HME is currently generating a significant interest in the pharmaceutical industry as the percentage of poorly soluble compounds continues to increase. HME thus enables such molecules to increase their solubility and bioavailability. The compendium covers the chemistry and applications of polymers in melt-extrusion to achieve the robust processing conditions and desired release profiles of poorly soluble drugs. Download and comment on the Compendium at **www.innovative-excipients.basf.com**

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Technology & Services Showcase

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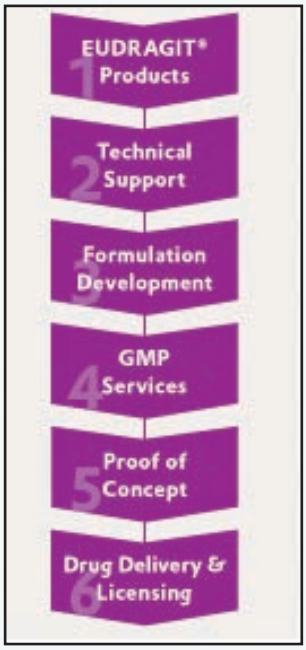
Pharma Polymers



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Analytical Testing

Heavy Metals Testing (USP<231>) Revisions: New Limits & Procedures for Elemental Impurities in Pharmaceuticals & Dietary Supplements

By: Jeff Grindstaff and Colleen Schroeder, Columbia Analytical Services, Inc.

Introduction

Changes to heavy metals test procedures for the analysis of pharmaceuticals and dietary supplements are under review with new standards set to be in place by mid-2013.[1] The intention of this review is to update current analytical testing historically performed using United States Pharmacopeia (USP) <231>. The revisions (USP<232>, USP<233>, and USP<2232>) are designed to set safer limits for public exposure and to reduce the environmental impact of dated methods. Many in the pharmaceutical industry have concerns about the new instrumentation, more stringent requirements, and the associated costs. Nonetheless, the revisions should have a beneficial impact on the industry by significantly improving specificity and analyte recoveries, as well as by yielding overall time-savings, resulting in safer, higher quality products.

Shift From Outdated Technology to Modern Methodology

First introduced over 100 years ago, USP<231> is a colorimetric procedure based on the precipitation of insoluble metal sulfides. The test is qualitative rather than quantitative. It is not an element-specific method, nor is it equally sensitive to each metal. The limits specified by the test are based on the ability to observe the precipitate, rather than on the analysis of toxicological data. The procedure does not necessarily detect all potential forms and/or valences of elements of concern when they are present as the oxo ions or in the organometallic form. Chromium and nickel are

