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VACCINE DEVELOPMENT AND MANUFACTURING 2017

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Making Vaccines Accessible

Agnes Shanley

Despite the challenges and high cost of development, vaccine innovation is at an all-time high, as new approaches aim to improve global access.

Vaccines make a huge contribution to improving the quality of human life. Over this decade, vaccines will prevent more than 23 million deaths worldwide, says Amy Finan, director of the Sabin Vaccine Institute, which focuses on improving access, as well as advancing basic research and innovation (see **Sidebar**). "That number can only grow if we continue to develop new vaccines and improve access to existing ones," she says.

Achieving this goal is far more challenging than it sounds, given the high cost and variability of vaccine manufacturing. But it remains crucial, since, every year, three million people still die from diseases that could be prevented by a vaccine, and over half of them are children under five years of age, according to the World Health Organization (WHO) (1).

Developing a vaccine the traditional way can take hundreds of millions of dollars and 5–12 years, says consultant James Robinson. According to the US Department of Defense, the 25-year lifecycle cost of a three-product vaccine facility is approximately $1.56 billion, and it usually requires seven years to go from design and build stages through commercial manufacturing (2).

Between 2000 and 2014, demand for vaccines has grown from $6 billion to $33 billion per year, according to the 2017 Access to Vaccines Index (AVI) (3). As global markets require larger quantities of workhorse vaccines and more new vaccines for unmet medical needs, reimbursement for vaccines has been depressed (4).

Vaccines for distribution to low-income nations are priced at the cents-per-unit level. Currently 65% of the pharmaceutical industry's vaccine production is sold to developed economies, 23% to upper middle income countries, 8% to lower middle income countries, and 4% to lower income nations, according to AVI.

Creative approaches

Manufacturers, including GlaxoSmithKline (GSK) (which bought Novartis' vaccines business in 2015), Merck, Pfizer, and Sanofi are using creative approaches and collaborating more actively with nonprofits and government agencies to bridge the supply vs. profitability gap, and to help improve access to vaccines. According to AVI, these four companies account for 80% of the global vaccine business (based on revenue).

Johnson & Johnson is another leading supplier (based on revenues), while Daiichi Sankyo and Takeda also maintain a strong market presence, according to AVI, which notes that the Serum Institute of India is a major manufacturer based on volumes produced.

Despite challenges, important breakthroughs have been made. The quadrivalent flu vaccine represented a major milestone when it first appeared, says consultant Robert Dream. In October of 2017, Bill Gates, co-chair of the Bill and Melinda Gates Foundation, said

INCREASING ACCESS AT THE MIDDLE LEVEL

Where the Vaccines Alliance (Gavi) works to ensure that low-income nations have access to vaccines, the Sabin Institute helps improve that access once nations move into middle-income status. Amy Finan, director of the Sabin Institute, discusses issues and progress.

PharmTech and BioPharm International (PharmTech/BioPharm): What progress has been made in improving overall vaccine accessibility?

Finan: Initiatives like Gavi (now known as the Vaccine Alliance), seek to create equitable access to vaccines by helping low-income countries introduce new vaccines. Gavi has helped developing countries avert nine million deaths since its inception in 2000. As countries transition away from Gavi support, Sabin is working with local decision makers to establish long-term immunization financing and policy solutions that will protect immunization systems for generations to come.

Over the past year, the global health community has come together to launch several new efforts to accelerate vaccine development. The formation of the Coalition for Epidemic Preparedness Innovations (CEPI) is dedicating global resources and attention to three diseases of epidemic potential, identifying new platforms to speed delivery to overcome the traditional development challenges. This is great news in the fight against CEPI's target diseases, but there are many more diseases that are significant global burdens and pose significant global threats that are not on their list.

The remaining challenge is to identify systemic solutions for developing vaccines against the bulk of diseases that lack commercial incentive. The newly-formed Bill and Melinda Gates Foundation's Medical Research Institute will no doubt accelerate the product pipeline for malaria and tuberculosis, as well as several diarrheal and enteric diseases.

In the public sector, FDA has introduced priority review vouchers to incentivize and accelerate vaccine development for historically overlooked diseases. All these efforts are tremendous, but they are still not enough. We must continue seeking solutions that incentivize investment in vaccine development for diseases that lack a commercial market.

PharmTech/BioPharm: Where do you see the greatest unmet need?

Finan: We need to improve early strategic thinking on vaccine development. Often, the need for vaccines is greatest in areas with limited health infrastructure. To make sure that we are developing vaccines that will be affordable for mass procurement, we must keep this in mind or we risk overlooking one of the greatest obstacles to meeting global immunization needs. We are challenged with not only expanding childhood immunization, but also adopting a life-course approach to vaccination in order to ensure that vaccines remain a part of healthy living throughout all stages of life. To meet this challenge, we must make certain that adolescent and adult vaccines are developed to meet the demands of these varied age groups.

Shifting our focus to consider, fully, the needs of older populations offers a cost-effective solution to fostering healthy populations across all ages. If we can adjust our strategic thinking accordingly, we will better be able to extend the benefits of immunization to all people.

PharmTech/BioPharm: What are your top goals for the Institute?

Finan: From supporting the introduction and uptake of specific vaccines, such as the new typhoid conjugate vaccine, to working with key decision makers to maintain immunization programs, we are focused on improving vaccine access and uptake. This includes everything from working with countries to take financial ownership of their immunization programs to supporting surveillance programs that provide much needed evidence to policy-makers to support national immunization decisions, as well as workshops on adolescent vaccine introduction, and more.

PharmTech/BioPharm: Where has the most progress been made?

Finan: Last spring, we welcomed Dr. Bruce Gellin to our team, who joined us after serving for 15 years as the deputy assistant secretary for health and as director of the National Vaccine Program Office at the US Department of Health and Human Services. He is expanding our efforts to champion sustainable, evidence-based solutions to prevent disease through vaccination and drive partnerships in the global health community.

The world has achieved new milestones for vaccine introduction. For every dollar spent on childhood immunization, there is a $16 return on investment. As of 2015, 99 low- or middle-income countries have introduced one or more new vaccines. These countries have introduced a total of 160 vaccines, which have contributed to the global decline of mortality for children under five, helping to bring under-five deaths down from 85 to 38 per 1000 live births.

There has been notable progress in developing malaria, dengue and HIV vaccine candidates. Now we need to expand coverage for existing vaccines and support the introduction of new vaccines. Vaccines are among the greatest public health achievements in history. Childhood vaccination alone saves up to three million lives every year. We can't afford not to continue to build on this success.

that efforts to eradicate polio through vaccination had already largely succeeded (5), two years ahead of schedule. In addition, a number of significant vaccines have been introduced recently, including:

- Bharat Biotech's Typhar-TCV, a conjugate vaccine against typhoid fever that can be given to children as young as six months old
- Sanofi's dengue fever vaccine, Dengvaxia (or CYD-DTV)
- GSK's Shingrix, a vaccine for shingles
- GSK's Mosquirix (or RTS,S), the world's first malaria vaccine, which was approved by the European Medicines Agency in 2015, and will undergo real-world testing in 2018.

With its new dengue fever vaccine, Sanofi broke with industry tradition and registered the product first in regions with the greatest need (e.g., Latin America and Asia) rather than those countries with the most stringent regulations (i.e., the United States, Europe, and Japan), according to AVI. This step may seem like a small change, but is expected to help speed access.

Improving existing vaccines

Roughly half of the vaccine R&D projects at Big Pharma companies now center around reformulation, according to the AVI. One major focus is improving temperature stability, to allow vaccines to be transported more easily to more remote areas with high ambient temperatures.

Merck took this approach with its human papilloma virus (HPV) vaccine Gardasil, in 2015 and received WHO's cold temperature chain (CTC) certification for the vaccine (6).

GSK is currently working on characterizing the thermostability of its pneumococcal conjugate vaccine, Synflorix, and Sanofi is doing the same for its cholera vaccine, Shanchol, according to AVI. The Gates Foundation has also funded research into making the adjuvant for GSK's malaria vaccine stable for three years at temperatures up to 30 °C.

Stability is also the goal of freeze-dried vaccines, such as the meningitis vaccine MenAfriVac, as well as a smallpox vaccine being developed by BARDA and Bavarian Nordic, a freeze-dried version of a vaccine that uses Bavarian Nordic's modified vaccinia Ankara (MVA) platform.

MVA and other platforms (see feature, p. 11) are also being evaluated for use in vaccines that could provide immunity against multiple diseases, and reduce the number of inoculations needed. GeoVax, for example, is using recombinant MVA to express antigens on virus-like particles (7).

Reducing fixed costs

Advances that improve manufacturing flexibility also address the gap between growing demand and low profitability by reducing fixed costs, the largest component in vaccine manufacturing economics (8). Traditional grass-roots facility costs can range from $50 million to $500 million per antigen, and up to $700-million for multiple vaccines, while cGMP space may run to $600 million per ft^2 and non-GMP space, to $350 per ft^2 (9). Some of these costs are shown in **Table I**.

Streamlined manufacturing solutions include facilities designed for quick installation and deployment, driven by research at the Defense Advanced Research Project Agency (DARPA) and at BARDA. Examples would be found at the Center for Innovation in Advanced Development and Manufacturing (CIADM), at Texas A&M. They involve the use of modular enclosed systems, featuring greater levels of automation and the use of isolators.

Closed aseptic manufacturing processes (see feature on p 31) would allow aseptic fill/finish operations to

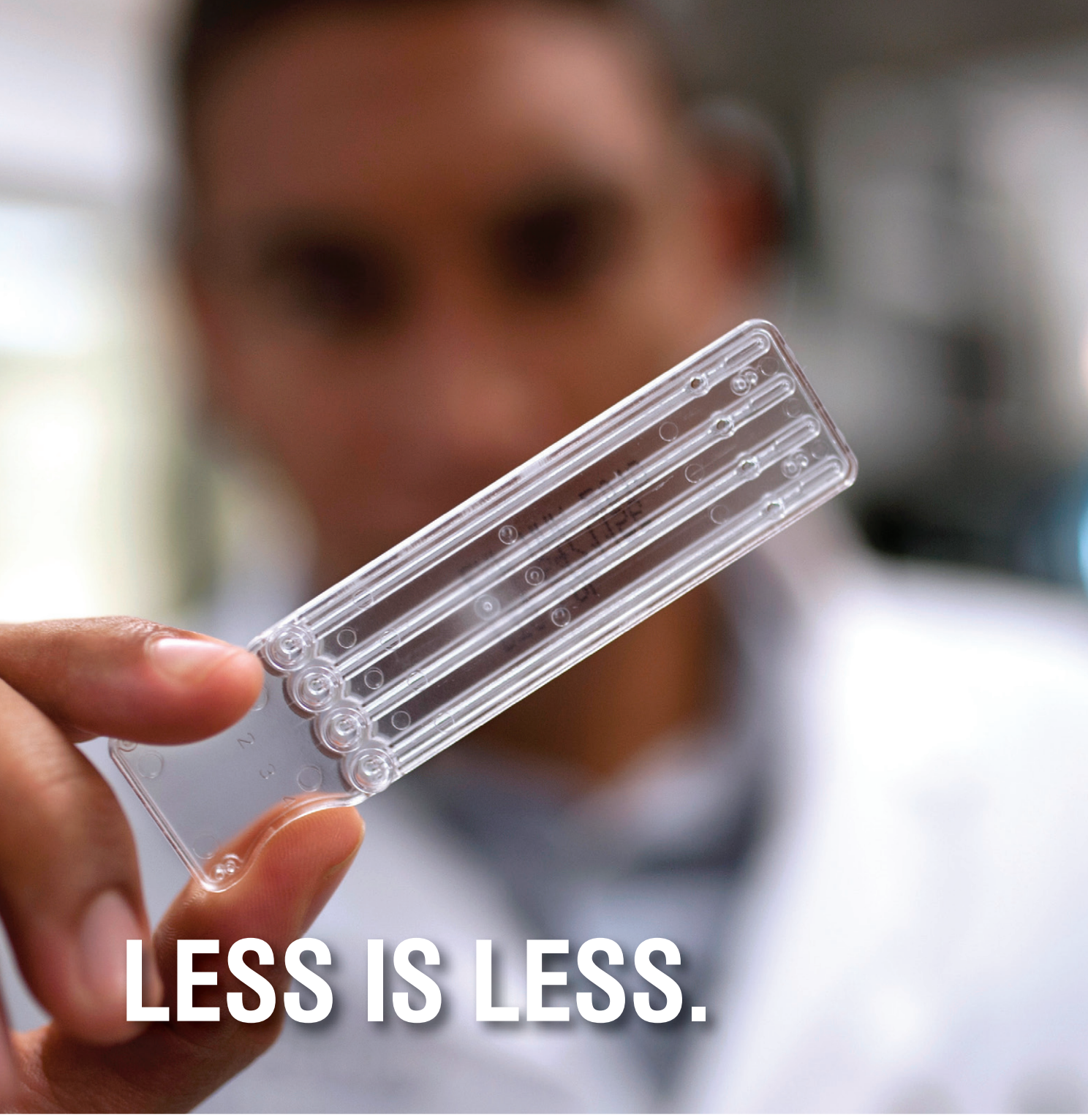

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Table I. Vaccine manufacturing costs

Facilities and R&D account for most of the spending on vaccine development and manufacturing	
• Fixed (i.e., product development, facilities and equipment, third-party financing and grants) • Variable (i.e., consumables, raw materials, biological and chemical agents, vials, stoppers and seals, labels cartons, and quality-control testing kits) • Semi-variable (direct labor) • Mixed (overhead, commercialization, and licensing costs)	
R&D	$500 million ($135 to $350 million, when adjusted for risk)
Facilities	$50 to $700 million
Direct Labor	Less than 25% of total manufacturing costs
Overhead	Up to 45% of the costs of raw materials and labor combined
Licensing	WHO site audit fee: $30,000
Traditional vaccine fees	
Evaluation: $35,000 to $100,000 Annual: $4800 to $140,000	
Complex or novel vaccine fees	
Evaluation: $69,500 to $232,800 Annual: $8400 to $250,000	

Sources: (8) Production Economics for Vaccines, a guide released by the Bill and Melinda Gates Foundation in 2016, and (9) S. Plotkin, et al., "The Complexity and Cost of Vaccine Manufacturing," *Vaccine 35* (2017), 4064.

take place in unclassified environments, eliminating the need for expensive cleanrooms. At the same time, single-use technology is being used to reduce vaccine manufacturing costs in platforms such as GE's FlexFactory and Novavax's platform (10). GSK has been using single-use technology widely in vaccine manufacturing for close to a decade (11). The resulting facilities can be brought online quickly, with lower HVAC requirements and without the need for water for injection (WFI) and clean steam systems, allowing for substantial capital and operating cost savings.

Shrinking and declassifying processing areas

"The use of closed systems has the potential to shrink and declassify production environments, with significant decrease in facility costs," says Robinson. To date, however, most large companies have not yet taken advantage of this technology for vaccine manufacturing, he says.

Innovation is also being seen in the drug substance area, says consultant Robert Dream. Sanofi, for example, is working on continuous processes for cell- and microbe-based vaccines. "We're starting to see an overhaul of vaccines, as manufacturers develop more efficient approaches that promise to make vaccines faster, in a more sustainable and cost-effective way," he says.

