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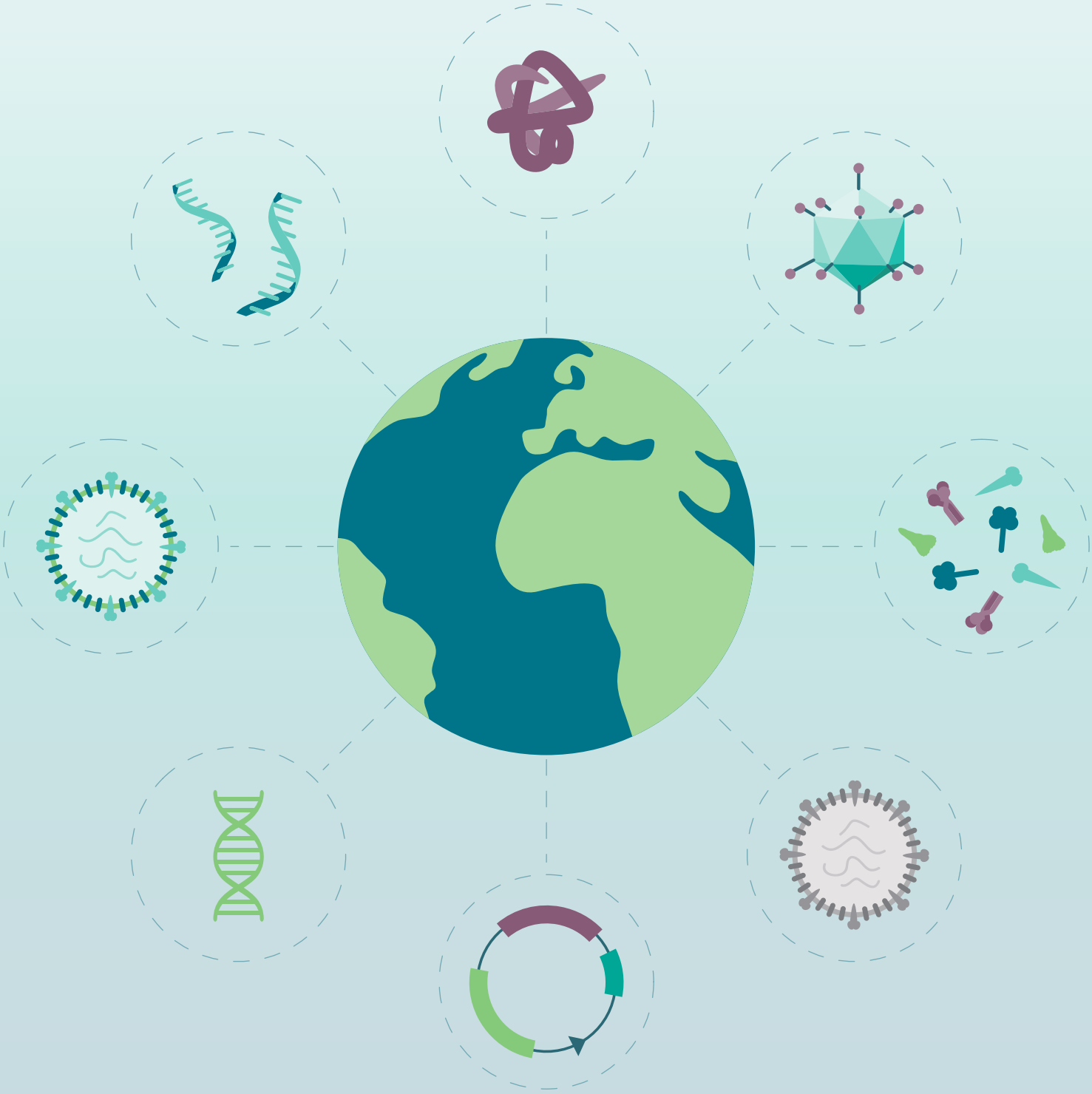
VACCINE INSIGHTS

SPOTLIGHT ON
Manufacturing: upstream and raw materials



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SPOTLIGHT

Spotlight on vaccine manufacturing

Cleo Kontoravdi, Simon Daniel, and Nilay Shah



"In this issue, we focus on steps needed to achieve global equity in vaccine access for both future pandemic situations and routine vaccination needs."

FOREWORD

Vaccine Insights 2024; 3(2), 77–80

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The unprecedented rate at which SARS-CoV-2 vaccines were developed and manufactured at a global scale has unveiled a new potential paradigm for vaccine development, while also highlighting the centralized fashion in which targets have been selected and vaccines produced to date. In this issue, we focus on steps needed to achieve global equity in vaccine access for both future pandemic situations and routine vaccination needs.

First, an interview with Olga Rovira, a Regulatory Affairs expert, discusses hurdles for vaccine manufacture for the global population, focusing on regional regulatory discrepancies and the need for guidance harmonization.

Weller and Hayes discuss the need for and challenges of transferring vaccine manufacturing technologies to countries and regions where the vaccine is most needed. They give the example of the oral cholera vaccine and review opportunities for local manufacture in Africa and Asia to control and eventually prevent local outbreaks.

An interview with Gaurav Gupta, a vaccine R&D expert currently working on strengthening manufacturing capacity in Bangladesh, discusses these challenges from the point of view of a low- and middle-income country manufacturer and highlights the sustained importance of established platform technologies that are low-cost and in which know-how already exists. In the same vein, Kathleen Hefferon's contribution brings to the forefront of the discussion the promise of plant-based vaccines as a cost-effective manufacturing solution based on advances already made in the space of plant-derived macromolecules, such as monoclonal antibodies. Finally, Wong and Ruxrungtham review existing manufacturing capacity in South East Asia, with an emphasis on Thailand, Malaysia, Indonesia, and Vietnam, and recent developments in the establishment of regional hubs in the area, and discuss priorities for achieving manufacturing self-reliance.

Taking a more general standpoint, Anubhaw Kumar Singh, a development and manufacturing expert, outlines key challenges in technology transfer and highlights the need for greater collaboration between industry and academia for the development of novel, IP-free solutions, as well as across the life sciences sector to remove barriers to tech transfer.

In closing, we look at manufacture in conjunction with procurement of raw materials and product distribution in an interview with Kilian Mullett, Senior Director of Commercial Supply Strategies at Pfizer, who discusses the logistics of maintaining warm-base manufacturing facilities and the conditions necessary for achieving equitable vaccine distribution.

BIOGRAPHIES

CLEO KONTORAVDI received MEng and PhD degrees in Chemical Engineering, both from Imperial College London, then joined Lonza Biologics as a Research and Development Scientist. In 2007 she went back to Imperial as a Lonza/RCUK Fellow in Biopharmaceuticals Processing, where she is currently Professor of Biological Systems Engineering. Her research involves the development of comprehensive platforms that synergize modelling with experimentation for bioprocess understanding, design, and optimization. She collaborates extensively with industrial partners, including GSK, AstraZeneca, Amgen, and vaccine manufacturers around the world.

SIMON DANIEL is a PhD student at the Sargent Center for Process Engineering, Imperial College London. After obtaining a MSc in Life Sciences Engineering from the Swiss Federal Institute of Technology in Lausanne (EPFL), he worked in the Future Vaccine Manufacturing

Hub. Simon created a new framework for RNA vaccines development and performed techno-economic modelling of the RNA manufacturing landscape. Simon then joined the CEPI-Wellcome Leap's R3 program, where he implemented process digital twins in rapid-response, continuous RNA vaccine platforms. His current research focuses on modelling novel production processes, the development of innovative soft sensors and data-driven approaches for vaccine formulation design.

NILAY SHAH has just completed a term as the Head of the Department of Chemical Engineering and was formerly the Director of the Centre for Process Systems Engineering (CPSE) at Imperial, and a Chemical Engineer by training. He has co-authored over 300 technical papers on process systems modelling and engineering, design, and optimization of low carbon industrial systems, biochemical processes, vaccine manufacturing systems, sustainable energy systems, hydrogen economy, supply chain modelling, process scheduling and optimization, and plant safety and risk assessment. Nilay Shah has received several awards and he is particularly interested in the transfer of technology from academia to industry. He has provided consultancy services on process optimization, innovation, and industrial applications of new technology to a large number of process industry and energy companies. He is a member of the UK Government's Hydrogen Advisory Council and the Vaccine Development Working Group.