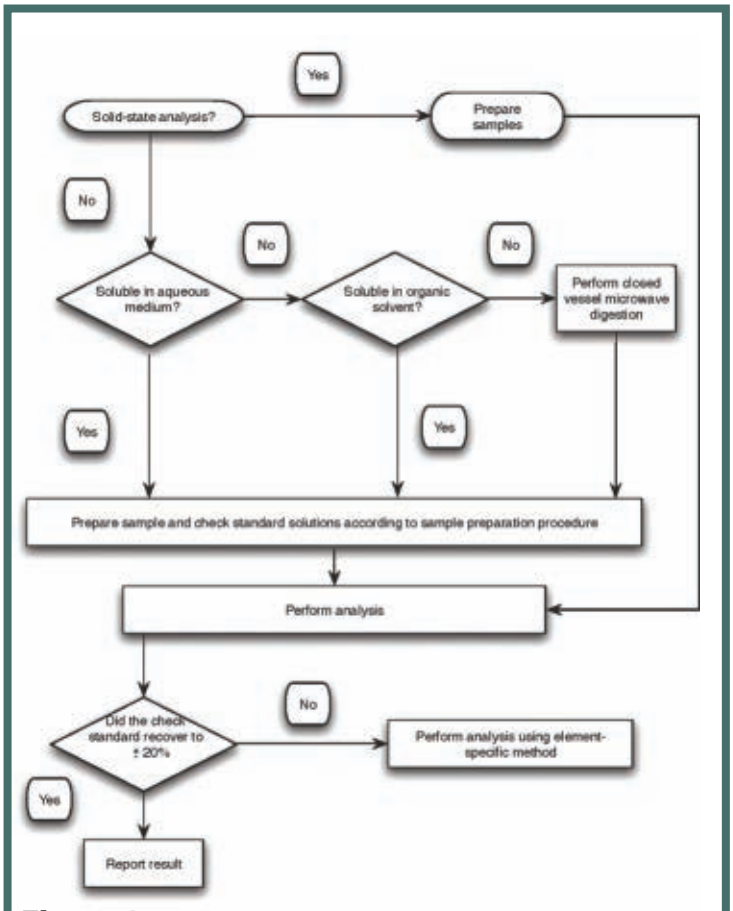

Figure 1. *Sample Preparation Decision Tree*

potential contaminants from modern stainless steel processing equipment and are not detected by USP<231>.[2] Other studies indicate inconsistent recoveries of monitor and standard solutions using USP<231> method II.[3,4]

Industry criticism of this dated method began around 15 years ago and sparked the revision process by the USP. After seeking public comment and advice from experts on metals toxicology, the USP is now recommending that USP<231> be revised to USP<232>, which will require the use of updated instrumental technology to improve selectivity and sensitivity. The change includes modification to the preparation and analysis methodology as well as the impurity limits of each analyte.

Revisions to the elemental impurities test will constitute a serious change for the pharmaceutical industry. The change will shift the testing from a relatively inexpensive procedure that requires minimal set-up and operator training to tests that require expensive instrumentation and highly skilled metals analysts. However, by employing modern instrumental methods, the USP's intent is to ensure safer products for the consumer as well as offer flexibility and efficiency during testing.

All drug products produced and sold in the US will have to comply with the limits set by USP<232>, and drug substances and excipients will have to be tested and reported for elemental impurities. Likewise, all nutraceutical products will have to comply with limits set by USP<2232>, which includes guidelines for speciating organic and inorganic forms of various elements. USP<232>, USP<233>, and USP<2232> are currently in a preliminary recommendation stage, and the limits described have not been finalized.

Improved Methodology for Identifying Discrete Elements

One of the main criticisms of USP<231> has been the inability of the testing to recover and identify individual elements. Previously, the elemental impurity list included arsenic, antimony, bismuth, cadmium, copper, lead, mercury, molybdenum, silver, and tin due to reactivity of these metals with the sulfide ion utilized in the procedure. The metals were reported inclusively as "heavy metals" due to the procedural inability to show them discretely. In addition, arsenic, bismuth, and molybdenum were not necessarily detected by USP<231> due to common occurrences of these elements in forms inert to the mechanism in the procedure. Because numerous instrumental procedures have been developed over the life of USP<231> that incorporate significant improvements in selectivity and sensitivity, the USP's proposal will require individual quantification of arsenic, cadmium, lead, and mercury (target elements considered most toxic to humans and the environment, see Table 1). If the presence of additional metals is suspected (for instance, if used in the manufacturing process as catalysts or if detected during previous testing), then those additional metals would be added to the target list. Each element screened will have individually distinct impurity limits, based on unique toxicity data.[5,6]

Element	Health Risks
Arsenic (As)	Inorganic forms of arsenic are particularly toxic and water-soluble inorganic arsenic is readily absorbed by the human digestive system. Symptoms include stomach and intestine irritation and skin disturbances, lung irritation and decreased white and red blood cell production. Very high exposure to inorganic arsenic can cause infertility, skin disturbances, declined resistance to infections, heart disruptions, brain damage, and death. Acute oral LD_{50} values range from 10 to 300 mg/Kg.
Cadmium (Cd)	Cadmium is more readily absorbed through the lungs than through the human digestive system. Exposure to cadmium can damage kidneys, the central nervous system and the immune system, as well as cause bone fractures and reproductive problems. Symptoms can include stomachaches, diarrhea, and vomiting. Oral LD_{50} values in animals range from 63 to 1125 mg/Kg.
Lead (Pb)	Exposure to lead can occur through ingestion and inhalation. No clear threshold has been established for lead; however, the USP is deferring to the FDA maximum allowable level for lead in bottled water (5 micrograms/L) to set the elemental impurities limit. Lead can cause: disruption of the biosynthesis of hemoglobin, anemia, high blood pressure, kidney damage, reproductive/fertility problems and brain/nervous system damage.
Mercury (Hg)	Prevalence of mercury in the environment leads to biomagnification in the food chain. Organic forms of mercury, such as methyl mercury, are more toxic than inorganic forms due to the ease of absorption into the human system. Symptoms of mercury poisoning include: kidney damage, disruption of the nervous system, damage to brain functions, DNA and chromosomal damage, allergic reactions, sperm damage, birth defects, and miscarriages. LD_{50} values are as low as 1 mg/Kg in small animals.