Collaboration and new models

Public-private coalitions are playing a much stronger role in vaccine development and distribution. WHO has established a Global Vaccine Action Plan to improve access to vaccines, and boost immunizations. The Vaccines Alliance (Gavi) has been taking the lead in ensuring funding while the Gates Foundation, through agencies such as PATH, has been stimulating development and tech transfer to bring safe, inexpensive vaccines to people in low-income nations. In 2017, the Gates Foundation gave PATH a $120-million grant to establish a Center for Vaccine Innovation and Access, to drive 30 different projects with the goal of eradicating 17 diseases through immunization.

Collaborative models that have led to success in the past have provided templates for current and future work. One example is the African meningitis vaccine MenAfriVac, which was developed by the Meningitis Vaccine Project (MVP) in a venture that involved 13

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The vaccine industry is challenged to produce large quantities of vaccines in a rapid and cost-effective way. Major changes to current bioprocesses are both difficult and very expensive to implement. Using HEK293 it has been demonstrated that addition of recombinant human insulin to commercially available chemically defined media, can be used as a supplement to increase VCD and specific viral yield.

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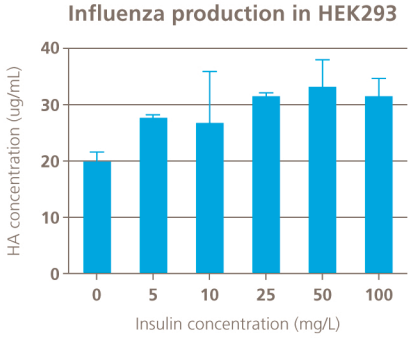

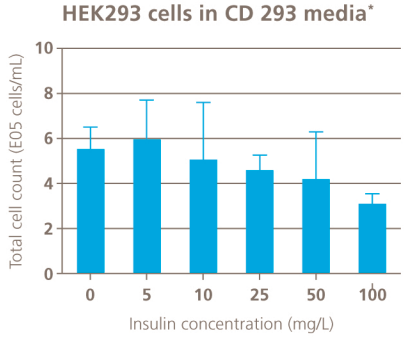

*Data kindly supplied by Aziza Manceur, National Research Council Canada. Hemagglutinin (HA) assay is used for quantification of Influenza (H1N1). Insulin Human AF used in the experiments is supplied by Novo Nordisk Pharmatech. CD 293 media is trademark of Thermo Fisher Scientific.

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African nations, WHO, and PATH, and was funded by the Gates Foundation. MVP wound up becoming a virtual company (12), outsourcing manufacturing and tech transfer.

At first, the project sought pharmaceutical company partners, but could not reduce price below $2 per dose. Eventually, MVP collaborated with SynCo Bio in the Netherlands, which supplied the starting material and process and transferred fermentation and purification knowhow to Serum Institute of India, which manufactured the vaccine for $0.50 per dose, one-tenth the cost of the average new vaccine. In polio vaccine manufacturing, Gates Foundation-funded programs have led to significant reductions in costs, says Robinson.

Collaboration will also drive the next steps in launching GSK's malaria vaccine, which, according to AVI, took $695 million and 28 person years to develop. The company is now working with the PATH Malaria Vaccine Initiative, with funding from the Gates Foundation and other nonprofits, to start testing the vaccine in target areas in 2018. The current goal for the vaccine's eventual price will be manufacturing costs plus 5%, with any profits being invested in the next generation of vaccines, according to AVI.

Ebola and Zika as wake-up calls

Recent Ebola and Zika outbreaks have spurred efforts to improve pandemic readiness. The Coalition for Epidemic Preparedness Innovations (CEPI) was established in 2017 to set new goals for advancing vaccines for underserved markets, including MERS and Lassa fever vaccines. CEPI supports development of platforms that would allow manufacturers, and regions, to respond more rapidly to unknown threats, Robinson says.

In the end, the most fundamental challenge to vaccine development is variability, due to the "nearly infinite combinations of biological variability in basic starting materials, the microorganism itself, the environmental condition of the microbial culture, the knowledge and experience of the manufacturing technician, and the steps involved in the purification process." (9)

Although quality-by-design-type methods cannot be applied directly to vaccines, research is looking into ways to make vaccine development more systematic. Including studies of a number of recombinant approaches for making proteins, particles, and nucleic acids coded for target proteins (RNA and DNA), as well as synthetic approaches, "Innovation is probably at an all-time high," says Robinson.

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Accelerating Vaccine Development and Manufacturing

Cynthia Challener

The use of approved platform technologies can reduce the time and cost required to generate new vaccines.

Established vaccine manufacturing technologies, whether for viral, microbial, recombinant protein, or polysaccharide vaccines, are complex, often highly customized, and typically performed in dedicated facilities. Development and approval times are therefore lengthy, and the entire process is costly. Rapid response to pandemic diseases and implementation of vaccine manufacturing in developing economies is a challenge. Standardization, or the use of vaccine manufacturing platforms that can be deployed across different vaccines and vaccine types, could ameliorate this situation.

Limitations of existing vaccine technologies

Unlike biologic drugs based on recombinant proteins, the biological compounds used in most vaccines are not fully chemically and physically characterizable using existing analytical methods, says Thomas Lingelbach, CEO of Valneva. "As a result, the manufacturing process itself is part of the licensure and must be fully established before a vaccine producer can seek regulatory approval," he observes.

In addition, some delays to clinical study starts can be attributed to aspects of the product development cycle, such as long lead times to develop a product-specific cell line, process improvements for enhanced yield and purity, formulation studies for improved stability, and analytical method development for release and product characterization, according to Tim Hahn, senior vice-president for global manufacturing operations with Novavax.

The manufacture of vaccines in large-scale, stainless-steel facilities also has its limits. "The construction and validation of very complex facilities increases the time-to-market schedule and the environ-

Cynthia Challener is a contributing editor to *Pharmaceutical Technology* and *BioPharm International*.

mental footprint, which adds to their already high CAPEX," says José Castillo, CTO of Univercells. He adds that the most widely used methods (roller-bottle, eggs) still require large workforces and large numbers of manual operations, creating possible risks in variability and contamination, and inducing the need for highly complex, lengthy, and expensive quality control processes. While single-use technologies have led to more flexible facilities with lower investment and production costs, their scalability is limited, according to Castillo.

Aside from direct development and manufacturing challenges, biological manufacturing consultant James Robinson notes that because vaccines against infectious diseases are generally administered to healthy individuals, and very often at young ages to prevent disease, safety is of utmost importance for their approval for widespread use. During clinical phases, the process is developed, optimized, and validated; analytical methods are developed and validated; production starting materials are produced and validated (master seeds, cell banks, etc.); and production facilities are designed, built, commissioned, validated, and used for commercial launch lots. This traditional approach can take many years, and although it does allow safety to be established, the process is not amenable to rapid development and validation of products to protect against an unknown epidemic threat.

Johan Van Hoof, global head of Janssen Vaccines & Prevention, part of the Janssen Pharmaceutical Companies of Johnson and Johnson, adds that, historically, vaccines were developed based on the virus itself; for viruses such as Ebola and human immunodeficiency virus (HIV), however, killed virus-based vaccines have not worked and live attenuated approaches have been considered too risky. "There is a need for new delivery platforms as a result," he says.

There are also further issues besides production complexity that need to be addressed, according to Van Hoof, and not just by the companies that develop vaccines, but also more widely by society and governments. "There is a collective need to think through what can be done differently in order to maintain the highest of safety standards while also optimizing our vaccine delivery to societies the world over."

Platform technologies developed based on time-tested methodologies and expertise that can demonstrate the ability to quickly and reliably produce consistent results can help reduce time, costs, and variability.

Advantages of a platform approach

Platform technologies developed based on time-tested methodologies and expertise that can demonstrate the ability to quickly and reliably produce consistent results can help reduce the time, costs, and variability that may otherwise hamper vaccine production, according to Sean Marett, chief operating officer for BioNTech. Kathleen Hefferon, a professor at the University of Toronto adds: "Platforms such as plant-based technologies are much less expensive to produce, are easy to upscale, and lack many of the complex purification procedures associated with mammalian cell cultures."

"Once a vaccine production platform is developed such that a new product could be made using the same manufacturing process and starting materials (cell lines, facilities, analytics), the manufacturing and quality control elements of this development can be expedited, and clinical trials may advance more quickly (up to 50% time savings in facilities alone) as products made from a proven platform may be perceived to have less risk from a safety perspective," Robinson explains.

In addition, Castillo notes that the development of more affordable and flexible manufacturing platforms would allow smaller players to enter the market, answering the growing need for quality vaccines, potentially coupled with local manufacturing.

Robinson further notes that platform processes that use a "plug-and-play" approach—change the starting material, but keep the process the same and still get the target product—are ideal platforms for rapid response. However, any need to further develop/optimize the process for target product takes time and additional validation, making it less ideal.

Many different platforms

A number of different platform technologies are currently under development for the production of vaccines. Many involve cell culture using a variety of cell substrates, including bacterial, plant-, and insect-based systems. Modern permanent cell lines, according to Lingelbach, allow the growth of viruses in chemically defined media under clearly characterized and reproducible conditions, affording both enhanced safety and quality. While switching to cell-culture production for existing vaccines is limited due to the effort required to demonstrate clinical equivalence, many new vaccines are being developed on modern cell substrates.

Castillo notes, however, that "the introduction of Cytodex (GE Healthcare Life Sciences) technology allows viral production on adherent cell lines in bioreactors and is a significant step toward disruptive improvement of vaccine manufacturing." He adds that fixed-bed bioreactors further improved on this technology by increasing cell-culture density. "Viral vaccines manufactured on classical technologies (roller bottles) can be easily adapted to such cell-based systems, which allows significant manufacturing improvement for existing vaccines. This can be seen as a path toward more affordable manufacturing of viral vaccines," he asserts. Univercells' cell-based platform integrates this high-density cell culture with in-line purification.

Hahn believes that an insect cell/baculovirus expression system platform is the most promising. "Through the use of this system, proteins are properly folded and acceptably glycosylated. The system also allows the use of a single master cell bank, and most importantly, it provides for the use of a template manufacturing process and analytical methods," he comments.

Hefferon develops plant-based vaccines, which she says are advantageous because they are less costly, easy to scale up, and free from human pathogens. They are unconventional, however, and can suffer from public perception issues (concern about genetically modified organisms). It can also be difficult to produce some proteins in plants, and yields can be low in certain instances. Recent advances in virus expression systems that can be easily delivered to plant hosts are helping address these issues.

Virus-like particles (VLPs) and enveloped-VLPs (eVLPs) are also promising to Hefferon. VLP and

Examples of platform technologies for vaccine manufacturing

BioNTech manufactures mRNA encoding antigens or neoepitopes for its cancer vaccines. mRNA lends itself to a vaccine technology because of its versatility, low manufacturing cost, and intrinsic immunostimmulatory (innate immune response) properties, according to BioNTech's Chief Operating Officer Sean Marett. BioNTech is using its mRNA to develop several cancer vaccine immunotherapy approaches from off-the-shelf vaccine immunotherapies that use larger batch sizes, as well as bespoke single-batch manufacturing for the individual patient based on his or her cancer genome.

mRNA encoding antigens

The company's mRNA vaccine approach includes a self-amplifying RNA-based amplicon vaccine platform. The platform has high flexibility and can be adapted to rapidly evolving viral strains. In addition, this synthetic RNA has short manufacturing times at low costs, according to Marett.

A key challenge with mRNA-based vaccines is delivery to the target cells, the dendritic cells, to trigger an appropriate immune response. BioNTech initially used an intra-nodal formulation of its mRNA vaccines and demonstrated early evidence of clinical effect in cancer using this formulation. Recently, the company completed development of a proprietary intravenous formulation that is universally applicable to mRNA vaccines targeting dendritic cells systemically, according to Marett. The formulation is straight forward to manufacture and can be conveniently administered to the patient to create the desired immune response.

Transient gene expression in unmodified (non-GMO) green plants

iBio Technology uses proprietary, transient gene expression in unmodified (non-GMO) green plants. The company says its short gene sequence-to-manufacturing timeline of as little as one month makes the technology an ideal manufacturing platform for rapid response to pandemic threats (1). In addition, because the growth of plants and the method for introducing the iBio Technology vectors are the same regardless of the desired product, a facility using the iBio Technology system can produce multiple proteins without the need for physical reconfiguration. iBio has demonstrated the feasibility of its plant-based expression platform for the production of vaccines against a range of viruses, bacteria, and parasites, including vaccine components that could not be manufactured using bacterial, yeast, or animal cell expression systems.

Adenoviral vector delivery

Johnson & Johnson's AdVac adenoviral vector delivery platform consists of specific low prevalaent serotypes of adenoviruses (a type of common cold virus) that have been modified to render them replication incompetent. This means the AdVac vector cannot replicate in the body after being administered. A certain part of the target virus for vaccination is added into the vector, aiming to induce both humoral and cellular immunity against the target pathogen, according to Johan Van Hoof, global head of Janssen Vaccines & Prevention, part of the Janssen Pharmaceutical Companies of Johnson and Johnson.

The company's cell-based production platform, the PER.C6 cell line, is based on an immortalized retina cell that has been genetically modified to enable its replication using recombinant DNA technology. During the past two years, Johnson & Johnson has produced close to 200 vaccine batches on the PER.C6 platform, with capacities ranging from 10L to 1000L in scale. The platform is being applied in the company's respiratory syncytial virus, HIV, and Ebola vaccine programs.

Insect cell/baculovirus expression systems

Novavax uses the insect cell/baculovirus expression system platform. In addition to properly folding complex proteins, a key advantage to this platform is the rapid timeline from discovery to clinical trial, according to Tim Hahn, senior vice-president for global manufacturing.

For example, Novavax was able to develop, manufacture, test, and release a glycoprotein nanoparticle Ebola vaccine within four months from gene sequence identity to GMP release. This achievement was preceded by the production of a clinical batch

eVLP technologies benefit from high product safety, according to Castillo, which can be reflected in the facility design, particularly with respect to containment and environmental risk limitation. "However," he says, "these novel products show limited or modified immunogenicity and thus reduced efficacy, which when associated with the lower productivity inherent to this type of vectors, causes limitations in production capacity."

DNA/mRNA systems have the potential to be truly universal vaccine manufacturing platforms, with only slight modifications of regions or epit-

of a vaccine candidate against the avian influenza A/Anhui/1/2013 (H7N9) virus in three months based on a flexible and agile VLP vaccine manufacturing platform. "With this platform process, the timeline for commercialization is largely limited by the clinical and regulatory plan," Hahn asserts.

Protein Sciences, which was acquired by Sanofi in August 2017, also uses baculovirus expression system to produce vaccines. Its Flublock vaccine was the first recombinant protein vaccine to receive FDA approval. The company also manufactures a recombinant GAD-protein, the main component in the diabetes vaccine Diamyd from Diamyd Medical, which is undergoing multiple clinical trials (2). The Baculovirus Expression Vector System generates large quantities of desired recombinant proteins, and the company says its Spodoptera frugiperda insect (expresSF+) cell line has been optimized to do so more quickly and less expensively than other production systems (3).