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VACCINE INSIGHTS

MANUFACTURING:
UPSTREAM AND RAW MATERIALS

SPOTLIGHT

INTERVIEW

Technical development and manufacturing of vaccines in the context of worldwide regulatory requirements

Getting safe and effective vaccines to market quickly is essential in a pandemic situation, but this can be made more difficult by differing regional regulatory requirements. **Casey Nevins**, Assistant Editor, *Vaccine Insights*, speaks with **Olga Rovira**, Regulatory Affairs Senior Consultant in Vaccines and Managing Director, KONTIVAX SRL, about how worldwide regulatory requirements impact the technical development of vaccines, and potential solutions to this challenge.

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Q How did you get involved in the vaccine space?

OR: Around 20 years ago, I moved from Germany to Belgium and simultaneously shifted from working in recombinant protein and monoclonal antibody R&D to vaccine regulatory affairs, specifically in CMC. The decision to pivot stemmed from a desire to gain a broader

"...greater global harmonization and collaboration between regulators across countries and regions is essential."

perspective on the development of medicinal products. I wanted to be involved with the whole lifecycle of a product. In my new role, I could be a part of the process from the discovery phase, through transitions into clinical development, Phase 1, Phase 2, Phase 3, licensing, and then post-licensing.

My work now revolves around two main areas. Firstly, I support vaccine development for indications like COVID-19, rift valley fever, chikungunya, tuberculosis, and influenza. Secondly, I support pandemic preparedness regulatory initiatives, developing mechanisms and tools that can be readily deployed in the event of a future pandemic.

Q What key lessons can we learn from the COVID-19 pandemic about getting safe and effective vaccines to market as quickly as possible?

OR: One crucial takeaway from the COVID-19 pandemic is the importance of having invested previously in technologies and R&D. It is evident that allocating multiple and diverse resources to emerging technologies (even if they initially appear to be unpromising) pays off. Prior to the COVID-19 crisis, mRNA technology was in development, but no vaccine product utilizing that technology had been marketed yet. However, due to prior R&D work generated, the first mRNA product became quickly available.

Another positive takeaway from the COVID-19 pandemic is the readiness with which some governments supported at-risk investments. Governments deployed significant funding to support companies to develop solutions, and this readiness to support such risky investments was instrumental in accelerating vaccine development.

Furthermore, the collaborative effort, relentless spirit, and hard work of people in the field were pivotal in the fast development and regulatory authorizations of the first COVID-19 vaccines. I feel honored that I was part of one of the many teams worldwide contributing to that endeavor. Though we were all very much under stress, that collaborative spirit was very rewarding and I would hope to see that attitude repeated should we ever face another public health emergency in the future.

Considering areas for improvement, greater global harmonization and collaboration between regulators across countries and regions is essential. Regulatory processes and requirements remain diverse across regions despite there being a unified scientific understanding supporting the development of vaccine products.

Additionally, there is a need to pre-establish certain protocols and procedures. Establishing frameworks for preliminary actions ensures that crucial steps have been assessed, discussed,

and agreed upon in advance. This proactive approach minimizes delays during emergencies, as preparations are already in place beforehand.

Q What are the most important principles of vaccine development as you see them?

OR: The most important principle of vaccine development is safety. Safety is at the forefront of everything we do, in all areas of vaccine development. However, it is important to understand that safety does not translate into a product that will never cause an adverse event. Instead, it entails an appropriate understanding and assessment of the overall benefit–risk associated with the vaccine product for the targeted population and medical indication.

Moreover, upholding high-quality scientific standards and transparency when developing these vaccines is key. From laboratory research to clinical trials, scientists in all working areas involved must be transparent about methods and results, even if those results may sometimes be unfavorable. Maintaining integrity assures credibility and trust.

Q How do different regional regulatory requirements impact the technical development of vaccines?

OR: Being exposed to different regional regulatory requirements calls first for having a deep understanding of regulatory diversity, followed by a comprehensive pre-planning of technical activities during vaccine development, in line with quality management systems. While these requirements may not always directly influence the technical aspects of vaccine development, they do shape the overall regulatory strategy and associated timelines. As an example, post-approval technical changes requiring regulatory approval have historically taken years to be completed for the same vaccine product registered in multiple countries/regions. Oftentimes, this has led to maintaining multiple manufacturing and supply options in parallel, which favors neither manufacturing capacity nor timely vaccine access.

Q What elements are desirable in the regulatory CMC landscape across different regions, and how would this benefit patients?

OR: Considering the different requirements in the regulatory CMC landscape across different regions is crucial for streamlining processes and ultimately benefiting patients. Achieving harmonization and/or a much higher level of convergence across different regions or

"I am a big supporter of platform technologies since they could allow for accelerated vaccine development, without compromising safety or quality."

health agencies is very desirable as this would facilitate a smoother and more efficient pathway for drug approval and access.

Harmonization entails ensuring that regulatory requirements are consistent across regions, thereby reducing the burden on both pharmaceutical companies and regulatory bodies. Achieving global harmonization remains a lofty dream. As an example, today there still exist countries that have not adopted international standards like those of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).

One potential, though idealistic, solution would be to establish a single worldwide regulatory body. While this solution would make harmonization efforts unnecessary, it is unlikely to occur since it undermines the sovereignty of individual nations to determine healthcare decisions for their populations.

More realistically, collaborative approaches and work-sharing initiatives could be applied more often, which would increase patient access to medicines. The centralized procedure in the European Union (EU) is an excellent example of how EU member states, together with the EMA, contribute to the assessment of human medicinal products collaboratively.

Q Are there other guidelines or regulatory evolutions related to vaccine products that you would like to see?

OR: I am very keen to see future guidelines around platform technologies. I am a big supporter of platform technologies since they could allow for accelerated vaccine development, without compromising safety or quality—elements that are needed in a pandemic situation. In the short term, I would love to see technical guidelines on how to enable efficient and flexible use of platform technologies while meeting product-related regulatory expectations. Many developers already know how to use these technologies from a technical perspective, but they seek guidance on ensuring regulatory acceptance, and also regulatory procedures for submitting these technologies irrespective of any specific vaccine product.

Another promising area where I would like to see regulatory progress for vaccine products is in modeling manufacturing processes. Increasing our understanding of how to develop, validate, and utilize such process models can lead to more efficient vaccine production development. By leveraging modeling tools, we can reduce reliance on empirical methods without compromising quality, safety, and efficacy. However, concerns about these tools may arise if they are perceived as being used to cut corners. Regulatory guidance is therefore crucial to support developers interested in moving in that direction.

BIOGRAPHY

OLGA ROVIRA has an academic background in industrial chemistry engineering from the University of Zaragoza, Spain and in structural molecular biology from the University of London, UK. Olga is currently working at KONTIVAX, her own regulatory affairs (RA) consultancy company, which serves small biotech companies, large multinational corporations, as well as partnerships such as Coalition for Epidemic Preparedness Innovations (CEPI) or TuBerculosis Vaccine Initiative (TBVI). Her expertise focuses on RA chemistry, manufacturing, and controls (CMC) for vaccines. She has a solid knowledge and understanding of technical development of vaccines throughout the whole development path up to registration and subsequent life-cycle stage and how this relates to worldwide regulatory requirements. She is member of The Organisation for Professionals in Regulatory Affairs (TOPRA).

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VACCINE INSIGHTS

MANUFACTURING:
UPSTREAM AND RAW MATERIALS

SPOTLIGHT

How can investing in technology transfers help build a sustainable manufacturing ecosystem? An example from cholera

Jeanette Hayes and Charlotte Weller
Wellcome

"New manufacturers with greater geographical diversity for oral cholera vaccine are required"

VIEWPOINT

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THE CHOLERA PANDEMIC

The world is currently facing an upsurge of the seventh cholera pandemic, with Zambia being the latest country to report unprecedented outbreaks with more than 12,000 cases and 467 deaths already in 2024 [1]. Cholera is an acute diarrheal disease that

spreads rapidly throughout communities faced with inadequate access to safe water and basic sanitation, with the greatest burden occurring in Africa and Asia [1]. This burden has been compounded in recent years by an intensification of the climate crisis, conflicts, political instability, and humanitarian emergencies [2]. Whilst long-term disease control will rely on access to safe water and sanitation, vaccination is key to immediate control.