Table 1. *Health Risks Associated With the Four Elements of Primary Concern*[5,6,12,13]

The USP is considering many factors to decide which elements will be tested and at what levels. The likelihood of contamination during manufacturing, possible additional environmental exposure, as well as reactions with other metals (co-exposure) during drug administration are factors influencing the review. Though rapid, accurate, simultaneous multi-element analysis of many metals is now possible at very low concentrations, the USP has preliminarily decided to base impurity limits on toxicologically relevant data in an effort to avoid burdening the industry with unnecessarily low limit requirements. The new limits will be based primarily on previously established guidelines for human and animal toxicological exposure and are dependent on route of delivery (Table 2). Screening will be required for all toxic metals that have been shown to be present, regardless of whether or not they are included in the impurities list. However, the USP will not mandate methodology. Each manufacturer will be able to choose the procedure(s) that best fits their processes.

Element	Parenteral or Inhalational Daily Dose (µg/day)	Oral, Topicals, and Dermal, Mucosal Daily Dose (µg/day)	
Inorganic Arsenic	1.5	15	**Required**
Cadmium	0.5	5	**Required**
Lead	1	10	**Required**
Inorganic Mercury	1.5	15	**Required**
Chromium	25	250	**Discretional**
Copper	250	2500	**Discretional**
Manganese	250	2500	**Discretional**
Molybdenum	25	250	**Discretional**
Nickel	25	250	**Discretional**
Palladium	10	100	**Discretional**
Platinum	10	100	**Discretional**
Vanadium	25	250	**Discretional**
Osmium	10 (Combination not to exceed)	100 (Combination not to exceed)	**Discretional**
Rhodium			**Discretional**
Ruthenium			**Discretional**
Iridium			**Discretional**

The above limits are derived from conservative calculations based on 50Kg (110lb) body weight and 10g daily dose, assuming a 70-year life span. Bioavailability assumptions: oral 10%, parenteral 100%. Compliance options may be demonstrated by analysis of the drug product at maximum daily dose and compared to limit level (modified daily dose permitted daily exposure) or summation of the impurity level in each of the components of the drug product.[1,5]

Table 2. Proposed List of Elements & Limits[5]

USP<233> Methodology

In moving from a chemical- to an instrument-based methodology, the USP has taken great care to allow for a flexible approach and is working closely with both the FDA and industry to ensure widespread agreement on interpretation of the revisions. The following are brief descriptions of the methodologies being proposed.

Sample Preparation - Sample preparations range from relatively simple acidification and direct injection to more complex total oxidations/dissolutions performed under elevated temperature and pressure in appropriate acid(s) to ensure dissolution of target elements. Sample preparations are intended to yield an aqueous digestate suitable for instrumental analysis via one or more instrumental techniques.[1,7] See Figure 1 for a decision tree on sample preparation and analysis.[5]

Instrumentation - The techniques typically utilized for the analysis of the sample digestates are Cold Vapor Atomic Absorption (CVAA), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP/OES), and/or Inductively Coupled Plasma-Mass Spectrometry (ICP/MS). Technical considerations beyond the scope of this discussion dictate the choice of procedures. As with any analytical technique, interferences (chemical and/or physical) exist with each technique. Intelligent decisions relative to the elements of interest and the sample matrix will indicate the appropriate analytical approach.

Although the majority of applications can be satisfied by the use of ICP/MS and/or ICP/OES, expert trace metals chemists recognize that alternative procedures are required at times to satisfy unusual analytical challenges. Careful examination of each application must be done from a quality assurance perspective. There are situations when multi-element techniques that utilize the plasma as an ion source or light emission source are capable of producing values that appear to be valid from a quality control standpoint, but are nonetheless invalid from a quality assurance standpoint. On these occasions, the following instrumental techniques still play a role in a fully functional trace metals laboratory: Purge & Trap Cold Vapor Atomic Fluorescence Spectroscopy (P&T-CVAFS), Graphite Furnace Atomic Absorption (GFAA), Flame Atomic Absorption (FLAA), and Gaseous Hydride Atomic Absorption (GHAA).