Univercells' cell-based technology involves densification of the cell culture and purification using a new type of single-use, high-density, fixed-bed bioreactor operated in perfusion mode integrated with cutting-edge, high-performance chromatography membranes, allowing a sequential-continuous purification process, according to the company's Chief Technology Officer, José Castillo. "This intensified and integrated manufacturing process leads to a low-footprint process that can be accommodated in an isolator, which has a tremendous impact on factory design. It is also flexible and designed to manufacture any type of viral vaccine at a very low cost, making it attractive for production of vaccines in emerging countries," he observes. First feasibility studies will take place in 2018, with large industrial deployment expected to follow.

Cell-culture based platform

Valneva's cell-culture based platform includes the widely adapted EB66 cell line, which serves as an alternative for the cost-effective manufacturing of vaccines currently produced in eggs or primary chicken embryo fibroblasts. With this cell line, proliferation occurs in suspension in stainless-steel and single-use bioreactors at high cell densities in chemically defined media. Valneva has generated fully controlled GMP EB66 cell banks, and a biologic master file describing the history, traceability of raw materials, and results from extensive quality controls was filed in 2008 with FDA. The cell line is licensed to the majority of players in the human and veterinary vaccine industry, according to CEO Thomas Lingelbach.

In July 2017, the company granted Emergent BioSolutions exclusive worldwide rights to its Zika vaccine technology ZIKV. Valneva and Emergent will co-develop ZIKV-VLA1601, a highly purified inactivated vaccine candidate against the Zika virus, which was successfully developed by Valneva using another of its established manufacturing platforms—an inactivated platform used to produce the licensed Japanese Encephalitis vaccine IXIARO/JESPECT (4).

eVLP Platform

VBI Vaccines' eVLP Platform allows for the design of enveloped vaccines with structures that closely mimic those of enveloped viruses, but without the viral genome, potentially yielding safer and more potent vaccine candidates. The technology is flexible, allowing VBI to rationally design preventative or therapeutic vaccine candidates by controlling the expression of both surface and internal target proteins of interest, according to the company. Its lead candidate is a vaccine to prevent cytomegalovirus infection, which is in Phase I. Other preclinical- and discovery-phase candidates include vaccines targeting glioblastoma multiform, medulloblastoma, Zika virus, and respiratory syncytial virus.

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opes in the expression system required to produce a wide variety of vaccine types. They are also fully characterizable using existing analytical techniques. "This technology will be a game-changer in the world of vaccines," Lingelbach states.

Marett considers mRNA technology to be among the most promising approaches. "The mRNA technology itself is highly versatile and hence applicable in many different areas, from cancer and infectious disease, to protein replacement therapies. mRNA can also be used to encode bi-specific antibodies without the need for time-consuming

and costly cell-line development and purification methodologies typically seen with protein-based antibody approaches," he says.

Achieving technological advances is not the only challenge on the road to commercialization of new vaccine production platforms.

Any recombinant expression system that can rapidly produce and release a master seed from pre-certified sources and then produce a competent immunogen using a standard/fixed process could be a useful platform process, according to Robinson. "In many cases, it is not the generic platform that matters (insect cell vs. plant cell vs. transfected cell line). But instead, it is important that there is established safety of the starting materials, the experience to reliably and quickly produce high-yielding starting materials (master seeds/cell lines), to rapidly execute the production in a pre-defined, high-yield process (not optimized for each new target), and then release product by established analytical methods, perhaps only changing the potency assay of the final product. If the platform has been used to support a previously licensed product, it has an advantage over one that is first-in-class, as regulatory agencies would also be more familiar with the technology, the product safety profile, and the production and testing methods," he explains.

Road to commercialization

Some of these technologies have already been used to produce approved vaccines, while others are still in clinical development. Novartis received FDA approval for its Flucelvax influenza vaccine produced using cell-culture technology in 2012 (1). The insect cell/baculovirus expression system is also used for licensed products, such as Cervarix from GlaxoSmithKline and Flublok from Protein Sciences (recently acquired by Sanofi).

Johnson & Johnson's swift response to the Ebola crisis in West Africa was based on its delivery and production platforms, according to Van Hoof. In 2014, when the World Health Organization (WHO) declared a public health emergency, Johnson & Johnson had an Ebola vaccine candidate in development based on AdVac technology from Janssen, while Bavarian Nordic A/S was working on an Ebola vaccine based on its MVA-BN technology (2,3). "At the time, we had just discovered that the combination of the two vectors gave superior immunity and protection. Although we had not produced a single clinical trial batch, by leveraging our PER.C6 line for AdVac production and in collaboration with Bavarian Nordic A/S, within one year we had produced two million vaccine regimens that remain at our disposal today for pandemic preparedness efforts," Van Hoof says. The PER.C6 line is also being used for the production of REKOVELLE (follitropin delta), a human recombinant follicle stimulating hormone for use in controlled ovarian stimulation for women undergoing assisted reproductive technologies, for which the European Commission granted marketing authorization to Ferring Pharmaceuticals in December 2016 (4).

DNA/RNA vaccines are in late-stage clinical development and could be available in a couple of years. BioNTech is currently using mRNA technology to develop and commercialize therapeutic cancer vaccines, and was one of the first companies to build and establish a proprietary mRNA platform

with an in-house GMP production facility to produce clinical-grade mRNA-based cancer immunotherapies, according to Marett. The company is adding a second facility that will focus on providing bespoke manufacturing of single clinical batches for each patient, based upon the unique genomic profile of the individual cancer patient's tumor. It has also begun, in collaboration with Siemens, to develop a fully automated, paperless, and digitalized commercial cGMP-production process for commercial manufacturing, according to Marett.

Achieving technological advances is not the only challenge on the road to commercialization of new vaccine production platforms. "Acceptability by key biopharmaceutical players will drive the emergence of such technologies. Support from regulatory agencies will also help bring innovation in this area. It is therefore necessary to enhance the acceptability of these new platform technologies by regulatory agencies in order to ensure they will reach commercialization and that the engaged development costs can be recovered," Marett asserts. "Required efforts should target increasing awareness of the technologies and their benefits, facilitating the adoption process by key industry players, and participation in the redaction of FDA/European Medicines Agency (EMA)/WHO guidelines," he adds.

Focusing on diseases common in developing countries would be beneficial, according to Hefferon. "Companies are less likely to develop unconventional vaccines that must compete with the gold standards that are currently on the market. Platform-technology vaccines could fill in some niches that have been overlooked," she notes.

The formation within the past 18 months of the Coalition for Epidemic Preparedness Innovations (CEPI) is a key development for new vaccine commercialization, according to Gunnstein Norheim, acting director of the group's vaccine science team. "This group was formed as an outcome of lessons learned from prior epidemic disease responses and has taken expert advisors from industry, academia, and regulatory agencies to understand and help mitigate the obstacles for rapid response to epidemics as well as facilitate preparedness for known threats of infectious disease," Norheim says. Two requests for proposal are underway, one to advance programs for three target diseases (MERS, Lassa Fever, and Nipah virus) on the WHO priority pathogen list and the second to advance production platforms that can respond quickly to newly emerging pathogens.

"Many of these diseases affect poor countries, and the incidence can vary widely over time, making them challenging targets for companies that operate for profit. The funding of these programs helps to remove that obstacle and reduce the risk of taking on the development of the products," he adds. CEPI has raised more than $600 million to fund these and other initiatives, with the goal to raise $1 billion for the next five years of program development. The leadership has come from Wellcome, the Bill & Melinda Gates Foundation, and the governments of Germany, Japan, and Norway (central to managing the Ebola response in 2014–2015).

Role of academia

Academic collaborations and private-public partnerships are crucial to optimizing vaccine development and large-scale access, according to Marett. Academic centers have the capacity to pursue basic research that can translate into clinical advances. "It is a well-understood paradigm that larger enterprises struggle to achieve really

breakthrough innovation, and that today most innovation comes from academia or small innovation incubators. The best system has academia pursuing really basic research and basic ideas and smaller companies doing translating those academic ideas into industrializable concepts," adds Lingelbach.

Academic researchers should, according to Marett, be encouraged to integrate small-scale industrial technologies (laboratory-scale bioreactors) during the discovery phase of novel products, ensuring smooth production scale-up without risk of product alteration. "Such efforts could be achieved by strengthening the bridge between academic research centers and industry players to facilitate the translation of innovative technologies into commercial products," he remarks.

Norheim agrees that much innovation starts in academic research centers, but they often lack the expertise to advance development and achieve commercialization of products. "The goal of CEPI is to help facilitate partnerships between these innovation centers and firms with the capabilities to navigate the clinical and regulatory pathways, as well as to facilitate access to production capabilities for creating clinical supplies and eventually stockpiles in preparation for outbreaks that many believe are just a matter of time to occur. There are many groups engaged already, and our goal is to see multiple successes, as there are many targets to address and having multiple approaches increases our odds of success. Key to reaching CEPI's goals is to link the full body of academic research on vaccines and disease protection with the vaccine industry and end users in affected countries," he states.

One example of a valuable group, according to Hahn, is the Macromolecule and Vaccine Stabilization Center (MVSC) at the University of Kansas. "Led by Dr. David Volkin, this organization is advancing the rapid development of product formulations to stabilize recombinant protein products, work that is particularly beneficial to start-up biotechnology companies that need the technology but may not be able to do the work on their own," he says.

Academic collaborations and private-public partnerships are crucial to optimizing vaccine development and large-scale access.

BioNTech closely interacts with national and international research institutes in Europe (such as TRON, the German Cancer Institute, Cancer Research UK) and scientific networks (such as CI3 and the Association of Cancer Immunotherapy), and also collaborates with universities and medical centers (Gustave Roussy in France, Vrije Universiteit Brussel in Belgium, University Hospital of Zurich in Switzerland, Uppsala University in Sweden, Southampton University Hospital in the UK, Heidelberg University Clinic in Germany). In the United States, the company is one of two European members of the Tumor neoantigEn SeLection Alliance (TESLA) headed by the Parker Institute for Cancer Immunotherapy and the Cancer Research Institute (CRI), a network that includes 30 of the world's leading cancer neoantigen research groups from both academia and industry.

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Importance of outside funding sources

Funding by groups outside of the vaccine industry is crucial for furthering the development of new vaccine platforms. Recently, according to Thomas Lingelbach, CEO of Valneva, foundations have come to understand that in addition to providing infrastructure for vaccine production and delivery in developing economies, investments in translational research and the industrialization of basic research is important for turning new ideas into product candidates, then clinical candidates, and ultimately actual products.

The Grand Challenge and similar programs are seeking to support significant changes and breakthrough technologies that will have large-scale impacts on global health, according to Gerard Cunningham, a principal with Innovations for Global Health. "The primary goal," he continues, "is to encourage entities to test and develop technologies that represent a major change for some aspect (e.g., cost, novel delivery systems, duration of response) of global health. One area of focus has been supporting the development of novel low-cost technologies for producing vaccines and therapeutics. It is anticipated that Grand Challenges and related programs will continue to help facilitate technological leaps that will enable faster, cheaper, and more effective interventions for future epidemics."

"Programs like the Grand Challenge from the Bill & Melinda Gates Foundation are immensely important to the development of outstanding technologies in commercial applications that may not otherwise advance," agrees Tim Hahn, senior vice-president for global manufacturing operations with Novavax. These types of grants enable the development of innovative platforms and technologies by supporting the R&D costs and reducing associated development and commercial risks, adds Sean Marett, COO for BioNTech. The sponsors often then serve as recognized prescribers, facilitating technology adoption and paying attention to the implementation of the platforms and ensuring they reach their intended objectives.

For Johnson & Johnson, according to Johan Van Hoof, global head of Janssen Vaccines & Prevention, a key learning from the 2014 Ebola outbreak in West Africa was that accelerating vaccine development requires partnerships and collaborations across all stakeholders, including vaccine manufacturers, non-governmental organizations, and governments. Solutions to challenges like HIV and Ebola will not come from one organization but from true team work. "We have established highly valued partnerships with many leading supporters of vaccine development, including the National Institutes of Health, the DoD [US Department of Defense], BARDA [the Biomedical Advanced Research and Development Authority, part of the US Department of Health and Human Services], and Europe's Innovative Medicines Initiative (IMI). The Gates Foundation is a great supporter of the work we do at Janssen, providing support with efforts such as our goal to develop and produce a low-cost inactivated polio vaccine. Janssen also strongly supports new cross-sector initiatives to improve the world's pandemic preparedness, such as CEPI," he comments.

In April 2017, the University of Kansas, the Massachusetts Institute of Technology, and University College London received a $17.6-million, five-year grant from the Gates Foundation to support the Ultra-Low Cost Transferable Automated (ULTRA) Platform for Vaccine Manufacture initiative, which is focused on developing and producing a low-cost vaccine manufacturing platform for the production of vaccines targeting diseases such as hepatitis B, HIV, human papillomavirus, malaria, and rotavirus in developing countries.

In December 2016, Univercells received a $12-million Gates Foundation grant to develop an intensified continuous vaccine manufacturing platform. Univercells is collaborating with Natrix and Batavia, who will respectively provide a single-use chromatography membrane platform and vaccine development and manufacturing capabilities.

Therapeutic Vaccines Target Cancer and Other Viral-Induced Diseases

Feliza Mirasol

The use of therapeutic vaccines presents a new way to manage diseases, such as cancer and sexually transmitted diseases.

As research into harnessing the immune system's natural ability to fight diseases advances, so does the development of therapeutic vaccines. Similar to traditional vaccines, therapeutic vaccines are used to stimulate the immune system against an infection. Unlike their traditional counterparts, however, therapeutic vaccines are used to treat disease (active immunotherapy), rather than as a prophylactic (1,2). Much of the attention in therapeutic vaccine R&D is aimed at targeted therapies for cancer, or cancer vaccines, but there is also substantial research going into therapeutic vaccines for other indications, particularly sexually transmitted diseases (STDs) (1).

Cancer vaccine developments

Cancer vaccines that aim to treat the disease in late-stages provide a new method for managing cancer. Beyond that, they open the path for a way to rationally design and optimize future vaccines with improved anti-cancer efficacy, as evidenced by the significant number of vaccine strategies being evaluated preclinically and clinically (2).

The approval of Dendreon's Provenge (sipuleucel-T), a cellular immunotherapy product, in April 2010 marked the first approval of a therapeutic cancer vaccine in the United States (3). The therapy is indicated for treating prostate cancer and is designed to be an active cellular immunotherapy, which stimulates a patient's own immune system to target and attack prostate cancer cells (4). Since then, the arena of cancer vaccine development has progressed. Over the past three to four years, there has been increased attention around what are known as checkpoint inhibitors, for example.