In 2013, the WHO created a global stockpile of oral cholera vaccine (OCV) [3]. Since then, demand for vaccines has grown, with Gavi estimating the average annual demand between 2023 and 2030 to be approximately 85 million doses [4]. The stockpile originally included Shanchol™ (Shantha Biotechnics) and Euvichol-Plus® (EuBiologics Co., Ltd), however Shantha Biotechnics ended production and distribution of Shanchol™ at the end of 2023 [5]. This left EuBiologics as the sole manufacturer of cholera vaccines. As outbreaks continue to rise and with new manufacturing capabilities taking years to build, a worrying and increasing mismatch between supply and demand is projected. As a short-term solution, in 2022 the International Coordinating Group (ICG; the body that allocates OCV), switched from a two-dose to a one-dose OCV strategy for outbreak response campaigns [6].

A year later, the one-dose strategy still prevails. In 2023, all 36 million OCV doses manufactured were delivered in one-dose reactive campaigns, with a remaining estimated 70 million annual dose gap [7]. New manufacturers with greater geographical diversity for OCV are required, but the question the world was faced with was 'how can supply be rapidly increased?'

WHAT ACTION IS BEING TAKEN?

Whilst cholera outbreaks were increasing, the world's attention was on the recovery from COVID-19 and preparation for future outbreaks, with equity at the heart of many of these discussions. Significant political attention led to the African Union and Africa Centres for Disease Control and Prevention launching the 'Partnerships for African Vaccine Manufacturing' (PAVM) in 2021. The goal was to strengthen the African vaccine manufacturing ecosystem, with the ambition of locally manufacturing 60% of Africa's routine immunization needs by 2040 [8].

In response, Wellcome commissioned a report in 2023 to better understand the perspectives of African manufacturers on the challenges to scaling up vaccine-manufacturing capacity and capabilities. The three main challenges (beyond market-access prerequisites) that were highlighted for support were [9]:

1. Access to finance: provision of specialized and cost-effective funding options with extended repayment periods;
2. Talent: provision of assistance to support African manufacturers in acquiring practical experience through secondments with seasoned manufacturers and facilitation of international experts in local manufacturing operations;
3. Transfer of technology: funding and collaboration with African manufacturers to facilitate the transfer of technology, enabling capacity development so these manufacturers become viable candidates for private partnerships.

Global discussions on increasing manufacturing capability and technology transfers have centered around mRNA vaccines, with multiple new initiatives (e.g., WHO mRNA hub, BioNtech/CEPI) [10,11]. The unanswered question is how to sustain these new initiatives. With the exception of COVID vaccines (where demand is waning), there are no vaccines licensed on an mRNA background. For manufacturers to build a sustainable business there needs to be a clear demand for the vaccine.

Gavi published a white paper in November 2022 highlighting a clear supply need for certain vaccines in their portfolio

and signaling possible market demand that would be attractive for manufacturers. This included cholera, measles-rubella, yellow fever, Ebola, and malaria vaccines [12]. Given that the African continent has the largest cholera burden, but that no cholera vaccines are manufactured on the continent, this emulates the exact vaccine equity that the PAVM was initiated to address.

A STEP CLOSER TO SUSTAINABILITY AND INCREASED OCV SUPPLY

Technology transfers are not straightforward—they can fail at many different stages and take significant time (e.g., the transfer of Hib vaccine to Fiocruz from GSK took 8 years) [9]. This is often a barrier to bringing a new platform, vaccine, or part of the process into a manufacturing organization, and where philanthropy is often called on to contribute. In November 2022, with funding from the Gates Foundation and Wellcome Trust, the International Vaccine Institute (IVI) entered into a technology transfer agreement with Biovac to manufacture OCV-s (simplified OCV) in Africa, with the long-term goal of WHO pre-qualification [13]. Whilst there are many barriers to face before this transfer starts to alleviate the strained stockpile, it is a start towards developing a diversified OCV manufacturing ecosystem.

The technology transfer brings with it the opportunity to build talent and expertise in the African continent by transferring know-how to Biovac, as well as the capabilities to develop and manufacture drug substance. OCV-s has a clearly defined regulatory pathway to licensure; the South African Health Products Regulatory Authority can build expertise in overseeing and regulating the manufacture of clinical trial products and building an ecosystem capacity beyond Biovac within South Africa.

The Global Task Force for Cholera Control (GTFCC) was endorsed by the World Health Assembly in 2018 to reduce cholera deaths by 90% and eliminate local transmission in at least 20 countries by 2030 [14,15]. Alongside a reliable, geographically diversified supply, other evidence gaps such as effective prediction of local outbreaks and increasing cholera incidence are critical to enable effective cholera control. This type of holistic approach is needed to connect research on transmission, vaccine duration and effectiveness, water, sanitation, and hygiene, together with policymakers at a global, regional, and national levels. These ambitious goals merit a concerted global effort to improve the reactive and unsustainable cholera control options many countries require today alongside cholera control in the future through preventative OCV campaigns.

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BIOGRAPHIES

JEANETTE HAYES is a Research Manager in the Vaccines team which sits within the Infectious Disease Health Challenge area at Wellcome. She joined Wellcome at the beginning of 2023 and has been involved in managing a portfolio of projects that largely focus on cholera, ranging from early product development for new cholera vaccines to later stage vaccine effectiveness studies generating new evidence required for policy decisions. Before joining Wellcome, she worked in public health in an evidence and intelligence team for a local council in the UK, carrying out data analysis which was used to help guide local health policy decisions. Prior to working Jeanette studied infectious diseases for 4 years at the University of Edinburgh before completing her masters at the London School of Hygiene and Tropical Medicine.

CHARLOTTE WELLER is Head of Prevention in the Infectious Diseases Area at Wellcome. Since 2016, she has led a team to develop new and improved vaccines and antibodies, gaining a better understanding of protective immunity and strengthening the connection between research and decision makers. Charlotte joined Wellcome in 2014 and led the funding response to the Ebola epidemic of 2014 to 2015 and managed the epidemics research activities. Charlotte was involved in founding CEPI and currently chairs CEPI Investors Council and contributes to a number of national and international advisory committees in vaccines and epidemics. Before joining Wellcome Charlotte gained over 16 years of research experience in both academic and pharmaceutical environments, ranging from host pathogen interactions to cellular and molecular immunology. Including leading target identification and validation in the respiratory disease area at Novartis. Prior to this, Charlotte investigated cell localization and function in health, respiratory disease and parasite infections as a postdoctoral fellow at Imperial College London. She holds a PhD in Immunology at Imperial College London.

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VACCINEINSIGHTS

MANUFACTURING:
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SPOTLIGHT

INTERVIEW

Advancing global vaccine production

With a career spanning multiple sectors, countries, and platforms, independent consultant **Gaurav Gupta** has a wealth of experience in vaccine research and development, manufacturing and CMC. **Charlotte Barker**, Editor, *Vaccine Insights*, caught up with Gaurav to discuss his current work on strengthening manufacturing capacity in Bangladesh, the prospects for animal-origin-free production, and why newer isn't always better when it comes to platform technology.

Vaccine Insights 2024; 3(2), 1–4

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Q How did you get your start in the vaccine field?

GG: I studied veterinary microbiology and immunology, but I was always most interested in applying science to real-world problems. I went on to join the pharmaceutical industry, working in research and development, process development, tech transfer, CMC, and manufacturing. Over the past 19 years, I have worked on live, inactivated, subunit proteins/VLPs and viral vectored vaccines for a range of companies, including Speransa therapeutics, Zydus Cadila, and Panacea Biotec. I have worked on most aspects of vaccine production, from transitioning to animal-origin-free processes to tech transfer of new vaccine platforms and setting up new GMP manufacturing facilities.