The revised quantitative methods, though of great benefit in terms of accuracy and recovery, are significantly more expensive than the qualitative USP<231>. Perhaps the main criticism of the revised testing protocols relates to the associated cost of new instrumentation and/or outsourcing for testing. Because atomic spectroscopy and ICP spectrometry are not yet widely used in the pharmaceutical industry, smaller manufacturers and excipient companies may not yet have the instrumentation in place and will need to either purchase the new equipment or send their testing to contract laboratories.

The various instrumental techniques each include advantages and disadvantages with respect to cost, sensitivity, selectivity, and ease of use. Some of the techniques are best suited for certain elements, but not for others. The same is true for certain sample matrices. For example, the analysis for lead and arsenic by ICP/OES or FLAA frequently represents a poor choice because of the associated high detection limits (DL). With these elements, sample preparation would have to be more complicated to offset relatively high DL for the instrumentation. Alternatively, GFAA or ICP/MS would be preferable choices.

The instruments listed in Table 3 are capable of performing analysis of some or all of the elements listed in USP<232>. This table compares the instruments and equipment most commonly required to meet the USP requirements. Approximate values representing initial purchase and ongoing operating costs as well as abbreviated summaries of strengths and weakness are also listed.

Instrument/ Equipment	1. Purchase Price 2. Operating Costs (annual)	Best Use	Advantages	Disadvantages
Graphite Furnace Atomic Absorption (GFAA)	1. $30,000 - $65,000 2. $5,960	Sensitive and selective; good for metalloids that suffer poor ionization and are weak light emitters.	Low detection limits and good selectivity when Zeeman BG used; proper temperature programming overcomes abbreviated digestions.	Single element technique; consumables are costly; higher skill level to operate.
Flame Atomic Absorption (FLAA)	1. $15,000 - $40,000 2. $5,600	Commonly used for alkali metals.	Easy and relatively inexpensive to operate; accurate and sensitive for alkali metals.	Single element technique; not sensitive for heavy metals; subject to uncorrectable interference.
Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP/OES)	1. $50,000 - $100,000 2. $6,250	Excellent multi-element technique with relatively good sensitivity and selectivity when configured correctly.	Rapid multi-element analysis produces relatively low detection limits; excellent for alkali and alkaline earth elements; large linear dynamic range; tolerance to high levels of dissolved solids; axial and radial viewing of the plasma provides high versatility; essential backup for situations where uncorrectable interferences exist for ICP/MS.	Occasionally stymied by uncorrectable spectral overlap; elements of significance to USP (As, Pb, Hg) are not sensitive enough for many applications.
Inductively Coupled Plasma-Mass Spectrometry (ICP/MS)	1. $130,000 - $180,000 2. $14,150	Multi-element ultra trace technique.	Superior sensitivity; selectivity excellent when configured correctly and applications investigated thoroughly; excellent for high mass elements; many polyatomic interferences can be removed via collision or reaction cell technology; rapid determinations possible.	Higher skill level to operate; initial and ongoing cost is high; occasionally stymied by uncorrectable isobaric interference.
Digestion	1. $500 for microwave digestion bomb; $40,000 for microwave system. $35 for oven digestion bomb; $4,000 for convection oven.	Use dependent on the matrix under test. Note that essentially equivalent, efficient and less expensive alternatives are available rather than dedicated systems. Variations in acid matrix and heating times fluctuate with material being digested. Near complete oxidation of organic carbon to CO_2 and water is important when ICP/MS is used to avoid enhanced ionization of certain elements and/or carbon-containing polyatomics.		

Table 3. Instrumentation

Validation of Quantitative Procedures

Verification of the compendial procedures indicated in USP<233> will be required prior to use. This can be completed by meeting the Procedure Validation Requirements outlined in USP<233>.[1] Two types of validations (limit and quantitative) will be permitted. The limit test validation will include limit of detection, precision, and specificity. The quantitative test validation will include performing accuracy, precision, specificity, limit of quantitation, range, and linearity. Both types of validations will need to be verified experimentally. In addition, sample preparation not specified in the monograph will also require verification. The compendial procedures encompass both ICP/OES and ICP/MS technologies, and the general instrumental and suitability requirements for each procedure are specified for users. Laboratories will be able to choose the appropriate technology that best fits their needs.