Checkpoint inhibitors are a form of passive cancer immunotherapy, according to Dr. Geert Mudde, chief scientific officer at OncoQR ML,

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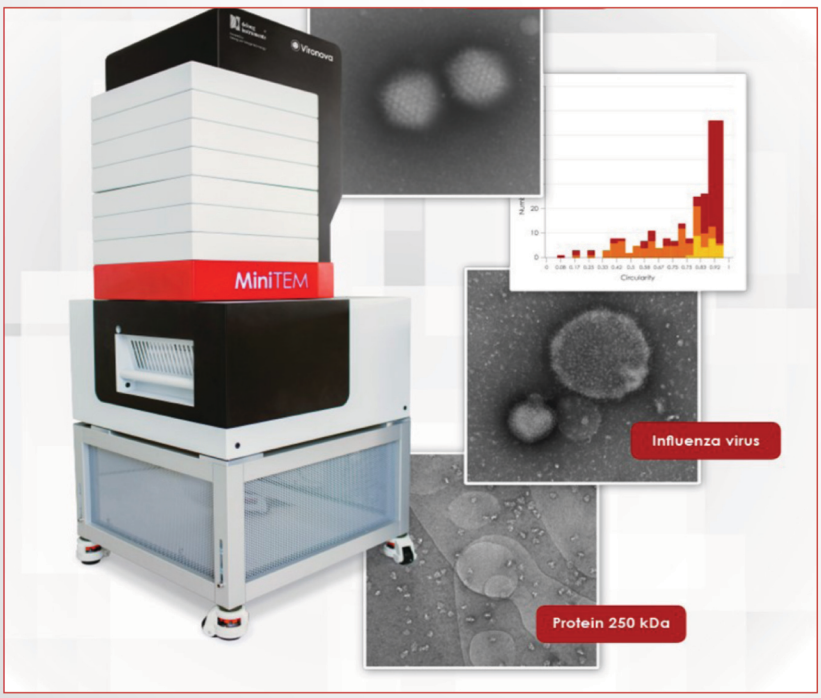

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an Austrian cancer-focused biotech company, and S-TARget therapeutics, an allergy-focused biotech company. Checkpoint inhibitors are effective in killing tumors of certain cancer types, but they have a small therapeutic window because they lack tumor specificity and "uncontrolled," un-specific T-cell activation, which results in "massive autoimmune reactions and cytokine storms," says Mudde.

OncoQR, which is focused on developing cancer immunotherapies, is using an approach known as active checkpoint control immunotherapy (ACCI). This approach mobilizes the full potential of the patient's own immune system by activating the body's four naturally available tumor-killing mechanisms, which results in strong tumor-specific humoral and cellular immune responses without any side effects (in >60 non-human primates), says Mudde.

"With therapeutic cancer vaccines based on this revolutionary form of active cancer immunotherapy, oncologists will be able to specifically control all the body's relevant immune checkpoints needed for tumor-specific responses, rather than just non-specifically inhibit one of them, as is the current mode of action within the passive checkpoint inhibitor immunotherapy approach," Mudde remarks. The ACCI approach can thus be considered more effective, tumor-specific, immunogenic, and safer than current passive cancer immunotherapies. ACCIs can also be combined with other existing cancer therapies, such as chemotherapy, as well as with passive immunotherapies, such as the checkpoint inhibitors, according to Mudde.

The path forward

The development of effective cancer vaccines remains a challenge, but the landscapes consists of many diverse therapeutic vaccination strategies either under development or being evaluated in clinical trials, says Mudde. Among these are cell vaccines targeting tumor or immune cells, protein and peptide vaccines, and genetic vaccines based on DNA and RNA, or utilizing viruses.

Funding for innovative cancer vaccine development is coming from a variety of sources, including government funds and grants, some of which are allocated for biotech start-ups; investments and/or licensing deals by major pharmaceutical firms; and traditional investments from equity investors, notes Mudde.

One example is Eli Lilly and Company's $1.8-billion deal with a German messenger RNA (mRNA) technology-focused company, CureVac, under which the two companies will develop and commercialize up to five potential cancer vaccine products using CureVac's proprietary RNActive technology (5). Lilly made the deal in October 2017, for which it paid $50 million upfront. The rest of the $1.8-billion comprises an equity investment of EUR 45 million (US$52 million) and potentially more than $1.7 billion in development and commercialization milestones if all five vaccines are successfully developed (5).

The companies will use mRNA technology that targets tumor neoantigens and delivers mRNA that ultimately directs the human immune system to target the encoded neoantigens. Neoantigens are thought to instruct the immune system to mount a selective and potent response to eliminate cancer (5).

CureVac has also raised approximately $420 million in equity investments since it was founded in 2000 with lead investors dievini Hopp BioTech holding GmbH & Co., a German private equity and venture capital firm, and the Bill & Melinda Gates Foundation (6).

In another deal, Boehringer Ingelheim entered a long-term collaboration in September 2016 with ViraTherapeutics, an Austrian biotech company that develops potent anti-cancer therapeutics based on oncolytic viruses. The companies are jointly developing a next-generation oncolytic virus therapy platform and investigating ViraTherapeutic's lead candidate, vesicular stomatitis virus glycoprotein (VSV-GP) as a monotherapy and in combination with other therapies (7).

In this instance, viruses are used to spark an immune response from the body by infecting cancer cells and breaking them down (7). This cell lysis releases tumor antigens normally hidden from the immune system inside the cancer cells, triggering an immune response to attack the tumor. ViraTherapeutic's lead candidate, VSV-GP, has a shorter replication time than other oncolytic virus platforms currently under development, according to the company.

What's more, it does not integrate into the host DNA and has been modified to avoid neural inflammation typically associated with wild-type viruses. The vesicular stomatitis virus glycoprotein in VSV-GP has been replaced by the lymphocytic choriomeningitis virus glycoprotein to conceal it from the immune system, which was shown in preclinical models to not induce virus-neutralizing antibodies. Thus, VSV-GP is not itself eliminated by the body's immune response. This suggests that it could potentially be administered repeatedly (7). ViraTherapeutics is currently conducting preclinical safety and efficacy studies and aims to move into clinical trials in 2018 (8).

With OncoQR, the company is developing ACCI cancer vaccines composed of a proprietary generic module ("warhead") and a proprietary, disease-specific module ("immunogen"), linked by high-affinity connectors. "In short, the warhead ensures specific delivery of the immunogen in a non-toxic manner to those cells that adjust and (re-)direct the patient's immune response (in particular plasmacytoid dendritic cells and B-cells). In addition, the warhead strongly boosts and defines the therapeutic effect of the drug. The modular concept allows the combination of the warhead with different immunogens, resulting in multipurpose cancer immunotherapies," says Mudde.

The company's research is funded by grants from the Austrian Research Promotion Agency (FFG), a national funding agency for industrial research and development in Austria, and Wirtschaftagentur Wien (the Vienna Business Agency), an agency in Vienna, Austria, that offers financial support, real estate, and urban development incentives (9).

Other therapeutic vaccine development

The notion of a therapeutic vaccine to treat disease extends beyond cancer. There is ongoing research into developing therapeutic vaccines for treating symptoms of other viral infections, including STDs such as human papillomavirus (HPV) (10). In the case of HPV, preventative vaccines are available, but these are not known to have a strong therapeutic effect on already-established HPV infections and lesions. The lack of treatment has prompted the development of therapeutic strategies, including therapeutic vaccines, for treating established infections and resulting diseases, but the road is challenging (10).

"Great progress has been made to develop and improve novel therapeutic HPV vaccines to treat existing HPV infections and diseases; however, there is still much work to be done. We believe that therapeutic HPV vaccines have the potential to become a widely available and successful therapy to treat HPV and HPV-associated diseases in the near future," Yang *et al.* noted in a published study that reviews HPV vaccines in development in the US (10).

Different types of therapeutic HPV vaccines have been evaluated in preclinical and clinical trials, including live vector, protein or peptide, nucleic acid, and cell-based vaccines, which primarily target the HPV oncoproteins E6 and E7. The aim is to deliver E6 and E7 antigens in various forms to antigen-presenting cells that would activate HPV antigen-specific CD8+ cytotoxic T-cells or CD4+ helper T-cells (10).

Approximately 20 clinical trials were conducted or are ongoing in the US to evaluate therapeutic HPV vaccines in the following indications: persistent HPV infection and low-grade squamous intraepithelial lesion; cervical intraepithelial neoplasia/high-grade squamous intraepithelial lesion; anal intraepithelial neoplasia; HPV-associated incurable solid tumors; head and neck cancer; and cervical cancer (10). Different forms of therapeutic HPV vaccines have been evaluated in clinical trials, including bacterial vector-based vaccines, viral vector-based vaccines, peptide or protein-based vaccines, nucleotide-based vaccines, and whole cell-based vaccines (10).

Yang *et al.* concluded that the current therapeutic HPV vaccines reviewed in their study each have advantages and limitations, and that further clinical studies are still necessary to verify that these vaccines provide anti-tumor efficacy. "With continued efforts to improve and develop therapeutic treatment strategies, we anticipate the continued success of therapeutic HPV vaccines over the next few years, and beyond. We believe that therapeutic HPV vaccines will become clinically available in the near future and be offered alongside other available therapies for the control of HPV-associated diseases," the study authors said.

Challenges on the road to development

The roadblocks to developing therapeutic vaccines goes far back and are based largely on past failures. According to Mudde, the failure of active vaccines nearly three decades ago due to a lack of immune checkpoint control has led to skepticism in the ability to develop such vaccines in general. As a result, there is limited trust and, subsequently, limited investment into new strategies and companies that may have solved the issues encountered in the past.

"Investments over the past 10 years or so, have consequently focused mainly on passive checkpoint inhibitors, leading to an enormous pipeline of competing monoclonal antibodies against a small number of targets currently in clinical development (Phase I–III)," Mudde says. In terms of manufacturing roadblocks or roadblocks to approval, peptide/protein-based vaccine strategies are not likely to encounter them, as long as they are safe, stable, and reproducible, according to Mudde. In comparison, cell-based vaccines will always have production issues, whereas genetic vaccines will likely be carefully monitored concerning safety and efficacy, he adds.

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As advanced therapies such as oncolytic viruses and gene therapy progress, a new type of facility is required to fulfill the contract development and manufacturing (CDMO) role. An advanced design for late-stage and commercial multiproduct operation is needed to support the array of products and processes. Ideally, the facility would be configurable to flexibly segregate production of these products by allowing a custom envelope for any given product's needs.

Live viral vaccines/products represent an important and rapidly growing segment of the global pharmaceutical market, with whole new classes of product in development. Several factors are contributing to this growth, as well as presenting new challenges for production.

Periodic influenza pandemics also drive a need for faster time to market, while emerging new disease threats (e.g., Ebola, Chikungunya virus, and avian influenza) require rapid development and manufacturing of new vaccines for use in remote parts of the world. Development of new therapeutics for cancer, genetic birth defects, and other diseases has created a need for production of a potentially large number of viral-based products at relatively small scale. These challenges require best practices in manufacturing to assure flexible, cost-effective, and rapid production of a myriad of new vaccines, most often under high-containment precautions in order to prevent release of infectious organisms.

Zone control

Efficient design of a flexible and high-containment vaccine manufacturing facility that can meet all the challenges presented by the

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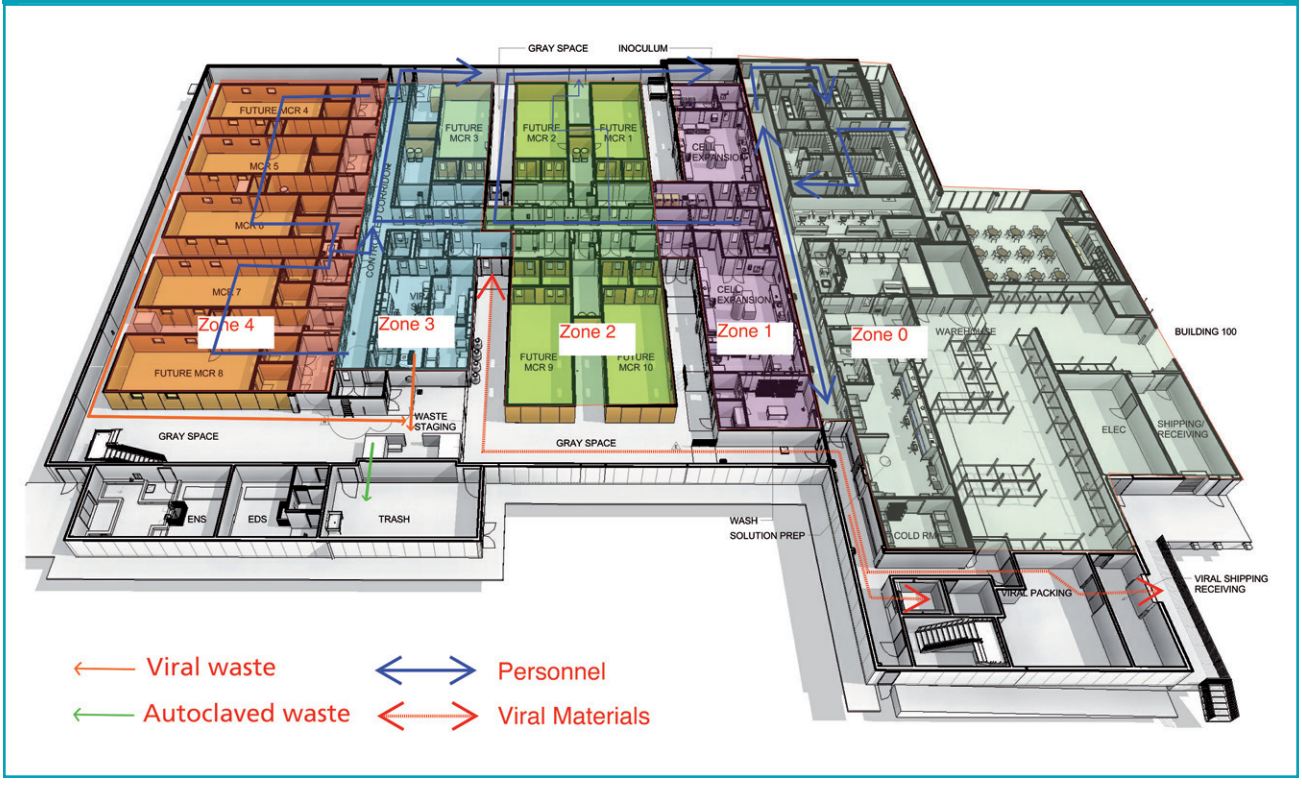
Figure 1: Zone control of a vaccine manufacturing facility, with the flow of air and personnel going from the lowest containment zone (Zone 0) to the highest (Zone 4, infectious agent processing). The blue lines show the flow of people; the red lines show the flow of viral materials; and the orange and green lines show the flow of waste through the zoned manufacturing facility.



market requires a design philosophy that includes all the key control concepts: air, people, equipment, product, and liquid and solid waste (1). Zone control provides for proper airflow, movement of personnel, and containment of live viral agents. It starts in a "clean" core, where office spaces, storage, and shipping and receiving reside (Zone 0, see **Figure 1**). The level of exposure risk increases across the subsequent zones, with Zone 1 housing routine lab functions, such as clean cell expansion. The last zones (Zones 2, 3, and 4 in **Figure 1**) are designed to contain live viruses or other agents. Layered and redundant segregation of zones and multilayer security and access controls are imperative, as are layered controls for containment.