More recently, I made a foray into academia at Oxford University's Jenner Institute, where I worked on designing virus-like particle malaria vaccines, and downstream process development for the ChAdOx COVID-19 vaccine. I then joined the UK Vaccines Manufacturing and Innovation Centre as a CMC lead, helping to set up labs' infrastructure for flexible manufacturing of different types of vaccines (mRNA, viral vectors, subunit proteins, conjugated, live, and inactivated). Now, I work as an independent consultant to the vaccine industry.

Q What projects are you particularly excited about?

GG: Right now, I'm associated with a scientific advisory team to the Bangladesh government, which is committed to setting up new vaccine manufacturing facilities in the country. Currently, Bangladesh has limited capacity in the private sector to produce vaccines locally, hence the government recognizes that they urgently need to further boost capacity in the country from discovery and development to high-value manufacturing. Their approach is to start by buying the drug substance and carrying out the fill-finish, to build a pool of trained staff, before expanding into full-scale manufacturing.

They also have plans to partner with US biotech company Dyadic International to leverage their high-yield fungal expression platform for producing protein. The platform can produce proteins at grams/liter, and could be an interesting solution for Bangladesh and other developing countries, allowing them to produce more product in a smaller space. It is a similar concept to China producing HPV vaccines using an *E. coli* expression platform, which has been successful in bringing down costs.

The Bangladesh government is also building the country's R&D capabilities, to allow research on priority pathogens such as Dengue and Chikungunya, to build future pandemic response. The aim is to produce relevant vaccines within a 100-day timeline. A major new animal facility is being planned to make the country a hub of drug discovery for preclinical testing, bridging the enormous potential of clinical trials with a population of over 170 million. At present, local institutions are forming a global consortium spanning numerous UK universities including Oxford and Sheffield, and partners from the Netherlands, Germany, South Africa, and India to name just a few.

Q What technologies will help the vaccine field progress?

GG: Protein-based adjuvanted vaccines are a long-established and safe platform, and I believe not enough effort has gone into improving formulation and manufacturing processes to get them into the clinic faster. Novel technologies such as mRNA are attractive to scientists because they can be rapidly modified. However, costs are high, the long-term safety profile is not yet available, and they require ultra-cold chain delivery [1].

Making improvements to longstanding vaccine platforms is important work, but often less attractive to large pharmaceutical companies, and smaller companies find it hard to get funding.

"...governments should be proactive and continue to invest in state-owned vaccine manufacturing facilities to be ready for the future."

Q You have previously worked on developing animal-origin-free processes. How close are vaccine manufacturers to the goal of 100% animal-origin-free?

GG: Work towards this goal is progressing well, but barriers remain, particularly in terms of cost. Recombinant sources of products like human serum albumin are often significantly more expensive than traditional animal sources. Recombinant excipients are also more expensive and high volumes are needed. The demand cannot always be met by suppliers currently, so scale-up is needed on the supplier side too.

There can be quality and efficacy issues with recombinant products too, due to endotoxin contamination, or the difference in formula compared with highly complex animal-origin products.

It is easier to apply these principles to new products than to make changes to an existing process. Regulators are supportive of post-approval changes to remove animal-origin components but the cost implications of pausing production to validate a new process are a barrier, especially for smaller companies.

However, with increasing regulatory pressure toward animal-origin-free products, and a real willingness to change within the industry, I think the challenges will be overcome.

Q How would you like to see the vaccine ecosystem evolve?

GG: I want to see industry and academia putting people above profit, putting aside rivalries, and concentrating on getting the best product into the clinic. When there is a pressing need, multiple companies should be pursuing different vaccine candidates, with the best and safest products across the board selected to go forward. Otherwise, there is a risk of becoming blinkered and committing too soon to a specific platform that might not be the right fit.

I would also like to see more consistent funding for pandemic preparedness and vaccine development. Now that the acute phase of the COVID-19 pandemic is over, government funding is being reduced, leading to the closure of pandemic vaccine manufacturing facilities such as VMIC. Ideally, I believe governments should be proactive and continue to invest in state-owned vaccine manufacturing facilities to be ready for the future.

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BIOGRAPHY

GAURAV GUPTA is a highly experienced senior researcher and leader with 19 years of experience and a PhD in Virology and Vaccinology. He has worked in both industry and academia, holding senior roles in biotech companies such as Biomed, Panacea Biotech, and Zydus Cadila. Gaurav contributed to the development of malaria and Oxford/AstraZeneca COVID-19 vaccines while serving as a Senior Research Scientist at the Jenner Institute, University of Oxford. As the CMC lead at VMIC, UK, from 2020 to 2022, he set up vaccine platform labs and managed client projects. Since April 2022, Gaurav has been an independent consultant, and worked recently on novel viral vectored COVID-19 vaccine development with Speransa Therapeutics in Germany. His expertise covers the entire vaccine development lifecycle, including design, execution, strategic and project management, with proficiency in various vaccine types. Gaurav's knowledge spans processes from conceptualization to market release, and he has successfully delivered diverse vaccine projects for clinical trials phases 1, 2, 3, and commercial production. He has also made significant contributions to the field with approximately 35 publications and 7 patents in immunomodulation, vaccinology, and immunodiagnostics.

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VACCINE INSIGHTS

MANUFACTURING:
UPSTREAM AND RAW MATERIALS

SPOTLIGHT

The power of plants as vaccine production platforms

Kathleen Hefferon
Forte Protein

"...plant virus nanoparticles are biodegradable, non-pathogenic toward humans, and can be scaled up rapidly."

VIEWPOINT

Vaccine Insights 2024; 3(2), 47–49

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For most of us, the old Hippocratic adage 'Let thy food be thy medicine' refers to leading a healthy life by taking advantage of the nutrients in plant-based foods. Indeed, plants have been a source of medicine since our earliest records of mankind. Over the past quarter century, however, this saying has taken on a new meaning for a group of scientists whose expertise resides in plant synthetic biology.

Today, the use of plants as platforms for the production of vaccines, monoclonal antibodies, and other therapeutic agents continues to develop as a discipline known as plant molecular farming [1]. Vaccines produced in

plants can be cheap to make, easy to scale up, safe from human pathogens, and, in many cases, can be maintained for lengths of time at temperatures that make them conducive for storage and distribution in low- and middle-income countries (LMICs) [2,3].

There are a growing number of examples of how plants have been harnessed to make vaccines. For example, during the COVID-19 pandemic, research groups from around the world worked to develop an effective vaccine from plants. One of these groups, in a Phase 3 multinational randomized and placebo-controlled clinical trial with 24,141 volunteers, demonstrated the high efficacy of virus-like particles (VLPs) expressing the spike protein of SARS-CoV-2 [4]. The work, initiated by the plant molecular farming company Medicago Inc., prevented illness from a spectrum of COVID-19 variants.

These vaccines represent a means to address global inequities that have become exacerbated during the current pandemic [5]. Inexpensive and highly accessible vaccines such as those produced from plants could be used to reach populaces that have no refrigeration and thus have no access to the storage temperatures required for the mRNA vaccines made by Pfizer and Moderna. Plant-made COVID-19 vaccines could also address vaccine hesitant populaces in the Global North, who have been reluctant to receive the newly established mRNA vaccines.

In addition to accessible COVID-19 vaccines, there are other ways that plants can provide a solution to the world's most pressing health problems. A tremendous example would be in the efforts made to generate effective and inexpensive monoclonal antibodies (Mabs) for HIV. For example, one research group has constructed three broadly neutralizing antibodies that can be used as an inexpensive alternative to antiretroviral therapy. These antibodies bind to sites on the virus that are needed for viral entry into cells [6]. In general, Mabs are prohibitively expensive, so making them in plants would enable a much-needed medicine to be provided to LMICs, where the prevalence of HIV is the highest.