Summary

Although USP<233> represents a major shift for the pharmaceutical industry, US Pharmacopeia has clearly stated they do not intend to create a system of unnecessary and complicated requirements.[5] The goal is simply to create standards for safer pharmaceutical products and dietary supplements, through the use of modern technology. While increased cost is a factor, and manufacturers will need to make certain adjustments, this shift represents an appropriate modernization that manifests itself by ensuring higher quality products. ◆

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Executive Summary

Mark Varney, PhD
President & CEO
Cortex Pharmaceuticals

Cortex Pharmaceuticals: Developing Novel Drug Therapies for the Treatment of Neurological & Psychiatric Disorders

The medical research community is engaged in a tireless quest to overcome a vast range of challenges: widespread ailments that range in symptoms and complexities from Parkinson's disease (PD) and Fragile X syndrome to ADHD and obstructive sleep apnea. Thus, it might seem incredible for the layperson to hear that a single pharmaceutical-based strategy could possibly be harnessed to address these four particular maladies. However, this is exactly what the leader of one pharmaceutical company is now betting on. Mark Varney, President and CEO of Cortex Pharmaceuticals, tells Specialty Pharma of a development that may very well offer hope to those who are experiencing the symptoms associated with PD, Fragile X, ADHD, and apnea. In particular, he describes the advent of a class of molecules called AMPAKINE compounds that are currently being developed by Cortex and that may provide protection from these ailments as well as a range of others.

Q: ***Before discussing their specific potential applications, can you tell our readers what AMPAKINES are and what effects they have on the human brain?***

A: AMPAKINE compounds are a class of proprietary pharmaceuticals that act through a two-pronged approach. They increase the strength of signals at connections between brain cells, and stimulate the production and release of certain growth factors in the brain. AMPAKINE molecules interact in a highly specific manner with proteins in the brain called AMPA receptors. These receptors are activated by the neurotransmitter glutamate, the most prominent excitatory neurotransmitter in the brain. AMPAKINE compounds facilitate the response to glutamate, essentially amplifying the normal level of signaling between neurons. Research by Cortex and its collaborators, including Professors Gary Lynch and Christine Gall from the University of California, Irvine, have demonstrated that AMPAKINE molecules also stimulate the production and release of certain growth factors in the brain, including brain-derived neurotrophic factor (BDNF). BDNF is essential for maintaining cell health in the normal brain, and plays an important role in restoring brain function following damage to the brain. Through elevating BDNF in damaged brain regions, AMPAKINE compounds may restore function to previously damaged areas.

Q: ***What is the status of Cortex's efforts in the Parkinson's disease arena?***

A: We have been awarded a grant by The Michael J. Fox Foundation for Parkinson's Research to test selected compounds from our AMPAKINE platform for their ability to restore brain function in animal models for PD. We aim to test our high-impact AMPAKINE drug candidates in the mouse model of Parkinson's, a well-validated model that exhibits many of the hallmarks of human PD and has been used extensively for drug development in PD. If successful, the work could lead to a neuroprotective treatment for the disease with the potential to slow or stop the course of the disease - something no currently available therapy has been proven to do. Current treatments for PD alleviate the symptoms but do not attack the underlying disease, or alter its course. Positive results will support moving selected compounds toward human clinical trials.

Q: ***Moving to Fragile X syndrome, how are you attempting to address this condition, which is the most common genetically proven cause of autism, with AMPAKINES?***

A: We have been granted an exclusive license by the University of California and a patent application for the combination of two substances that have shown promise in alleviating Fragile X symptoms, which can range from fidgeting and impulsive actions to epilepsy, OCD, and autism or autistic-like behavior. The first of these two substances are AMPAKINES, which, as I noted earlier, serve to increase the strength of signals at connections between brain cells and increases levels of BNDF. The second class of substance bears the name "metabotropic glutamate receptor type 5 antagonists," better known as mGluR5 antagonists. These appear to amplify the positive effects of the AMPAKINES in alleviating Fragile X symptoms. Early clinical studies with mGluR5 antagonists have shown promising results in Fragile X patients, and in animal studies, the combination of these agents with our AMPAKINE compounds has been seen to provide additional benefit via a synergistic mechanism. If these effects hold up in clinical studies, the combination could be an important treatment option.

Q: ***Can AMPAKINES also prove useful for those with the symptoms of ADHD?***

A: Yes. Again, the key attribute here is the ability of AMPAKINES to increase levels of neurotransmitters in parts of the brain that help people focus and control impulses, and to activate brain regions that are sluggish so that they regulate cognitive activity at a more normal level. Currently, there are a host of drugs already on the market, including Ritalin, Adderall, Concerta, and Vyvanse, which operate on these principles. However, these medications belong to the class of drugs known as stimulants and have an increased risk for addiction, and in some patients, lead to unacceptable increases in heart rate and blood pressure. In contrast, AMPAKINE compounds have the potential to be unique agents in treating ADHD because they lack the side-effect liabilities of existing treatments. Cortex is hoping to initiate clinical trials of its AMPAKINES for treatment of ADHD in late 2010.