Flow of personnel through the zones is unidirectional, from lower to higher zones, with airlocks separating the zones. Exit from any zone is through gray space to locker rooms and not to any lower numbered zone (see **Figure 1**). The air pressure in each space of each zone can be independently controlled. All equipment in Zones 2, 3, and 4 are captive per campaign—once a piece of equipment goes in, it does not come out until cleaned and sanitized. Local engineering controls are used in appropriate spaces to ensure that no open work is done with infectious materials. Liquid waste is decontaminated chemically within the process space and put into doubly contained systems for further treatment and/or disposal. All liquid waste treatment is performed via heat kill in a double air-locked in/out space with HEPA filtering of both intake and exhaust air (see **Figure 1**).

FIGURES COURTESY OF THE AUTHOR

Figure 2: Interior of mobile cleanroom (MCR), before coatings are applied, showing the welded floors and walls, freeboard, and built-in down ramp to the floor of the MCR.

Transactional controls are put in place between workspaces. Pass-throughs with airlocks are used to transfer materials from one space to another, and all transactions are electronically monitored. Zones 2 to 4 are constructed of mobile cleanrooms (MCRs) that can be easily and rapidly reconfigured to adjust to the needs of the manufacturing program.

Controlling spills

Handling spills in a high-containment viral manufacturing facility must be adequately planned for. Design of the facility should intrinsically limit the impact of spills. Cleanrooms should have fully welded floors and walls, as well as down ramps and freeboard at all entrances to contain infectious materials, as shown by **Figure 2.** Absorbents and sanitizing agents should be available in all spaces containing infectious materials. Procedures and training must be in place to quickly and safely recover from any upset condition. Measures must be in place to notify the multi-functional response team.

Design with procedures in mind

Each manufacturing facility is bound by company procedures that assure worker and environmental safety, as well as compliance with government regulations. All the senior stakeholders for those procedures should be involved in the design and required to sign off on it, stating that the planned design incorporates and follows corporate procedures. This will help prevent expensive and time-consuming errors that require further debate or remediation when the facility is under commissioning.

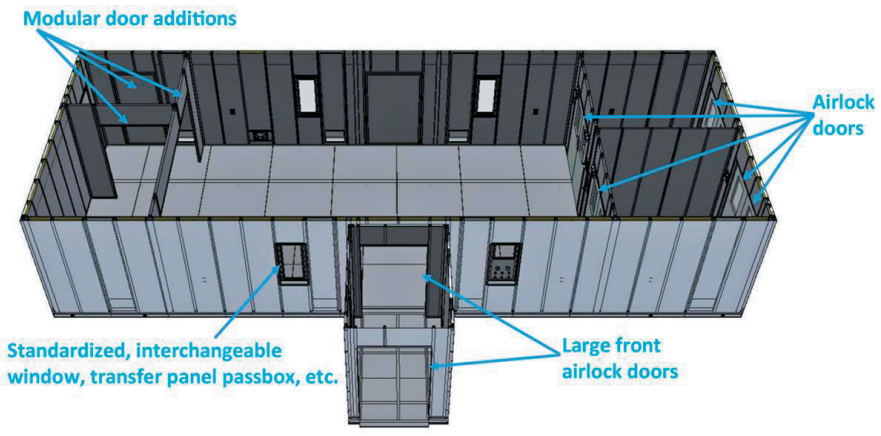
Mobile cleanrooms as equipment

The pharmaceutical industry has gained sizable benefits from standardized, single-use process equipment. It is logical to assume that similar benefits could be accrued by standardizing the mobile cleanroom. Having prequalified, prefabricated, and standardized MCRs available for high-containment manufacturing could dramatically decrease capital and regulatory risk.

MCRs are also essential to provide the flexibility, scalability, and high-containment levels required by today's virus manufacturing facilities. They can be prequalified before use as high-containment facilities and can provide lower risk than facilities constructed by conventional means, given the quality of materials and construction methods

used to build them. The use of modular cleanrooms enables products and multiple classes of product to be manufactured simultaneously, as each MCR can be customized and optimized to the needs of the product being handled, including air pressure, number of air changes per hour, and biosafety level (BSL), for example.

Figure 3: Third-generation FUJIFILM Diosynth Biotechnologies mobile cleanroom (MCR).



Modular and mobile cleanrooms can be constructed in parallel to the base facility or placed in an existing fallow space. As a result, they can enable flexible capacity management, thus reducing upfront capital investment. MCRs can be quickly added or deleted to meet manufacturing needs, and quickly started up or shut down. Their standardized construction makes them easy to prequalify and ship all over the world, as needed.

Figure 3 shows the third-generation MCR design used at FUJIFILM Diosynth Biotechnologies. It features a fully welded "bathtub" design that is airtight, passes a room pressure decay test, and provides leak-proof containment of spills. The MCR can be set up to provide a negative pressure cascade, as is often used in high-containment, or a positive cascade (if used for clean cell expansion or non-viral purification), and it can handle BSL 1 to BSL 3 requirements.

This MCR also features 10-foot ceilings, standardized and interchangeable transfer panels for air-locked pass-throughs, standardized windows, and redundant HEPA exhaust. The interior space can be configured to meet the needs of a multitude of development and manufacturing programs. All the rooms in the MCR have their own air and pressure controls, so that any of them can be used as a deeply negative pressure area for higher, nested containment, for example. Using transfer ports, consumables such as buffers can be added to the MCR, and equipment sitting in the grey space can be used to meet the needs of the manufacturing program, without having to move it into the high-containment space.

Layered controls ensure containment and prevent cross-contamination between MCRs. The primary control is closed processing or housing of open operations within engineering controls, such as isolators. The room acts as secondary or tertiary containment. The base level of control is passive. This is accomplished through airtight construction, which prevents release of airborne pathogens from the MCR. To this passive level of control are added layers of active control, such as pressure monitoring and control, alarms on all pressure controls, HEPA filtration of outgoing air, and, finally, a backup generator to ensure that all active controls work in the event of a power failure.

A vaporized hydrogen peroxide (VHP) decontamination system is an essential feature of this MCR, with 100% duct coverage of the HVAC system and through-HEPA filter kill. Test strips are used in the MCR to verify VHP coverage. The ceiling of the MCR holds LED lights that can be replaced without entering the MCR, as well as transfer panels that can be customized to bring in utilities, such as electrical and gas supplies. The HEPA filters are replaced from inside the MCR. Each configurable space in the MCR has its own FM200 fire suppression unit, which eliminates dead leg spaces that cannot be cleaned and cross-connection risks.

This MCR is modular and configurable to enable scalability. Reconfiguration can be done by moving the walls and doors. It can be used as one large room by removing the interior walls and doors, or it can be used as a suite of any number of rooms up to five. The front airlock doors can be removed so that the airlock can be used as part of the main room for cross-connected MCRs.

Using this modular and mobile MCR enables management of the entire lifecycle of a viral production program. The MCR starts at a "neutral" state that presents no contamination risk, but also contains no equipment. Next, the manufacturing environment is set up by choosing the number of MCRs to use and how to configure them. Equipment is then brought in and set up, using as much single-use equipment as possible. Single-use bioreactors shorten setup, decontamination times, and changeover times. The pressure cascades in the MCRs, the number of air changes per hour, and the flow patterns for product and personnel through the MCRs are then set to optimize the manufacturing program. Once the program has been run, the MCRs are decontaminated and the equipment is removed, enabling rapid and rigorous changeover from one manufacturing program to another.

Conclusion

The most successful manufacturers of the near future will avoid the urge to cut costs by customizing designs to meet only the demands of the current projects. Once the facility lifecycle is considered, allowing for redirection becomes crucial. By embracing standardization of production facilities using modular and mobile cleanrooms, a variety of process platforms and scales will be enabled over the lifetime of the facility. Control of the environment in each configurable space of the MCRs will make this possible—thus rooms become dispensable, not the entire facility.

Production times will be slashed by using prequalified MCRs that can be rapidly configured to meet the needs of multiple manufacturing projects. Capital costs will be reduced using standardized MCRs, as they can be quickly added or deleted to meet manufacturing needs, and quickly started up or shut down. Multilayered security controls and containment controls will assure safety for the workers in the facility, as well as people living near the facility. The end result will be accelerated availability of a large number of new vaccines to counter newly emerging diseases and to develop new therapeutic approaches for existing diseases, as well as rapid and effective response to new pandemics of diseases that have long plagued mankind.

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Moving to Closed Systems for Aseptic Processing

James Agalloco and Leonard Mestrandrea

Alternatives to time-consuming, error-prone operations promise to reduce vaccine manufacturing costs and improve facility flexibility.

Aseptic processing continues to challenge vaccine manufacturers. The operation, which involves filling a container with vaccine, and then sealing the container in a pristine environment, requires highly trained personnel and entails substantial costs, both for infrastructure and for everyday operation. The formulation, container, closure, and processing equipment used for aseptic processing must be sterilized individually, and substantial precautions taken to maintain their sterility throughout filling and sealing operations (see **Figure 1A**). As FDA explains in its aseptic processing guidance (1), the overall process involves more variables than terminal sterilization, and each step requires validation and control.

As the guidance states, "Each process could introduce an error that ultimately could lead to the distribution of a contaminated product. Any manual or mechanical manipulation of the sterilized drug, components, containers, or closures prior to or during aseptic assembly poses the risk of contamination and thus necessitates careful control."

Operators have long been identified as the predominant source of microbial contamination in aseptic processing (2). In fact, the very term "aseptic processing" represents a compromise, acknowledging that truly sterile process conditions remain unattainable, given the people and equipment required, and their potential to contaminate product. Best aseptic processing practices can at least ensure that the environment is free of pathogenic microorganisms that might put patients at risk if they wound up in the product.

Unfortunately, instances of contamination continue to occur, and regulators have penalized a number of vaccine manufacturers for failure to maintain a truly aseptic environment in filling and other

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operations. At times, these issues have led to shortages of crucial vaccines. At the same time, aseptic processing contributes to the complexity and high infrastructural and operating cost of vaccine manufacturing (3), at a time when prices and profitability for vaccines have remained depressed (4)

If aseptic processing is to continue to improve, compliance will have to be engineered into equipment design. Simpler, more elegant designs will be required.

This article will look at aseptic processing and the development of closed systems designed to prevent operators from coming in contact with the process, and will outline the evolution of one closed system technology for aseptic processing, describing how it works and summarizing results that have been seen in media fills performed both at the developer's facilities as well as those of its licensing partner.

Central to closed system performance for aseptic processing is the means to connect one closed system to another without contamination ingress. While closed systems have been used in pharmaceutical and biotechnology for some time, they have typically used a limited number of connections between their separate components. The closed system described in this article provides a means for closed system transfer from a closed filling system to pre-sterilized closed containers without exposing the product to environmental conditions and potential contamination.

Eliminating human contact with the product

Over the past few decades, aseptic processing performance has improved substantially. However, manufacturers still face significant difficulties, especially in aseptic processing lines in older facilities (5). Most advances have focused on the singular goal of separating operators from the process, or eliminating excessive or direct operator contact with sterile materials (6).

Many of these improvements have centered around the use of isolators or Restricted Access Barrier Systems (RABS). Concurrently, global regulators have mandated extensive environmental and procedural controls in attempts to increase the safety level in aseptic processing. These extensive controls are described, in exhaustive detail, in 21 *Code of Federal Regulations* (CFR) 211, FDA's 2004 Aseptic Processing guidance and EU *Eudralex* Annex 1 (1,7,8) .

Nevertheless, concerns about the safety of aseptically manufactured sterile products persist. If aseptic processing is to continue to improve, compliance will have to be engineered into equipment design. Simpler, more elegant designs will be required than the past decade's state-of-the-art, in order to ensure the safest products possible. Building compliance into equipment will be especially critical in emerging markets where the infrastructure and trained, skilled workforce required for reliable aseptic processing are often lacking.

Closed systems have become the Holy Grail of aseptic process development. The Parenteral Drug Association (PDA) defines them as systems that are or can be (9):

- Sterilized while closed prior to use
- Pressure and/or vacuum tight
- Used without breaching system integrity
- Adapted for fluid transfers in and/or out while maintaining asepsis

Figure 1A, 1B, 1C: Open containers/open needle; closed container/open needle; and closed container/closed needle configurations.

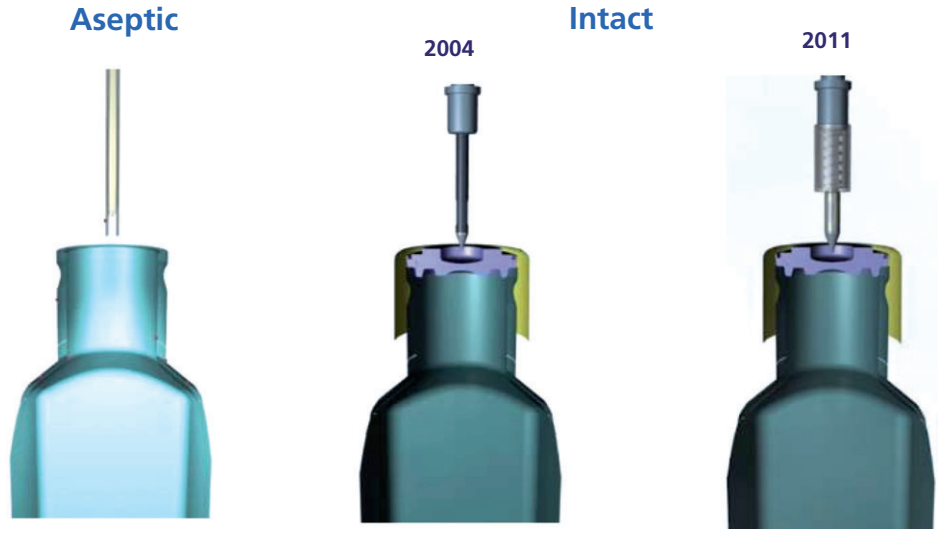

Table I: Medinstill 2003 media fills.

Open needle / Closed vial	Background environment	Fill environment	Media	Media fill results	
				# Units tested	# Units contaminated
2-ml vial,	Grade B	Grade A	TSB	31,752	0

- Connected to other closed systems while maintaining integrity of all closed systems
- Used with sterilizing filters that are integrity tested and traceable to each product lot.

The move to closed systems

A number of companies are working toward this goal, taking different approaches to separate operators from product. One approach taken by the Canadian manufacturer VanRx, works from the outside in. Based on best practices in the semiconductor industry, the platform uses robots to fill nested syringes, vials, and cartridges automatically in enclosed gloveless isolators, which shield the entire process and product from any exposure to outside contaminants (10).

Working from the inside out are processes that were developed by MedInstill Technologies (MedInstill). In 2003 and 2004, the company first successfully demonstrated an aseptic filling technology in which the closure on a sterile closed vial was penetrated by a non-coring needle and the opening in the container then re-sealed by using a laser to re-melt the closure (see **Figure 1B**) (11). This technology eliminates the need for operators to prepare and aseptically handle both container and closure (see **Table I** for a summary of media-fill test results of this initial technology.) Tests were conducted in an ISO Level-5 cleanroom at the PDA Training and Research facility.

The use of an open-eye filling needle mandated that the environmental controls associated with traditional aseptic processing be maintained in the background environment as well as over the filling needles. Aseptic Technologies (originally a GSK subsidiary, now owned by Skan AG) licensed the technology, and one product filled with this closed-vial technology has already been approved

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Table II: Aseptic Technologies' media fills.