Plant viruses can also be engineered to express pharmaceutical proteins and are designed to express epitopes on their surfaces in a regular pattern, so that a robust immune response can be elicited. Because of this, plant viruses have been engineered into virus nanoparticles (VNPs) for a variety of functionalities, including for cancer. Cancer cells can be identified in the body via specific epitopes and thus be targeted by plant VNPs [7,8]. Conventional nanoparticles are expensive to create, and thus new versions based on plant viruses have been explored for biomedical applications [8]. In addition, plant VNPs are biodegradable, non-pathogenic toward humans, and can be scaled up rapidly. The potential to use of plant VNPs for the treatment of both infectious and chronic diseases such, therefore, is tremendous.

The existing literature illustrates a drastic increase in publications and research groups who are involved in the field of plant-made vaccines. Similarly, the number of startup companies who work in this discipline now reaches over a dozen, showing the relevance of molecular farming today. As plant-made pharmaceuticals evolve, they will contribute to addressing the many urgent issues that we face in global health for many years to come.

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VACCINE INSIGHTS

MANUFACTURING:
UPSTREAM AND RAW MATERIALS

SPOTLIGHT

COMMENTARY

Vaccine security and self-reliance in South East Asia

Tuck Seng Wong and Kiat Ruxrungtham

Vaccine security and self-reliance must be a priority for all nations if they are to escape the worst health and economic impacts of future pandemics. For low- and middle-income countries, this is best achieved with a wider regional approach. Existing geopolitical and economic alliances and a strong baseline of biopharmaceutical manufacturing mean that South East Asia is well-positioned to become a global leader in vaccine production. The newly launched UK-South East Asia Vaccine Manufacturing Research Hub will support this ambition by addressing key priorities including technology transfer, IP, standardization, and supply chain.

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INTRODUCTION

The devastating impact of COVID-19 left many nations grappling with overwhelmed healthcare systems. Throughout the pandemic, socio-economic disparities were linked to higher and disproportionate burdens of COVID-19, along with glaring inequities in access to vaccines. Despite global initiatives like the COVID-19 Global Vaccine Access (COVAX) program aiming at equitable sharing, vaccine nationalism has largely overshadowed efforts for global equity. These disparities hold immense consequences for the economic recovery and future well-being of low- and middle-income countries (LMICs). As the immediacy of COVID-19 fades and life returns to normal, it is crucial to reflect on this global-scale crisis and fortify our preparedness for the potential recurrence of pandemics or epidemics.

Promoting equitable access to medicines is a central theme in the United Nations' Sustainable Development Goals (SDGs), with SDG 3.8 specifically emphasizing "access to safe, effective, quality, and affordable essential medicines and vaccines for all" as a fundamental element of universal health coverage.

Undoubtedly, global health diplomacy plays a significant role in bridging socio-economic gaps and realizing the recognition of health as a fundamental human right. However, the current reliance on wealthier nations must be re-examined. It is mandatory to shift towards self-reliance and assert control over vaccine security to break free from dependency.

The pursuit of vaccine security and self-reliance is markedly more effective in LMICs when adopting a regional approach, as opposed to isolated efforts. This involves a unified, collective effort by countries within the same region, proactively contributing to shared goals and benefits.

SEA COLLABORATION

This regional approach finds optimal applicability in South East Asia (SEA), driven by the existing geopolitical and economic framework that binds the countries in this region. The Association of Southeast Asian Nations (ASEAN), established on August 8, 1967 and currently consisting of 10 member states, serves as a prime embodiment of this framework. Operating under the guidance of the Secretary-General, the ASEAN Secretariat acts as a central coordinator, facilitating efficient decision-making within and among various ASEAN bodies.

ASEAN was instituted with a multi-faceted agenda, aiming to expedite economic growth, social progress, and cultural development through collective initiatives, foster active collaboration and mutual assistance on shared interests including scientific advancement, extend support through training and research facilities in educational, professional, technical, and administrative domains, and enhance effective collaboration to stimulate further growth in sectors such as agriculture, industry, and trade, among other objectives.

The ASEAN leaders' declaration on ASEAN Vaccine Security and Self-Reliance in 2019, spearheaded by Thailand's National Vaccine Institute, and the subsequent announcement in 2020 to establish the ASEAN Centre for Public Health Emergencies and Emerging Diseases stand as tangible manifestations of the region's commitment to fortifying ASEAN's health security.

Upon assuming office as the 15th Secretary-General of ASEAN on January 9, 2023, Dr Kao Kim Hourn outlined 'The Six Ps' as his focal priorities for the organization over the next 5 years: Peace, Prosperity, Planet, People, Partnership, and Potential. Placing the well-being of 'People' at the core of ASEAN community building, his vision revolves around ensuring a healthy, compassionate, and sustainable ASEAN community. This involves promoting healthy lifestyles, adeptly responding to all hazards and emerging threats, fortifying health systems and access to care, and ensuring food safety. Dr Kao actively advocates for 'Partnership' with advanced nations, such as the UK, USA, and Japan, promoting collaborative efforts to collectively realize the full health 'Potential' of the ASEAN peoples. This collaboration aims to establish resilient health systems that safeguard against public health threats and various other forms of challenges.

EXISTING VACCINE MANUFACTURING CAPACITY IN SEA

Crucially, several SEA countries, such as Thailand, Malaysia, Indonesia, and Vietnam, already boast established biopharmaceutical manufacturing infrastructures. The region is not initiating this endeavor from a baseline of ground zero. The political and economic stability prevalent in this area provides a conducive environment for continuous development and substantial investment in vaccine development and production. Furthermore, individual countries in the region are actively contributing to this common goal.

Thailand has emerged as a frontrunner in the development of COVID-19 vaccines, showcasing a diverse range of domestically developed vaccines that leverage three distinct technologies—egg-based, mRNA,

and recombinant protein. Among these, HXP-GPOVac, utilizing inactivated Newcastle disease virus genetically engineered to produce a stable form of the coronavirus's spike protein [1], is in the final stages of its third round of clinical trials. Produced using egg-based technology by the Government Pharmaceutical Organization, this vaccine holds promise as a cost-effective, locally manufactured booster option, poised to alleviate the financial burden associated with imported vaccines. Beyond immediate use, it plays an essential role in enhancing vaccine security and preparedness for future pandemics. Chula VRC, the leading vaccine R&D center at Chulalongkorn University, has adeptly acquired mRNA vaccine capabilities. Demonstrating remarkable efficiency, the center achieved the entire process from antigen design to the establishment of an animal model in less than 8 weeks. Importantly, this proficiency extends beyond COVID-19 vaccines, encompassing R&D for various infectious diseases. ChulaCov19 [2,3], an mRNA vaccine, has completed its Phase 2 enrolment in Thailand and Australia. Additionally, Baiya SARS-CoV-2 Vax 1 and Vax 2 [4], plant-based recombinant proteins developed by Baiya Phytopharm, a startup founded by two faculty members from Chulalongkorn University, have entered Phase 2 clinical trials. These endeavors collectively underscore Thailand's commendable strides in vaccine research, positioning the country at the forefront of global efforts against infectious diseases. In 2021, Malaysia initiated the National Vaccine Development Roadmap, assigning the Malaysian Genome and Vaccine Institute the central role of transforming the country into a hub for vaccine production and boosting confidence in vaccine usage. In a similar vein, Cambodia and China inked a Memorandum of Understanding for the construction of a COVID-19 vaccine filling and packaging factory in Cambodia. The Cambodian government has committed to procuring vaccines from this facility for a 3 year period between 2024 and 2026.

SEA benefits significantly from its proximity to nations at the forefront of vaccine development and manufacturing, such as China, South Korea, India, and Japan. This strategic geographic positioning facilitates numerous cross-border partnerships, especially evident during the COVID-19 pandemic. Taking Malaysia as an example, Solution Biologics engaged in an agreement with CanSino Biologics, based in Tianjin, China, covering market authorization, manufacturing, and commercialization. This partnership focuses on the supply of vaccines, specifically the COVID-19 vaccine Convidicea (Ad5-nCoV, a viral vector vaccine), into Malaysia. Duopharma received conditional registration approval from Malaysia's Drug Control Authority for the Sinopharm COVID-19 vaccine (BBIBP-CorV, inactivated virus), developed by China National Biotec Group (CNBG). Additionally, Pharmaniaga inked a local manufacturing agreement with China's Sinovac Life Sciences for the procurement of ready-to-fill bulk products of the COVID-19 vaccine (CoronaVac, inactivated virus).