Q: ***Cortex believes AMPAKINES will find use in treating the symptoms of obstructive sleep apnea. Could you discuss this endeavor?***

A: People with obstructive sleep apnea experience multiple interruptions of breathing that last 10 seconds or more while they sleep. The interruptions usually occur when the relaxation of upper airway muscles decreases airflow to the lungs, lowering the oxygen level in the blood and waking sufferers as they struggle to breathe before falling back asleep. This can happen

many hundreds of times every night. One of the most effective treatments is a machine called a Continuous Positive Airway Pressure (CPAP). This involves sleeping with a large facemask connected to a machine that pumps in air under pressure. Because of the uncomfortable nature of this treatment, patient compliance is low. This demonstrates the great need for a better solution.

The efficacy of AMPAKINES to treat obstructive sleep apnea was discovered after their use in treating psychiatric and neurological diseases was recognized. It turns out that in addition to the other effects on the brain I have mentioned thus far, AMPAKINES can also lead to the stimulation of a unique brain stem structure believed to play a major role in the modulation and generation of the respiratory drive. Put in a more simple way, AMPAKINES work by telling the brain to keep on breathing, and also by amplifying the signals from the brain to the upper airway muscles to maintain muscle tone.

CPAP devices represent a significant business, with sales of at least $1 billion dollars annually. We certainly hope that as our molecules undergo further clinical testing on the road to market, that they will one day gain the prominence among healthcare providers and obstructive sleep apnea patients as CPAPs currently enjoy.

Q: ***Who do you see as your chief competitors, and what is the current status of the clinical testing involving Cortex's AMPAKINES?***

A: At this time, we at Cortex do not see any genuine competition in our quest to develop molecules that can be used as widely and as effectively as our AMPAKINE compounds. As for clinical testing, we currently have one compound in clinical development: CX1739, which is targeted for obstructive sleep apnea and ADHD. We also have a robust portfolio of earlier-stage compounds that are awaiting clinical development.

Q: ***What would you say to our readers who experience the symptoms of Parkinson's, Fragile X, ADHD, or obstructive sleep apnea, or know someone who does? And what message do you have for potential investors in Cortex?***

A: Those who currently have one of these conditions or who have friends or family with them should be aware of cutting-edge research developments, such as the ones we have been discussing. Although currently there is no firm timeframe for bringing Cortex's AMPAKINE compounds to market, we are all looking forward to a day in the not-too-distant future when these compounds may offer a very substantial alternative or supplement to existing strategies. An important message should also be sent to healthcare providers, who stand to reap potentially big savings if AMPAKINE technology becomes standard. We believe our AMPAKINE compounds will tempt physicians to take a fresh look at the treatment options available to them. Keep in mind the total cost of these ailments involves more than just the immediate price of existing treatments themselves. Dealing with the complications that sometimes result from these conditions can be more expensive than anyone expects. When hospitals see the potential savings involved in switching to our AMPAKINE compounds as a standard treatment, we believe they will be enthusiastic.

As for potential investors, I would stress the fact that Cortex Pharmaceuticals' goals are broader than merely improving the lives of those with any one specific condition. We are a neuroscience company focused on the discovery, development, and commercialization of novel drug therapies for the treatment of a variety of neurological and psychiatric disorders. While we have been discussing specific areas of AMPAKINE therapy here, the fact is that they can potentially do much more. It is a very exciting time to be working in this field, and we at Cortex are looking forward to developing our technology and giving a wide range of patients new hope for the future. ■

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Controlled Release Society	31	651-454-0766	www.controlledreleasesociety.org
CMO/CRO	11		www.CMOCRO.com
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Wolfe Tory Medical, Inc.	18	888-380-9808	www.wolfetory.com
Xcelience	7	608-643-4444	www.xcelience.com

THE ADVANTAGES

OF MULTI-PHASE, MULTI-COMPARTMENT CAPSULES ARE CLEAR

Deliver Incompatible Compounds
Deliver incompatible compounds in a single dosage form with different release profiles.