Open needle / Closed containers	Background environment	Fill environment	Media	Media fill results	
				# Units tested	# Units contaminated
Various	ISO 8	Grade A	Various	74,538	0
Various	ISO 5	Grade A	Various	14,100	0

Table III: Medinstill 2011 media fills.

Open needle / Closed vial	Background environment	Fill environment	Media	Media fill results	
				# Units tested	# Units contaminated
250-ml. bottle	>1 x 10^2 CFU/m^3	>1 x 10^2 CFU/m^3	TSB	4,000	0

for use, while others are awaiting approval by FDA and EMA (12).

Over the next nine years, Aseptic Technologies ran a substantial number of media-fill tests to support their filling technologies and client container requirements (see **Table II**).

Meanwhile, designers at Medinstill sought a way to develop a sterile transfer system for filling closed containers, one that would prevent exposure of the sterile drug and product contact surfaces to surrounding non-classified environments and contact with operators within that environment.

With this goal in mind, media fills were performed using different variations of the closed vial technology, in background environments that ranged from ISO Level 7 to unclassified (see **Table III**). The filling enclosure was supplied with high-efficiency particulate (HEPA)-filtered air, but filters were switched off in some runs, which were designed to simulate worst-case conditions that might exist in some processing environments.

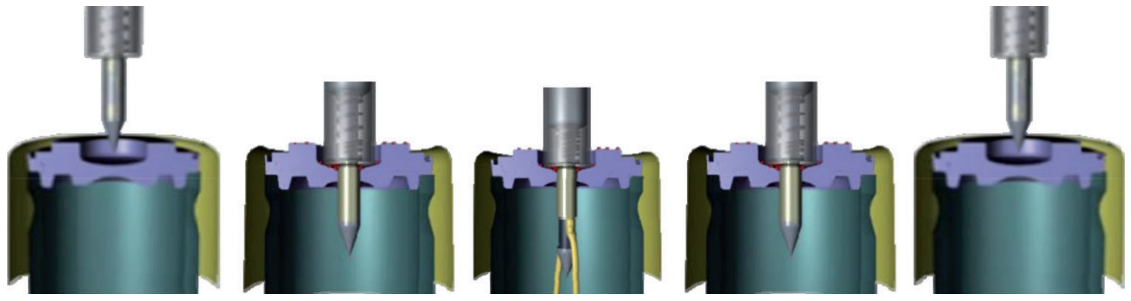
Development aimed to eliminate the need for environment control to protect sterilized product, fill components, and filling parts so that the resulting process would exceed the capabilities of the best existing separative designs. Equipment such as RABS or isolators still rely on environmental controls to protect exposed product containers, elastomeric closures, and filling heads. The basic goal of this work was to create a reliable means for truly closed sterile transfer in aseptic processing that would not rely on environmental controls of any type.

Closing off the fluid pathway

Ultimately, the designers applied closed system considerations, not only to the container but to the entire fluid pathway at all critical points in the process (see **Figure 1C**), at the point of fill, and where the filling system connects to the outlet of the sterilizing filter. The result was ISCON (short for Intact self-closing-opening needle) technology, in which a closed needle penetrates a sterile closed container, only opens once inside that container, transfers the fluid, and then self-closes within the container before it is withdrawn from the container. After its withdrawl, the pierced septum self-closes (see **Figure 2**).

This approach was taken to assure that sterilized product and all product contact surfaces are never exposed to the environment or the operator. A combination of materials science knowhow, closed system technology design, and automation permits reliable aseptic transfer without the typical environmental controls associated with other forms of aseptic operation.

Figure 2: Process sequence for Intact self-closing needle (ISCON) filling.



Intact filling has been successfully demonstrated in a controlled not-classified (CNC) environment for the filling enclosure and the surrounding room, an unclassified room where closed processes and their immediate support systems may be located (13). To support its application for use for filling of sterile products, a draft appendix to FDA's *Guideline on Sterile Drug Products Produced by Aseptic Processing* has been published (14).

Since these media fills were run, Medinstill's development team has improved septum design, as well as needle shape, dimension, and external finish. The company has successfully completed sterile media fills through microbial populations of 10^6 colony forming units (CFU)/mL on both the needle and the septum (15).

In the technology's latest design, microbes are excluded by frictional forces that are created where the septum and needle meet at the point of penetration, and which prevent microorganisms from entering the container. These same forces come into play as the needle is removed from the container, preventing any liquid from remaining on the surface of the needle.

The septum's self-closing design also results in the creation of frictional forces along the needle's conical tip so that, even after the needle has been completely withdrawn from the container, the pin hole left in the septum is difficult to discern visually.

In order to ensure container integrity, the tiny pin hole left by the needle in the septum's self-retractable material is immediately re-sealed within the filling enclosure, using silicone drop, hot melt, or laser-heat processing. This step eliminates the need for cap sterilization, as well as for related component transfers, and saves the capital that would be required to invest in a high-speed capping machine. Hot melt resealing, in particular, has the added benefit of assuring tamper-evident sealing of the filling port.

Although the process has been engineered to ensure complete isolation of the product from the filling process, several procedural controls have been added to further mitigate the microbial contamination risk (see **Figure 3**), including:

- Positioning of the ISCON filler in a non-classified restricted access controlled area, using a filtered air supply
- Use of a filtered air supply immediately over the filling zone, and excluding operators from the filling zone while filling is taking place
- Built-in routine monitoring of the total number of particles that are present in the room, to assure control of conditions in the background environment

Figure 3: ISCON filling enclosure.

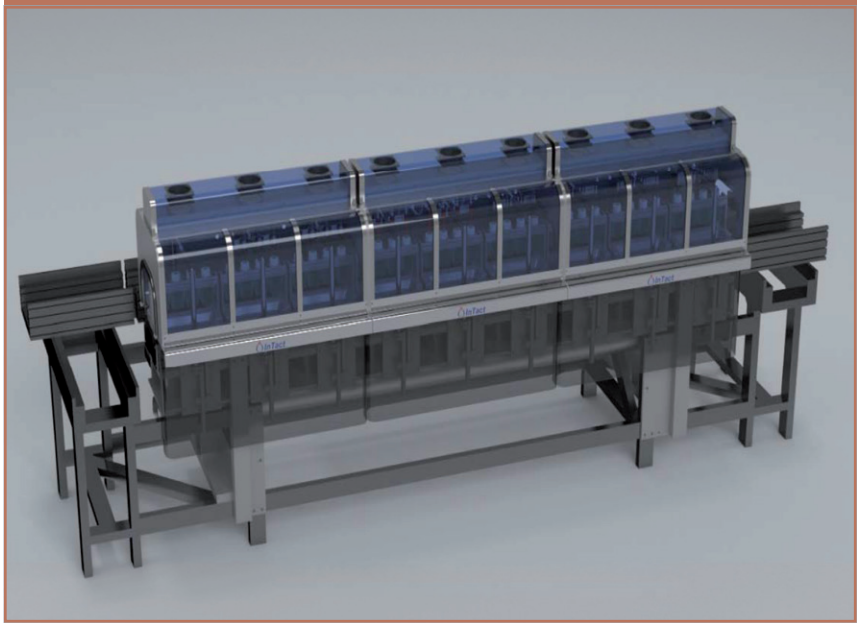
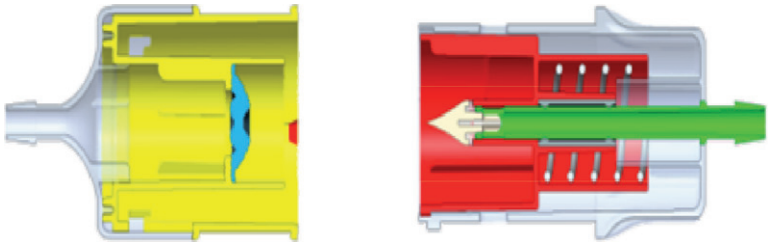


Figure 4: ISCON connector.



- Using radiation to pre-sterilize the disposable filling kit assembly (consisting of ISCON tubing, and sterile ISCON and septum-like connector) and the pre-closed container so that both are delivered to the filling system in sterile bags that are opened in the non-classified environment immediately before use
- Automated removal of the protective needle cap within the fill enclosure
- Visual confirmation of proper container position prior to enclosure entry.
- UV decontamination of the septum surface within the enclosure just prior to filling, in case of manual loading of the pre-sterilized closed containers
- Resealing of the pin hole in the septum created by needle withdrawal within the enclosure using controlled means
- Optional use of a protective over-cap on the septum in a separate enclosure, a step that is not needed when the container is hot melt resealed
- Use of disposable components for product contact throughout the aseptic process.

These measures serve to prevent any contact between the product and the processing environment. The closed, single-use fluid path also eliminates exposure of the product to the operator, so that the ISCON filling process meets Biosafety Level 3 (BSL-3) requirements.

The same ISCON mechanism in the Intact connector facilitates near-continuous aseptic manufacturing by avoiding the need for lengthy changeover procedures between batches (such as clean- and sterilize-in-place operations, environmental decontamination, and line clearance).The filling system has also been designed to fill multiple container types (whether vials, bags, or bottles) with minimal changeover time and can be transported to and installed in new sites, within days.

Use of closed transfer system principles eliminates nearly all of the facility design and operational considerations associated with conventional aseptic processing. In addition, it obviates the need

Table IV: Intact media fills.

Closed needle / Closed vial	Background environment	Fill environment	Media	Media fill results	
				# Units tested	# Units contaminated
Various	CNC	CNC	Various	17,331	0

Table V: Intact media fills with microbially contaminated septum.

Closed needle / Closed vial	Background environment	Fill environment	(CFU/septum)	Media	Media fill results	
					# Units tested	# Units contaminated
Various	Non-classified	Non-classified	4 Log and higher	Various	1,718	0

Table VI: Intact media fills in non-classified environment

Closed needle / Closed vial	Background environment	Fill environment	Media	Media fill results	
				# Units tested	# Units contaminated
Various	Non-classified	Non-classified	Various	54,828	0

for environmental classification and monitoring; environmental decontamination; and the proficiency of personnel in aseptic gowning, filling machine, and line setup and operation.

The filling system's aseptic processing performance has been demonstrated through the execution of a number of rigorous challenges (16, 17). Successful media fills have been performed in a variety of background environments starting with the planned controlled non-classified environment envisioned for commercialization as well as other less closely controlled environments (see **Table IV**). The background conditions for these media fills were intentionally performed under microbiological conditions that are more challenging than those typically used to test conventional aseptic filling systems.

The media fills cited in **Table IV** exposed individual septa to microbial contamination prior to the fill. Additional fills were performed on a limited numbers of units in which the target locations on the components were exposed to microbial populations of over 10^6 CFU (including *S. marcescens, B. diminuta, E. aerogenes, C. albicans and S. epidermis* strains) prior to filling (see **Table V**). Background environments used for these trials varied from ISO Class 7 to unclassified.

Table VI summarizes all the sterile media fills done that have been performed on the filling system to date in non classified environments, including worse-case media simulations. The Intact and ISCON filling technologies have demonstrated the ability to achieve microbial exclusion at levels that have not yet been seen in traditional aseptic processing operations, at conditions that could not be used with other technologies, including Blow Fill Seal, FFS, and robotic filling in isolators.

ISCON would also permit aseptic filling to be accomplished in non-classified environments. This, in turn, would eliminate the need for conventional environmental and other controls.

Potential impact on global health

By eliminating critical surface exposure, the key concern in aseptic processing, closed systems such as Intact could be used in pandemic response and just-in-time medical countermeasures. In addition, the ability to fill vaccines and other therapeutics into pouches and to deliver multiple-dose syringes using an anti-retro-contamination dispenseing valve could make the following possible:

- Filling one billion doses in three weeks at a cost of less than $0.10/dose. Current US government-funded capacity is approximately 50 million doses of preserved vaccine in 12 weeks (17), leaving millions of Americans and billions worldwide unprotected.
- Implementation at a very low capital cost, enabling dedicated lines with the flexibility to respond to pandemics with no interruption of routine filling essential medicines during a global threat.
- Simplified logistics and mass vaccination campaigns with one pouch and syringe (changing needles) for each 50–100 patients.

Tests for applicability for pandemics

The technology is currently being tested to demonstrate its ability to work in pandemic responses for the following:

- Pneumococcal vaccine using a single dose closed vial (18)
- Attenuated virus vaccine using a multi dose closed vial (19)
- Virus-like particles vaccine using a multi-dose closed vial and a multi-dose closed pouch (20).

In short, closed systems such as Medinstill's promise to play an increasingly important role in reducing the cost of vaccine manufacturing and improving facility flexibility, especially as companies in developing markets build their own local manufacturing plants.

As they continue to evolve, closed systems are proving to be disruptive technologies with the potential to change the way that vaccines and other sterile drug products are manufactured in the future. This change promises to bring the pharmaceutical industry closer than it has ever been to sterile processing.

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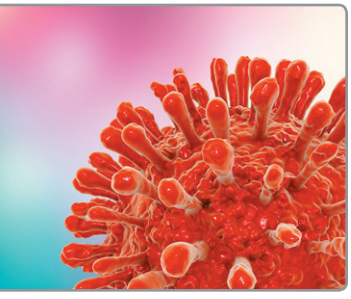

Achieving Intensification and Flexibility in Virus Purification with Next-Generation Chromatography Tools

Renaud Jacquemart, Melissa Vandersluis, Mochao Zhao, James G. Stout, and Sarah K. Wootton

Improved vaccine manufacturing technologies can enable more efficient and cost-effective processes. This article discusses the potential of using high-productivity membrane chromatography to achieve intensified and flexible virus manufacturing.

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Viruses, widely used in prophylactic vaccines, are gaining popularity in therapeutic fields. Viral vectors are being used as delivery vehicles in more than two-thirds of gene therapy clinical trials (1). Oncolytic virotherapies, with two commercially available and 48 currently known to be in clinical pipelines, represent the next potential breakthrough therapeutic modalities for cancer treatment (2). Despite the proven success of viral vaccines or therapeutic promises of emerging gene and viral therapies, there are still multiple barriers to extending access to these products around the world (3). The lack of advancements in manufacturing technology is one of them. While current viral vaccine manufacturing methods are, in some cases, effective for small-scale production, these methods share several shortcomings: they are too slow, too complicated, lack robustness, and require expensive specialized facilities and equipment for large-scale production. The lack of modern, efficient production technology is restricting progress on several fronts (4). Because of the high capital and development costs, low product profitability, and limits on product pricing, niche vaccine opportunities are regarded as financially risky, with low probability of breakeven or sufficient return on investment. Promising new vaccines, developed from large viruses, remain under-developed due to the lack of viable processing capabilities to handle large-sized virions. Likewise, progress in the development of oncolytic virotherapies is limited

because large doses of highly purified material are required, but efficient manufacturing processes are lacking (5). Meanwhile, viral vectors have been widely exploited for gene delivery approaches, further increasing the demand for large-scale production of highly purified viral material (6). In all cases, progress is directly limited by gaps in manufacturing technologies.