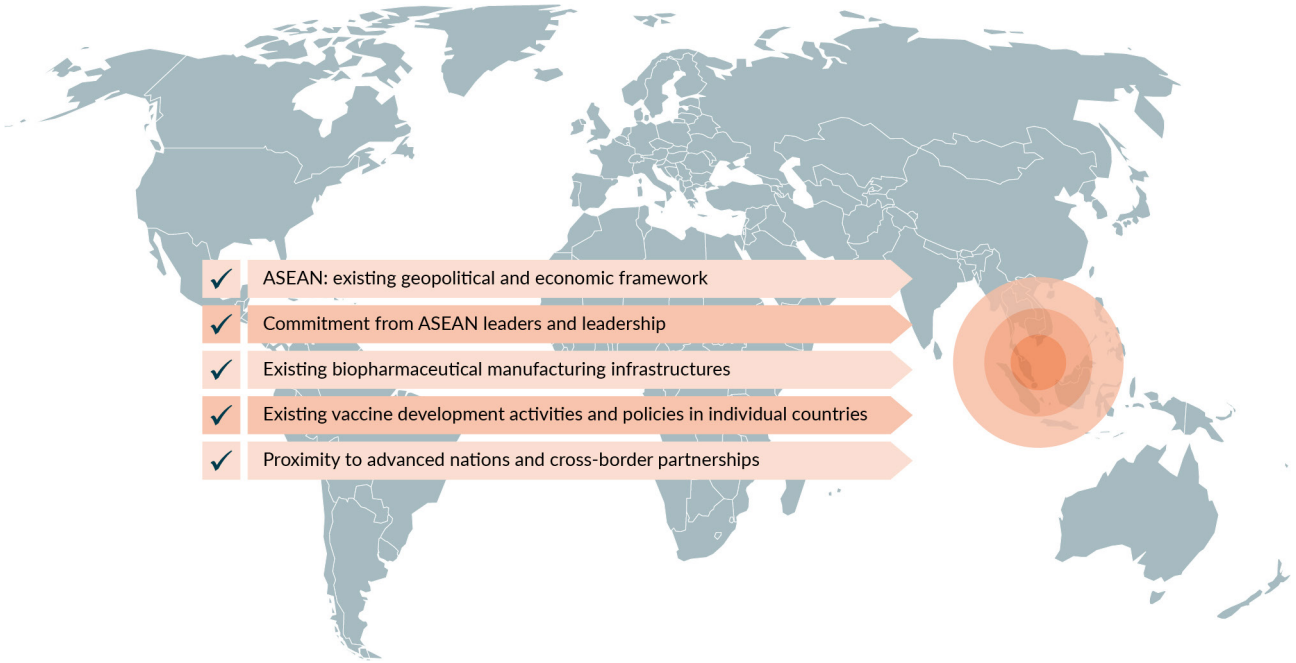
SEA: THE NEXT POWERHOUSE IN VACCINE MANUFACTURING?

Together, the aforementioned initiatives and evidence strongly indicate that SEA possesses the critical components necessary to emerge as the next powerhouse in vaccine manufacturing **(Figure 1)**.

Indeed, global health entities, such as the World Health Organization (WHO) and the Coalition for Epidemic Preparedness Innovations, recognize the substantial potential within SEA for vaccine manufacturing. Bio Farma, Indonesia's leading vaccine manufacturer, and Polyvac, a state-owned vaccine production company in Vietnam, are recipients of mRNA technology from the WHO's mRNA vaccine technology transfer hub. In a strategic move, the Coalition for Epidemic Preparedness Innovations and Bio Farma have forged a decade-long partnership aimed at enhancing the swift manufacturing of outbreak vaccines. This collaboration is vital in

FIGURE 1

Factors underlying South East Asia's potential as the emerging hub for vaccine manufacturing.



introducing state-of-the-art mRNA and viral vector rapid response vaccine manufacturing technologies to Indonesia and the wider ASEAN region. Moreover, it secures manufacturing capacity to supply countries in the Global South during future outbreaks and pandemics, thereby addressing the inequities witnessed during the COVID-19 response.

The UK government, represented by key entities such as the Department of Health and Social Care, Foreign, Commonwealth and Development Office, UK Mission to ASEAN, and UK Health Security Agency, has contributed significantly to health security in the SEA region. This commitment was underscored on August 5, 2021 when the UK became an ASEAN Dialogue Partner, marking the first addition in 25 years. Health security remains a central focus in this partnership, reflecting a shared dedication to enhancing the region's resilience against health threats.

A noteworthy manifestation of the UK's commitment is the recent establishment of the UK-South East Asia Vaccine Manufacturing Research Hub (UK-SEA Vax Hub), jointly funded by the Department of Health and Social Care and the Engineering and Physical Sciences Research Council. This initiative not only showcases the UK's dedication but also serves as a clear illustration of its prominent global leadership in healthcare technology. The UK-SEA Vax Hub functions as a research consortium involving four UK universities (Sheffield, Cambridge, York, and Kent) and one Thai university (Chulalongkorn). The collaborative efforts extend to 11 partners in SEA, encompassing vaccine manufacturers, academic institutions, research bodies, and governmental agencies.

While SEA has a solid foundation to align itself with the caliber of its neighbors in vaccine manufacturing, there is still much to accomplish and numerous challenges ahead. This realization serves as the impetus behind the establishment of the UK-SEA Vax Hub (Figure 2).

THE ROAD TO SEA VACCINE SELF-RELIANCE: CHALLENGES AND PRIORITIES

Vaccine confidence in the Philippines experienced a drastic decline after the 2017 dengue

FIGURE 2

Establishing a sustainable vaccine development and manufacturing ecosystem in low- and middle-income countries requires long-term commitment and support.

vaccine controversy involving Sanofi Pasteur's Dengvaxia. This incident triggered outrage and political turmoil within the country. Exposure to misinformation, coupled with a belief in its accuracy, has further heightened vaccine hesitancy and diminished the willingness to undergo vaccination. Enhancing vaccine acceptance emerges as a critical undertaking. Alongside improved public education, there is a pressing need for continuous monitoring of public perceptions and opinions regarding vaccination and related services, necessitating partnerships with behavioral researchers.

The transfer of technology to LMICs, a key objective of the UK-SEA Vax Hub, inevitably faces the challenge of navigating the intricate IP landscape in vaccine technologies. Ongoing legal disputes exist among entities involved in mRNA vaccine development. However, this challenge is not insurmountable, and there are several effective strategies for managing it. The Medicine Patent Pool, for example, has been a valuable partner for WHO, offering expertise in IP management. This includes providing IP analysis, defining terms and conditions, and negotiating agreements. In some instances, IP may not be filed in certain countries, granting the freedom to manufacture. It is feasible to develop highly efficient vaccines using technologies with the freedom to operate or those with lapsed IP. Additionally, negotiating cross-licensing deals or leveraging IP as a negotiation tool can create win-win scenarios.

Recognizing the importance of cross-disciplinary insights, we acknowledge the need to draw from other fields and integrate their best practices. Take synthetic biology (or engineering biology) as an exemplar – this field has witnessed rapid evolution, attributed in part to its early emphasis on the standardization of biological parts. This commitment to standardization has facilitated broad accessibility to these standardized parts. In vaccinology, where time and resources are critical factors, standardization assumes paramount significance. The imperative for agility in vaccine development and manufacturing, particularly when confronted with emerging diseases or newly identified pathogens, underscores the pivotal role of standardization. Indeed, numerous facets of vaccine development and manufacturing are amenable to standardization. These encompass, among others, vaccine platforms, genetic constructs, sequence design, assays, manufacturing processes, operating procedures, risk assessment, and regulatory approval procedures. Beyond standardization, the optimization of workflows and the implementation of process automation, such as continuous manufacturing, are equally crucial. These measures serve to minimize errors and waste, especially in regions where the workforce may have varying levels of training.