Multiple Release Profiles
Incorporate one or more release profiles into a single dosage form such as immediate, enteric, targeted, chronotherapy and pulsatile.

Higher Perceived Value
Consumers view multi-phase, multi-compartment capsules as having a higher perceived value than ordinary tablets, capsules and soft gels.

Choice of HPMC or Gelatin Capsules
With multi-phase, multi-compartment capsules you are not limited to just gelatin (animal-based product) but have the option of natural HPMC (hydroxypropyl methyl- cellulose) and alternative capsule materials.

Better Visual Appeal
Multi-phase, multi-compartment capsules have none of the dust and residue associated with powder capsules. Better visual product appearance translates to higher perceived value.

Increased Absorption and Bioavailability
Liquids naturally offer faster and increased absorption and availability of active ingredients.

Increased Profit Potential
Add up all the advantages. Expect higher sales...and high margins!

Multi-Phase System
Compounds can be delivered with the most advantageous pharmacokinetic profile such as liquids and solids

Faster Development
Multi-phase, multi-compartment capsules reduce the development time compared to bi-layer tablets to get a new product into clinical trials faster.

Smaller Capsules
Hard-shell capsules have thinner wall construction, allowing them to contain more ingredient in a smaller capsule versus thicker-shelled soft gel capsules. Hard shells have faster and more complete dissolution than soft gels.

Less Odor and Less Irritation
Reduces unpleasant ingredient taste and odor commonly found with tablets and traditional capsules. And, liquids provide less irritation than traditional delivery methods.

Tamper Proof Sealing
Band sealing reduces tampering and provides a non-permeable barrier to retard oxidation and increase shelf-life.

Unique Appearance
This new delivery system stands apart from look-alike products that crowd retail shelves.

Compounds
Deliver Pharmaceutical, bio-pharmaceutical and nutraceuticals in a single dosage form.

InnerCap™ Technologies, Inc.

Patent Pending US-2005-0008690-A1

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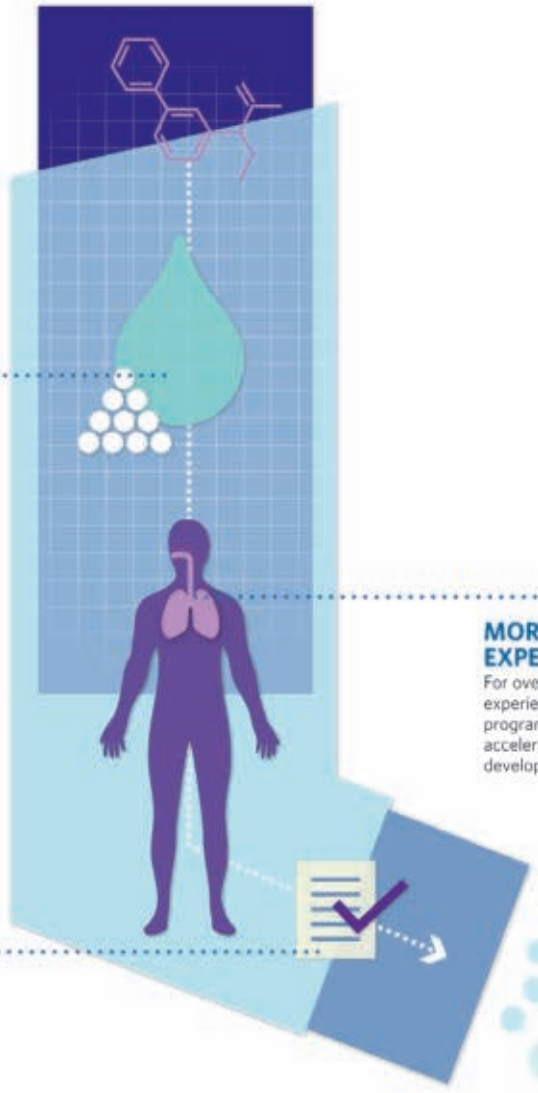

BROADEST DEVELOPMENT CAPABILITIES
With our complete range of dosage forms and comprehensive, integrated services, we create customized solutions that ensure superior results.

MORE INHALATION EXPERIENCE
For over 20 years, our deep experience and effective program execution have accelerated inhalation drug development time to market.

PROVEN TRACK RECORD
Our global project and supply chain management capabilities and proven regulatory expertise ensure high quality service and on-time delivery.

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