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Next-generation processes

State-of-the-art virus production processes and technologies hold the promise of enabling production of new vaccines as well as viral vector platforms for other applications, while also streamlining and strengthening current processes. These technologies, many of which are being used successfully in biotherapeutics manufacturing, can also create dramatic cost and productivity improvements for virus manufacturing (7). A new generation of highly productive, disposable processing technologies offers opportunities to simplify process architecture and reduce facility footprint. In upstream processes, stainless-steel bioreactors and microcarriers are being replaced by microfiber-based reactors that can achieve cell density up to 150 million cells/mL (8, 9). For example, the next-generation, fixed-bed continuous perfusion bioreactors developed by Univercells can reach high cell density with 50–70% volume reduction (10). In downstream processes, small-scale, high throughput disposable filtration units such as single-pass depth filters (11), high-viscosity tangential flow filters (TFF) (12), or single-pass TFF (13) also contribute to a smaller footprint and improved process efficiency, while the latest advancements in chromatography technology provide several performance and cost advantages over the traditional purification techniques (14, 15).

With more efficient upstream and downstream units, the facility design can be significantly improved and capital and operating costs can be reduced. When combined, new single-use, high-productivity process technologies can overcome the manufacturing constraints. Replacing traditional large, inflexible, and complex stainless-steel operations with small footprint facilities enables quick batch-to-batch turnover and rapid changeover between different products (16). The smaller, intensified process architecture allows viruses to be processed in small facilities across multiple geographies, with the promise of eventually placing production at the point of care. In addition, simplified, small footprint processes enable flexible processing and allow a new level of agility for responding to changes in demand and handling multi-product manufacturing (7).

Membrane chromatography is a new purification technology that combines high binding capacity and rapid mass transfer to improve

purification productivity. High-productivity membrane chromatography with common modalities (e.g., cation exchange [CEX], anion exchange [AEX], or mixed-mode) has been available and implemented in many biotherapeutics manufacturing processes. In some cases, such membranes with high selectivity can deliver sufficient purity levels in one step, making single-step purification a viable solution. In addition, efficient separation can be realized by combining affinity ligands with membrane chromatography to enable intensification of complex operations (17). With this powerful capture tool, complex process architecture can be simplified to fewer unit operations and still maintain critical quality attributes, leading to significant cost savings and highly intensified and productive processes. Membrane columns in single-use format further reduce validation, cleaning, and storage-related expenses while improving productivity. The ease of scalability of membrane columns also enables smooth transition from lab scale to the manufacturing site. The following three case studies demonstrate process intensification using these new chromatography tools.

Case study one: Newcastle disease virus single-step purification using AEX membrane

In this example, engineered Newcastle disease virus (NDV) produced for oncolytic virotherapy is amplified in embryonated eggs and purified from the allantoic fluid. The conventional centrifugation-based purification scheme, described in **Table I,** is slow, non-scalable, labor-intensive, has low virus recovery and partial impurity removal leading to non-optimal process economics, and high failure rates in manufacturing (18). Consequently, the manufacturers of NDV are faced with high cost of goods and the challenge to reliably meet market demand for this promising oncolytic agent (19, 20). This approach forces a trade-off between product purity and product recovery.

The new membrane chromatography method for purifying engineered NDV, shown in **Table I,** increases purification productivity and process reliability. An anion exchange hydrogel membrane (NatriFlo HD-Q) captures the virus while impurities flow through, enabling a single-step purification process (21). This fast, scalable method reduces purification time from nine hours to only 30 minutes and achieves high virus recovery (>90%) with >99% purity (21). The next-generation process greatly improves product recovery while maintaining product purity and reducing the production size and labor requirements for upstream operations.

Case study two: Influenza virus single-step purification using affinity membrane

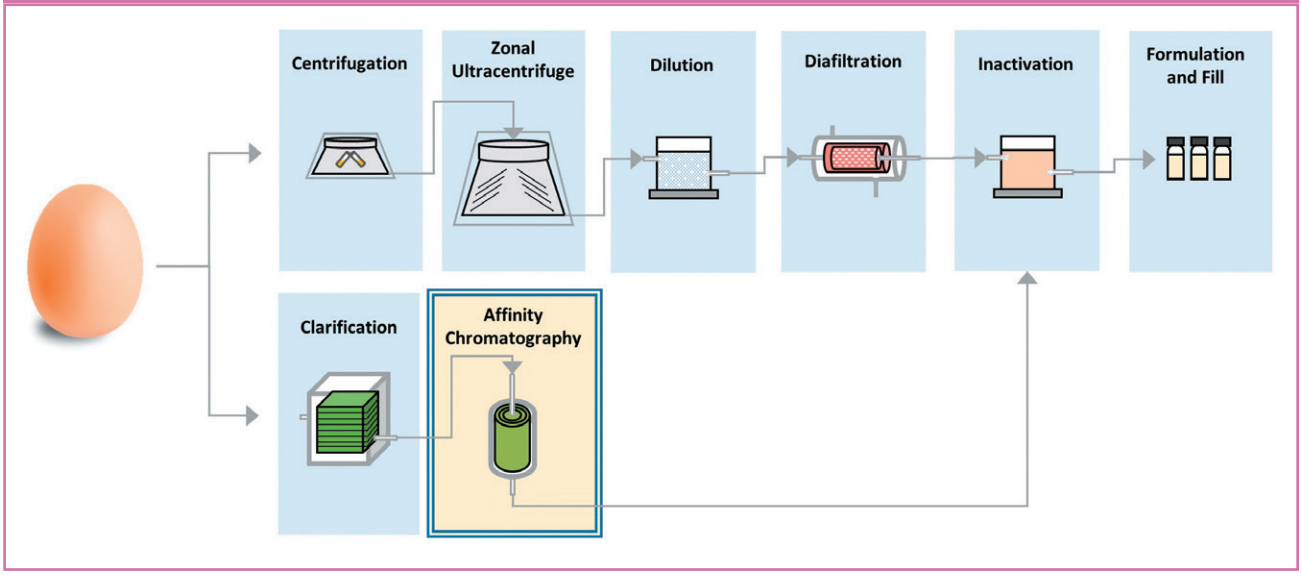
Traditional influenza virus manufacturing processes are complex and slow, often using centrifugation as the main purification operation. **Figure 1,** comparing an industrial process and a proposed next-generation process, demonstrates an example of how process intensification can be achieved by implementing affinity membrane chromatography (22).

In this case study, affinity membranes were used to purify influenza vaccine from allantoic fluid harvest. The use of affinity membrane resulted in successful purification after a single step (99.9% host cell protein [HCP] clearance, 87% yield, and 30-fold concentration) (23). The prototype affinity

Table I: Comparison of traditional versus new methods of Newcastle disease virus (NDV) purification for use in oncolytic virotherapies. TTF is tangential flow filtration. UC is ultracentrifugation. AEX is anion exchange.

	Traditional process	Next-generation process
Architecture	Depth filter → TFF → UC	Depth filter → AEX
Process duration	9 hours	0.5 hours
Product recovery	65-70%	>90%
Scalability	UC difficult and expensive to scale up	Depth filter and AEX easily scalable

Figure 1: Innovative affinity chromatography can enable process intensification of a traditional influenza purification process.



Membrane chromatography is a new purification technology that combines high binding capacity and rapid mass transfer to improve purification productivity.

membranes achieved performance equal to or exceeding published data on affinity resins as shown in **Table II** (23). Similar results were seen in terms of binding capacity and product recovery, although the affinity membranes had cycle times that were up to 45 times faster.

Compared to a typical commercial resin process, this affinity membrane enables a 100-fold reduction in column size for the same batch size. A 500-mL membrane column could purify allantoic fluid harvest from 1 million embryonated chicken eggs in 1 shift (8 hr, average feed concentration of 104 hemagglutinating units [HAU]/mL, and 5 mL of allantoic fluid harvested per egg) (23).

Case study three: Modeling a VLP-based vaccine process

To evaluate the quantitative and qualitative outcomes of implementing membrane chromatography into an industrial process, an intensified

FIGURE 1 IS COURTESY OF THE AUTHORS.

Table II: Comparison of influenza virus purification using affinity resins and affinity membranes. HAU is hemagglutinating units. CV is column volume.

	Impurity clearance			Productivity		
Media	Binding capacity (HAU/mL)	Recovery	Virus in flow through	Flow rate (CV/min)	Cycle time (min)	Productivity (HAU/L.h)
Affinity Resin #1	1×10^6	96%	2%	0.7	315	1.9E+08
Affinity Resin #2	7×10^5	87%	4%	1	220	1.9E+08
Affinity Resin #3	2×10^5	74%	NA	2.9	76	1.6E+08
Affinity Membrane Prototype 1	2×10^6	81%	10%	33	7	1.8E+10
Affinity Membrane Prototype 2	2×10^6	95%	3.6%	33	7	1.8E+10

process was modeled and compared to an existing commercial process. The study involved re-engineering a traditional vaccine purification process for a Saccharomyces-based virus-like particle (VLP) vaccine to investigate the potential of using new technologies to replace the traditional process (24). Natrix HD-Q and HD-Sb membrane columns were tested with original process feeds to gather the information needed to develop the economic model. The proposed new membrane-based process is anticipated to accomplish the purification in three unit operations, as compared to more than 10 operations in the baseline process (24). The simplified process resulted in much greater product recovery, which enabled downsizing of the required upstream operations to simpler, single-use bioreactors. Overall, the modeled modernized process would permit drastic cost reductions as well as facility flexibility. The new process model significantly reduced the capital investment (six-fold reduction), while also decreasing the vaccine cost per dose by up to 90% (24).

Perspectives

The concept of introducing state-of-the-art chromatography methods to replace traditional approaches can be expanded to an entire biopharmaceutical process. As illustrated by the collaboration (supported by the Bill and Melinda Gates Foundation) between Univercells, Batavia BioSciences, and Natrix Separations to develop the next-generation Sabin inactivated polio vaccine, a holistic approach to designing the upstream and downstream processes can enable efficient, cost-effective processes in which each operation is fully optimized (25). Applying a quality-by-design approach to process development results in maximized robustness and reduction in the number of out-of-specification (lost) batches. Though new media or innovative equipment are more expensive, the benefits of simplified, robust, flexible, and automated manufacturing lines outweigh the costs. Higher productivity technologies and sin-

Enclosed in modular cleanrooms, small footprint manufacturing of viral-based vaccines and therapies can be deployed anywhere in the world to provide greater access to lifesaving medicines.

gle-use solutions provide smaller footprints and improved flexibility for smaller batch production and facility utilization, thereby reducing the costs associated with building and maintaining a GMP plant. Furthermore, the addition of straight-through or continuous processing strategies result in even faster and more compact processes. New facility concepts including open (ballroom concept) or modular assemblies make it possible to realize the value provided by the new processing paradigms. For example, the POD design from G-CON is a prefabricated cleanroom system that can be used as a total process containment system for vaccine production (26). Small footprint processes can be entirely contained within modular cleanrooms that can then be operated in any geographic location. Lastly, online quality control strategies can now provide the benefit of real-time batch releases, hence, eliminating inventory costs and supply delays and further improving manufacturing efficiency.

Conclusion

The high productivity of single-use chromatography membrane technology enables favorable process economics compared to the labor- and capital-intensive reference processes. State-of-the-art chromatography tools and methods aid in achieving highly flexible and intensified processes. Holistic process development combined with modern technologies provides simplified architecture for the potential of fully continuous, disposable, and closed (aseptic) operation. Enclosed in modular cleanrooms, small footprint manufacturing of viral-based vaccines and therapies can be deployed anywhere in the world to provide greater access to lifesaving medicines.

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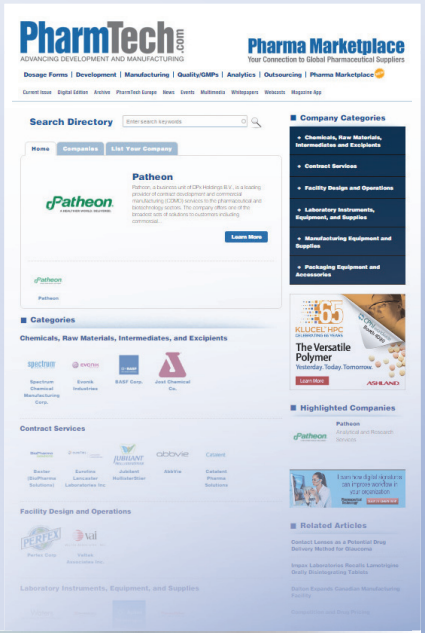

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Cold Chain: Delivering Vaccines to Patients

Amber Lowry

This article explores some of the challenges, services, and technologies that go into ensuring that vaccines are properly temperature controlled and maintain product integrity for delivery to patients.

With advancement in vaccine development comes an urgency to optimize the cold-chain delivery of these increasingly condition-sensitive biological products. This sensitivity has added new degrees of complexity to already complex supply chains and global regulations, resulting in the need for more precise approaches toward transporting vaccines. The idea of what it means to transport vaccines has changed, with specialists in cold-chain shipment approaching the delivery process as a mobile form of storage rather than a simple transaction (1). This article explores some of the challenges, services, and technologies that go into ensuring that vaccines are properly temperature controlled and maintain product integrity for delivery to patients.

Varying requirements

Being particularly sensitive to environmental factors, vaccines need to be shipped and stored in exact accordance with standards set by the manufacturer. For this reason, understanding and adhering to the unique complexities of each vaccine shipment is crucial, explains Christine Noble, director of global marketing at Marken. For instance, some vaccines are sensitive to strong or fluorescent lighting. Others such as bacille Calmette-Guerin for tuberculosis and meningococcal for measles, mumps, and rubella, are extremely sensitive to heat, while some vaccines like diphtheria and tetanus are more sensitive to cold. Any temperature or lighting excursion outside of the manufacturer's indications will impact vaccine stability, which in turn can damage the potency or safety of the vaccine once administered to the patient, according to Noble.

According to Ben VanderPlas, global project manager at Sonoco ThermoSafe, temperature requirements of each vaccine shipment

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are also highly dependent on the companies' risk profile, stability data, and geography. He states that because of regulatory implications, "the same vaccine could be shipped with different temperature requirements depending on the region's requirements. In some cases, stability data can be leveraged, and in other cases the temperature control must be within the storage specifications on the label."

Areas of improvement

When it comes to the cold-chain delivery of vaccines, constant efforts toward improvement are crucial for finding suitable solutions to temperature-control issues, as well as risk management. Performing a detailed risk assessment, including lane verification, helps to ensure a less risk-sensitive process, says Noble. Packaging solutions, including active and passive thermo-regulated products, are essential to ensure shipments remain within proper temperature ranges while in route and storage, in addition to the use of products including dry ice and liquid nitrogen, added Noble.

Investing in phase change materials, insulation, services, and technologies to enhance performance while driving simplicity is key in the pursuit of cold-chain improvement, says VanderPlas. One such solution from Sonoco ThermoSafe is ChillTech, a range of 2 to 8°C pulse code modulation systems that provide temperature control for two to six days for payloads from 4–40L, compared to conventional shippers on the market with a maximum of four days of temperature control (2). The solution uses the company's patented Zero Bench-Time technology, enabling systems to be packed out directly from the freezer. Each solution has the same pack-out configuration and can be used year-round. Additionally, shipment duration can be changed using the outer insulated shipper from 48 hours to 96 hours to 144 hours.