When evaluating self-reliance, it's crucial to look beyond the mere process flow diagram. An in-depth analysis should extend to identifying critical components essential for vaccine manufacturing, such as glass vials and filters, and understanding their supply dynamics. As part of the UK-SEA Vax Hub's work program, the mapping of region-specific supply

chains and the development of a tailored supply chain model become paramount. These endeavors enable us to forecast manufacturing outcomes and consequences under various scenarios, shedding light on unforeseen challenges. For instance, let's consider the gene synthesis phase, which is typically outsourced to companies with manufacturing facilities in China and the USA. Evaluating the potential impact on regional vaccine manufacturing becomes imperative if outsourcing ceases to be a viable option. While mapping all these factors may seem like a monumental task, it is an essential undertaking to ensure a comprehensive understanding of the dynamics at play.

Undoubtedly, enhancing regional vaccine capabilities in SEA demands substantial investment. This investment extends beyond the capital expenditure for manufacturing facilities. It encompasses a multifaceted approach involving investments in education—encouraging the younger generation to consider STEM as a future career, investments in people including the provision of training in biopharmaceutical manufacturing, and investments in R&D through funding for research activities and safeguarding arising IPs. A compelling business case, demonstrating sustainability and a return on investment, is fundamental in persuading investors, donors, and decision-makers. It is essential that these diverse investments are aligned synergistically, avoiding siloed approaches for maximum impact.

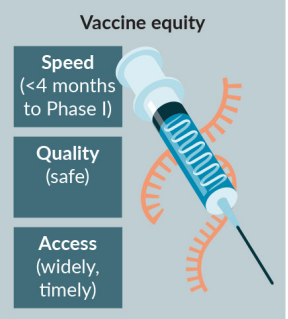
Efficiently managing manufacturing facilities to prevent unnecessary overproduction of vaccines is a pertinent consideration. With a significant decrease in demand for COVID-19 vaccines, it becomes obligatory to repurpose manufacturing capabilities for the production of vaccines addressing other diseases and various biopharmaceuticals to ensure a return on investment. It's crucial to emphasize that vaccines serve purposes beyond prophylaxis. An increasing focus is directed towards the development of therapeutic vaccines for conditions such as cancers, tumors, and AIDS, introducing a distinct dimension to the invested manufacturing capabilities in SEA.

CONCLUSION

The compelling need for an 'unconventional' approach is unmistakable in tackling the existing challenges. Conventional market-driven strategies have worsened disparities, affecting the accessibility of essential vaccines like HPV, herpes-zoster, and pneumococcal vaccines in LMICs. Recent advocacy articles [5,6] propose a paradigm shift centered on 'a common good for global public health need' (Figure 3). This approach advocates for transformative R&D and manufacturing in LMIC regions, prioritizing regional vaccine development over nationalism. It also emphasizes innovative funding mechanisms, the establishment of collaborative R&D networks focusing on

FIGURE 3

The pathways towards an enhanced preparedness for the next pandemic and vaccine equity in low- and middle-income countries.



1	**Funding:** government, regional, global, and donation
2	**Freedom to operate:** secured licensing and patents
3	**Manufacturer readiness and QC:** individual country and regional capacity
4	**Materials, consumables, and equipment:** supply and stock
5	**Harmonized regulatory system:** standardized and timely
6	**Good quality clinical trials:** regional network
7	**Sustainable R&D centre:** local, regional, and global diseases

regional/global unmet vaccine needs, and the creation of vaccine technology transfer hubs, among other strategic initiatives.

Investing in SEA is not just wise; it is a strategic imperative. We are confident that the positive impacts stemming from investments in this region will endure over the long term. By fostering collaboration and consolidating regional efforts, we can realize the four 'A' pillars of successful vaccine endeavors: Availability, Accessibility, Acceptability, and Affordability. This collective commitment ensures not only a resilient response to current challenges but also a sustainable foundation for the future of vaccine development and manufacturing in SEA.

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VACCINE INSIGHTS

MANUFACTURING:
UPSTREAM AND RAW MATERIALS

SPOTLIGHT

Overcoming challenges in technology transfer within the vaccine industry

Anubhaw Kumar Singh
Serum Institute Of India

"Aligning scientific vision with operational and management goals is imperative for successful technology transfer."

VIEWPOINT

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Technology transfer of vaccine manufacturing is vital to ensure global access to vaccines but has often proved challenging. What are the greatest roadblocks to successful vaccine technology transfer—and how can the path be cleared?

Manufacturing vaccines presents unique challenges distinct from other pharmaceutical products, with strategies unfolding over decades rather than months or years. Establishing trust among organizations and regulators, as well as ensuring ease of operation, hinges on proven concepts. Vaccine technology transfer, crucial for broadening access and ensuring quality, safety, and efficacy, involves transferring knowledge and technology from developer to recipient. Some organizations adopt a hub-and-spoke model in which technology licensing and transfer occur at two levels: corporate level and manufacturing level (project level). For example, the World Health Organization established a vaccine technology transfer hub at Afrigen in South Africa. The spokes of this system are the many worldwide vaccine manufacturing organizations to which the technologies are being transferred, like Biovac, Sinergium Biotech, and Bio-Manguinhos.

At the corporate level, management focuses on program-level strategies, creating robust business models, and negotiating the scope of agreements involving elements like intellectual property rights, royalties, and territory rights. The manufacturing level, on the other hand, deals with implementation and execution. This level of technology transfer is routine within an organization, occurring between various manufacturing departments such as R&D to manufacturing science and technology (MSAT) or between commercial units. There is no set formula for success. However, learning from other vaccine technology transfer firms is essential.

One significant challenge in vaccine technology transfer is aligning technology with commercial value. Bridging the gap between academia and industry is crucial, as many technologies lack commercial appeal due to a mismatch with vaccine manufacturing setups. Despite available drug delivery systems, traditional methods often dominate, hindering innovation. Additionally, vaccines targeting less lethal diseases may have lower demand, impacting their technology transfer and commercial viability.

Exclusivity rights conflicts present another obstacle. Vaccine technologies are often developed in high-income countries, leading to challenges in transfer due to conflicting interests and varied intellectual property rights at different production stages. These conflicts complicate sublicensing agreements and hinder technology dissemination. For instance, technologies like proprietary adjuvants may be obtained from different locations, which each have different intellectual property rights.

Manufacturing scale differences also pose significant challenges. Transitioning lab-scale technologies to commercially viable stages is arduous due to differences in operating economics between sending and receiving units. Variations in equipment selection and facility design further complicate technology transfer, requiring careful planning and adaptation.

Trade barriers add another layer of complexity. Utilization of specific-grade raw materials, equipment, and quality testing animals, coupled with trade embargoes and sanctions, creates obstacles to technology transfer. For example, a barrier was created when the USA placed economic sanctions on Iran and Russia. In addition, the Halal requirements for vaccines being administered in Islamic countries represent another barrier. These barriers affect the macroenvironment, creating an unhealthy climate for successful technology transfer to occur.

Communication, collaboration, and vision alignment are crucial for overcoming these challenges. Aligning scientific vision with operational and management goals is imperative for successful technology transfer. Effective project management and collaboration are essential for navigating research and development, process improvements, and commercial aspirations.

In conclusion, addressing these challenges requires concerted efforts and collaboration to facilitate seamless technology transfer, ensuring vaccines reach those in need

effectively and efficiently. Despite the complexities involved, overcoming these obstacles is essential for advancing public health and combating global health threats.

BIOGRAPHY

ANUBHAW KUMAR SINGH is an esteemed leader with a distinguished career spanning manufacturing, strategic planning, development, and management roles within multinational organizations. With over 18 years of experience, he is a recognized expert in the global manufacturing and commercialization of vaccines. Anubhaw excels in harmonizing technical and commercial aspects to drive optimal value creation. His areas of specialization include overall vaccine (global strategic alliance development, technology transfer, and project management). Anubhaw has successfully overseen the commercialization of numerous vaccines, from conception to market reality, demonstrating his ability to deliver tangible results at the highest levels.