Greatest challenge to cold chain vaccines

Ensuring that vaccines maintain proper temperatures throughout the cold chain process sits at the heart of challenges associated with getting vaccines to patients. This issue becomes more severe when it comes to global distribution. "The greatest challenge for [the] temperature-controlled distribution of vaccines is the last mile in developing nations," states VanderPlas. "Solutions exist for long distance to remote locations, but the challenge is maintaining temperature control from the remote hub to the patient. Many new technologies are emerging, but this remains today's biggest challenge."

While technological advancements in the cold chain delivery of vaccines can provide the most obvious solutions to delivery problems, utilizing the network capabilities established by global logistics providers plays a crucial role in maintaining a successful cold chain delivery process, according to Noble. Noble also notes that understanding the regulations and collaboration with regulatory bodies are necessary to ensure that vaccines reach the patients who need them most.

The role of new technologies

Working with new technologies to increase supply chain connectivity and advance the safe and less expensive shipment of vaccines is vital as pharma moves toward a more temperature-exact future with vaccines. Part of ensuring a successful delivery process is taking analytical measures to reduce the amount of errors made in manual data collec-

tion. One technological approach to tackling this is the use of radio-frequency identification (RFID) technologies, which can help enable the various track-and-trace steps that promote accuracy in data collection, explains Noble. Marken is working with pharma partners to explore how RFID can be used in supply chain solutions.

Technologies that track shipments include Marken's Maestro information technology system, which records clinical milestones in real time and is linked to tracking devices that provide live updates continuously from any location globally, according to the company.

Geo-fencing technology, which is used to define geographical boundaries along the vaccine cold-chain transport journey, can be used at airports or during the full lane that vaccine shipments travel to monitor the delivery process. Marken's Sentry GPS device can customize geo-fencing waypoints and status updates for each validated shipping lane, and transmits data in real time by communicating through customized cloud-based software, which integrates directly with the company's Maestro IT system. According to the company, the device and software are approved for use on more than 95% of all commercial airlines around the world.

Additionally, the company's 24/7/365 Global Control Center monitors shipments for company regions internationally. The center transmits data through the Maestro operating system, which uses the company's Sentry GPS tracking device. Noble also revealed the future release of the Sentinel device, which will provide tracking for individual components within each shipment, such as vaccine vials.

Data-gathering solutions are becoming a key factor in the successful shipment of vaccines. "As the number of high-value vaccinations for diseases such as cancer continues to climb, the demand for real-time data will continue to grow. This capability allows service providers to step in and mitigate potential risks to protect shipments from temperature deviations or theft, and hold their supply chain partners responsible if a deviation from the shipping plan occurs," states VanderPlas.

A system suited for this purpose is Sonoco ThermoSafe's PharmaPort 360 active system, which has an integrated telemetry system with data capabilities that include the ability to check in and monitor the temperature of a payload in real-time through a web portal and cell-network communication. Available data include internal and external temperatures, GPS location, remaining battery life, and the current speed of the container, added VanderPlas. Connection automatically shuts down when traveling by air, but resumes and communicates the in-flight data as the plane reaches its destination. The system also has geo-fencing-based security features and alert thresholds for a range of data that can be customized based on user request.

While the future of the cold-chain delivery of vaccines seems unwaveringly complex, the realization that technological innovation, as well as precision and cooperation from the various levels of supply chain, will drive manufacturers and global logistics providers to create more protective environments for vaccines, which will result in less waste and more vaccine potency for the patients who need them.

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Using Human Challenge Trials to Develop Flu Vaccines

Adrian Wildfire

Challenge trials may increase in coming years as new and improved challenge agents better emulate "natural" disease states.

The earliest recorded incidence of healthy humans being deliberately exposed to a virus dates back more than a thousand years, when people in China were challenged with mild strains of the variola virus in an attempt to ward off severe and disfiguring outbreaks of smallpox. However, it was not until the 20th century that formal challenge studies started, with one of the more familiar examples being the Common Cold Research Unit (CCRU) situated in the United Kingdom at Porton Down. The CCRU was set up in 1948 in a former army barracks, and volunteers were infected with rhinoviruses in an attempt to find a cure for the common cold. When the unit was shut in the 1980s, the model and technologies moved to a private venture (1).

Although a cure for colds remained elusive, the challenge technique attracted wider attention, and researchers elsewhere started to use live pathogens to research treatments for cholera, malaria, and norovirus. These human challenge trials are now routinely used by biotechnology and Big Pharma companies as Phase Ib and IIa proof-of-concept studies in the development of drugs and vaccines for various diseases, but particularly influenza.

Trials involving live challenge agents are best performed in specific trial units, where strict infection control requirements can be met. The design of these trials is slightly different from a traditional trial in that the duration of subject isolation, schedule of assessments, and nature of the samples required will depend both on the agent employed and the therapeutic being tested.

A human challenge study offers several advantages when testing drugs or vaccinations for infectious diseases, because proof of efficacy can be measured in humans across a defined period of infection as the exact time at which exposure to the infectious agent is known.

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Usually this knowledge as to when infection takes place is unknown, and therefore it is also undeterminable as to where on the disease cycle the subject is. Sampling events are scheduled to take specimens at critical periods, and symptomology is carefully monitored throughout. The ability to schedule interventions is important to assess whether the timing of doses is critical to the therapy's effectiveness.

It is important that the challenge agent being used should be as close as possible to the currently circulating strains so that the naturally occurring disease is mimicked. The challenge agent should be able to induce disease in healthy adults, have a known route of transmission, and demonstrate a well-defined disease progression in terms of the severity of symptoms and the signs and timing of their emergence.

Correctly designed and executed, a human challenge trial can act as a cost-effective bridge to wider field trials, allowing alterations to the therapeutic dosage or dosing schedule to be made before expensive large-scale trials are embarked upon.

As yet, there are no definitive rules for the manufacture of challenge agents, but adherence to current good manufacturing practice (cGMP) guidelines is expected. Batches of manufactured virus need to be tested for purity and the absence of adventitious agents (i.e., other viral or bacterial contaminants), toxicology or safety studies in animals carried out, and a characterization or a titration study run in humans to identify the number and degree of both virus and host changes observed before it is authorized for use in a clinical trial. A Clinical Trial Authorization (CTA) in Europe, or investigational new drug (IND) status in the United States, will only be approved for a challenge agent if it can be shown to be a quality consistent with cGMP and safe (producing neither serious, immediate, or short-term adverse events nor long-term issues [sequelae]) (2).

There are many considerations to be taken into account when planning a challenge trial. For example, subjects must not previously have been exposed to the strain of virus that is being used as the challenge agent if a high attack rate is to be achieved (i.e., they should be broadly sero-naïve); their immune status is assessed via a serum antibody assay as part of the pre-enrollment screening process. They must also be quarantined at day 2, prior to challenge to allow any incubating illnesses to develop. This pre-trial quarantine helps prevent co-infections in subjects that could compromise the trial data and stops any unplanned virus from entering the unit and being spread to other subjects.

The human challenge model in influenza

Speed is of the essence when developing and testing seasonal influenza vaccines even in a normal flu season; an effective vaccine is paramount in reducing the disease burden especially in the elderly. But in a pandemic season, where a particularly virulent and pathogenic strain predominates, the ability to quickly and effectively protect the general population is vital in counteracting the rapid global spread of a disease with often high accompanying mortality rates. A human challenge study allows the relative efficacy of both live and inactivated candidate vaccines to be established in the context of real infections. The studies give a rapid read-out of both viral kinetics and host dynamics and these may be related to proven correlates of protection where known.

Human challenge trials are principally used for dose finding and proof of concept, but more commercial aspects of the vaccine can be assessed too. In the right setting, it has a number of advantages

over a more traditional Phase II field study, as the number of required cohorts for a challenge study is typically 60–100 subjects, compared to much larger groups of 250–300 in even a small field study. In the field, the attack rate is typically low and dependent on prevalence, also there is no way of knowing when the subject may have contracted the infection. In a challenge study, the attack rate is purposely high, and the inoculation date is known precisely.

The environmental and clinical conditions of a challenge study are strictly controlled, and a trial typically takes 28 days; whereas, in the field, the environment is uncontrolled and trials are likely to last at least one to two years, covering both hemispheres, depending on the incidence and the seasonality of disease. Kill/no-kill decisions regarding a candidate drug or vaccine can be made much earlier with a challenge trial as extensive data analysis is unnecessary and results may be used to predict how a field trial might best be designed.

This all amounts to a significant cost saving, with a challenge study typically ranging from $2–3 million. In comparison, a field trial is likely to cost at least twice as much with 10 times the number of subjects (3).

Strain selection options

Selecting the optimal strain is an important part of achieving a successful trial for influenza therapeutics and vaccines, and the prevention of future pandemic diseases. Although it is not required by good clinical practice (GCP) or noted as essential by competent authorities (4), in reality, the use of a newly circulating strain, with characteristics close to Wild Type is more likely to provide relevant data regarding vaccine efficacy. Over the past century, most pandemics have arisen from either H1N1 or H3N2 strains of influenza A, and currently circulating, seasonal strains are related to such previous pandemic strains. For example, the 2009 swine flu was an H1N1 strain with roots that can be traced back to the 1918 Spanish flu outbreak.

Most currently circulating influenza A strains are local varieties of H1N1 or H3N2 and retain a pandemic potential. Influenza viruses routinely mutate and occasionally re-assort (i.e., take genes from other related flu strains), in a process commonly referred to as drift and shift. Such changes to their antigenic makeup may be sufficient to cause new outbreaks of disease. Other, phenotypic changes may occur; notably the H3N2/Switzerland 2013 strain that is currently circulating has lost the ability to agglutinate red blood cells, which has prevented its detection by standard screening assays (i.e., the hemagglutination inhibition [HA or HAI] assay).

Both H1N1 and H3N2 seasonal strains are appropriate as challenge agents, whether in influenza-specific trials or for more general studies regarding infections of the upper respiratory tract. For such a trial, a strain that has little chance of becoming highly pathogenic should be chosen. Any virus selected for use in clinical trials (regulated by GCP) must be grown according to cGMP; either in cell lines or in eggs. If the virus is manufactured correctly and does not become egg or cell-line adapted, healthy volunteers who are inoculated with the virus in human challenge trials will have an 80–90% chance of becoming infected. This high attack rate (AR) greatly reduces the number of subjects that would need to be enrolled in a challenge trial compared to one based in the community, where infection rates from local, wild type virus are 5–10% at best.

However, it is not just high ARs and good shedding profiles (i.e., the measured amount of virus shed by

the subject following infection) related to the virus that are important. Host characteristics, such as clear and consistent symptomology, related to the viral infection are essential for measuring drug and vaccine efficacy against disease severity. Those who succumb to infection are likely to experience a raised temperature (pyrexia), sore throat, aching limbs, headaches, and a feeling of lethargy or tiredness. Seasonal strains typically do not cause severe disease states; however, newly emergent pandemic strains are dangerous because, in often otherwise fit and healthy patients, they may trigger an uncontrolled cytokine cascade that could potentially lead to rapid hospitalization and even death. Strains chosen for a challenge trial are sufficiently "attenuated", or weakened by a period of circulation in the populace so that they have lost those parts of their genome that give rise to dangerous immune reactions and severe symptoms.

H1N1 and H3N2 both have their advantages and disadvantages as challenge agents. Seasonal strains of H3N2 usually lead to more severe symptoms than H1N1, and H3N2 has a mean age of attack that is about a decade younger than H1N1. Also, the broader viral shedding period and clearer symptoms that are common with H3N2 can be advantageous, as it makes it easier to estimate the effect that therapeutic interventions are having (i.e., the delta or δ effect). However, all this does not eliminate H1N1 as a viable challenge agent. For example, H3N2's loss of ability to hemagglutinate makes it more difficult to identify as a circulating virus using traditional testing models, and the greater stability and long-term persistence of the antigenic structure of H1N1, allied to its equally long-lived circulation, means it remains relevant as a challenge agent.

Because of the tendency of the prevalent circulating viruses to change over time, it is important to develop new challenge agents to maintain such relevance. SGS has developed a new challenge strain named A/Belgium/4217/2015 [H3N2], which has now been successfully tested in both a first-in-human and a commercial challenge trial (following approval for use granted in late 2016). The challenge agent, a drifted strain of the Swiss-2013 virus, was grown in eggs and passed exacting standards for purity (i.e., the presence or absence of adventitious agents, namely common viruses, spiroplasma, and mycoplasma species in addition to contaminating toxins and allergens).

When testing the virus in the first-in-human trials, three cohorts of 12 subjects who had not previously been exposed to the Belgium strain were challenged with increasing doses of the H3N2 strain: the first cohort with 10^5, the second with 10^6, and the third 6.78×10^6. Measurements of virus, subject symptoms, and clinical laboratory safety tests were made in order to assess the optimal dose level of the virus (high attack rate, safety, and tolerability).

The highest dose gave a 100% attack rate in subjects with no pre-existing protective antibodies and the degree of viral shedding could be seen to increase in proportion to the challenge dose of the virus (i.e., giving more virus resulted in higher peaks and longer shedding during infection). Subjects started to manifest symptoms of influenza approximately 36 hours after shedding was detected and 24 hours following the peak in detectable virus. The virus and host measurements, as well as the biomarkers examined, were consistent with a mild, wild-type influenza infection. Such a retention of wild type characteristics is an important consideration when developing any challenge agent.

Where next for human challenge trials?

Various US bodies have expressed interest in opti-

mizing the potential of the human challenge model to shorten drug and vaccine pipelines, including the National Institute of Health, the National Institute of Allergies and Infectious Diseases, and the Biomedical Advanced Research and Development Authority (5). Such trials are becoming increasingly popular in drug development for respiratory illnesses, and in particular in acute, potentially fatal viral infections in pediatric populations and the elderly, where the need to accelerate drug development is significant. There is also potential to apply the model to other types of infectious agents, such as bacteria and parasites, once it is possible to manufacture attenuated strains, according to cGMP (6).

There are many diseases that might, in time, be applicable to such proof-of-efficacy studies, or even a pivotal study in place of a community-based Phase III proof of efficacy (PoE) trial, particularly when the healthy trial patients closely resemble the target population for the therapeutic. It is likely that the size and diversity of the subject cohort might have to be expanded to better reflect the natural patient population, involving less stringent exclusion and inclusion criteria.

While the regulatory authorities are broadly becoming more comfortable with the concept of the human challenge trial, it still remains more of an exception than the rule and is currently limited to the development of vaccines and drugs for influenza and a small number of other, largely respiratory or diarrheal illnesses. FDA is increasingly reluctant to license new influenza treatments solely on the basis of biomarker studies and requires evidence of a positive effect in sick people with tangible reductions in symptoms and improvements in quality of life, rather than surrogates or correlates of efficacy. Observing such effects are frequently more cost effective and faster to achieve in a challenge trial than in community-based studies, and it is likely that the adoption of appropriate challenge trials will only increase in coming years as new and improved challenge agents better emulate "natural" disease states.

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