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MANUFACTURING:
UPSTREAM AND RAW MATERIALS

SPOTLIGHT

INTERVIEW

Maintaining 'warm base' capacity for pandemic preparedness

The COVID-19 pandemic put immense pressure on vaccine manufacturing facilities to rapidly scale up processes to meet demand—could warm base facilities be a solution for future pandemics? **Casey Nevins**, Assistant Editor, *Vaccine Insights*, speaks with **Kilian Mullett**, Senior Director of Commercial Supply Strategies, Pfizer, about challenges and solutions associated with establishing and maintaining a warm base vaccine manufacturing facility.

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Q What are you working on right now?

KM: I am part of a team known as Pfizer Global Supply, which is responsible for the supply, operations, and technical aspects of Pfizer's entire product portfolio. Within that group, I specifically work in three main areas: pandemic preparedness, equity initiatives, and product localization strategies in different international markets.

As a result of the COVID-19 pandemic, I have been heavily involved in considering what it means for industry to quickly produce vaccines in the event of an emergency. For example, we announced an agreement with the European Health and Digital Executive Agency to ensure manufacturing capacity remains active to help close gaps between initial manufacturing and sufficient vaccine supply in the event of a public health emergency for the EU.

Q What did we learn during the peak of the COVID-19 pandemic about worldwide vaccine manufacturing capabilities?

KM: One of the main things we learned is that, in order to quickly advance manufacturing efforts in a pandemic situation, you need to invest at risk and collaborate widely. Industry, government, and academia had to collaborate very quickly to figure out how we could rapidly scale up manufacturing within and across continents. Pfizer collaborated with BioNTech and with multiple contract manufacturing organizations during the COVID-19 pandemic, which was a huge part of our success. We worked with a very large array of suppliers globally, partly through our leverage and our scale.

Another key thing that we learned is that we needed to relentlessly focus on efficiency. Increasing capacity in our production timelines made a huge difference. Our approach at Pfizer was to scale up centrally in terms of the key technology around mRNA and then to scale out globally to a host of different partners. Much of our capability to achieve this was due to our site's infrastructure, our partners, and our human resources.

Q How would you define warm base manufacturing capacity, and what are the logistics of maintaining a warm base facility?

KM: Warm base manufacturing is the practice of maintaining the capacity and capability to scale up a medical countermeasure in a very short period of time. This requires certain key elements that allow for the maintenance of the infrastructure of a process. For example, when you look at a process like the mRNA platform, there are various steps required for success. There are the initial plasmid steps, making the mRNA, encapsulating the mRNA, fill/finish, and quality release. All of those manufacturing nodes need to be kept up and running in a warm base facility.

You need resources to maintain that infrastructure, including an adequate number of well-trained people. You also need to keep critical materials available and stored to allow for rapid scaling.

Maintaining this infrastructure in a non-pandemic situation, where there is not adequate demand for these manufacturing processes, is difficult. A key requirement for a production facility in any industry is product demand. If demand wanes, typically, companies will scale back their processes, so that the facility is efficient. In the case of vaccines, this would mean that a typical facility would scale back capacity to what is needed to produce endemic vaccine supplies. A warm base facility, however, cannot scale back in this manner and so is, in some respects, inefficient.

Q What are the main roadblocks in establishing warm base facilities, and how can they be overcome?

KM: The main roadblock is demand. Today, there is very little demand because we are in an interpandemic period. One potential solution for this problem is advance purchase agreements, which create demand to maintain a capacity reservation.

"One of the main things we learned [during the COVID-19 pandemic] is that, in order to quickly advance manufacturing efforts in a pandemic situation, you need to invest at risk and collaborate widely."

The 100 Days Mission, first articulated by CEPI, challenges vaccine manufacturers to develop safe, effective vaccines for viral threats within 100 days of recognition of a pandemic pathogen. Keeping manufacturing capacity 'warm' and leveraging proven platform technologies, like mRNA, could make the ambitious goals of the 100 Days Mission more realistic.

Manufacturing capacity routinely available in interpandemic times may not be adequate to provide the scale needed to supply vaccines to global populations quickly. To move quickly, we need to have manufacturing capacity, resources, and expertise 'warm' and ready in an emergency. This requires special planning and consideration now for how we meet this challenge.

Q What is the role of localized manufacturing in combatting future pandemics?

KM: Local manufacturing seeks to overcome barriers around unequal vaccine distribution and trade restrictions experienced during the COVID-19 pandemic. One of the challenges associated with the localization effort is the amount of infrastructure that is required, whether it is making the active ingredients, the intermediates, or the finished product. Enabling that infrastructure, which requires, at the very least, the purchase of equipment and the building of facilities, is very expensive. If you do not have the demand to underwrite those costs, you can end up with quite high product manufacturing costs, which can lead to higher pricing.

The largest barriers for new manufacturers are cost and demand. Manufacturers building new facilities may need to price vaccines higher than global competitors to cover high start-up costs, and buyers may need to be prepared to absorb the premium despite limited financing. Keeping manufacturing facilities warm and sustainable is also directly tied to demand for vaccine production—if demand is limited, local production will be threatened.

During the COVID-19 response, we did see some benefits to a more centralized manufacturing approach. There are speed efficiencies in having single-site manufacturing for plasmid DNA and mRNA drug substances, with a strong relationship to drug product manufacturing.

Efforts to further localize manufacturing in low- and middle-income countries may take years to build up, so while these efforts evolve, a priority must be to sustain and utilize what capacity we have now.

BIOGRAPHY

KILIAN MULLETT is a highly accomplished pharmaceutical professional with a rich history spanning back to 1994 when he commenced his journey with esteemed organizations like Schering-Plough, Washington Group, Wyeth Biotech, and his current role at Pfizer. Educationally, he holds a degree in industrial biology, complemented by an MBS in Strategy and Finance, as well as a postgraduate diploma in Executive Leadership. Throughout the early phases of his career, he excelled in leadership roles, spearheading teams in the

establishment of greenfield pharmaceutical plants, overseeing capital projects, and demonstrating effectiveness in operations management. Over the years, his trajectory led him to become the global portfolio leader for Pfizer's localization initiatives, where he has played a pivotal role in facilitating the global transfer of vaccine and small molecule technologies to emerging and developed markets. In his most recent position, Kilian has assumed a leadership role in both technical and supply aspects within Pfizer's vaccines business development, while also steering small and large molecule business development endeavors for emerging markets, with a particular focus on emerging markets and China. Currently, Kilian is instrumental in crafting and executing commercial supply strategies for Pfizer's equity, pandemic preparedness, and global product localization programs. He is the Pfizer global supply representative on the IFPMA Africa manufacturing committee

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VACCINE INSIGHTS

LATEST ARTICLES

VACCINE INSIGHTS

EXPERT INSIGHT

Impact of glycosylation of vaccine antigens on quality and performance

John F Cipollo and Diane McCarthy

Glycosylation of proteins used as vaccine antigens can impact the safety and efficacy of the vaccine and thus can be a product quality attribute. The term glycosylation refers to a group of post-translational modifications whereby oligosaccharides are linked to amino acid residues within proteins. In therapeutic glycoproteins, these post-translational modifications can affect protein stability, direct protein folding, modulate drug serum half-life, and influence the partners to which a glycoprotein binds. The composition and position of glycans within the protein structure can also affect function, as well as trafficking to cellular sub-compartments and specific tissues.

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INTRODUCTION

In terms of host–pathogen interactions, glycosylation can be important in a range of other processes, including but not limited to antigenic masking, interaction with immune system lectins [1], and serving as a component of antigenic sites [2]. Furthermore, the glycosylation status of the protein antigens is dynamic in nature, as a component of the pathogen. As the pathogen evolves over time, the glycosylation sites may shift position via mutation and change in number, and the glycans may change in composition and finer structure. For antigens, such as those of rapidly evolving viruses, their peptide sequence may be monitored for *N*-glycosylation state by sequon (*N*-glycosylation recognition sequence) identification in the protein sequence and analysis of the intact protein(s) (for instance, by gel electrophoresis). More comprehensive analysis may improve vaccine quality and efficiency. Vaccine antigen glycosylation will be discussed in this article, including its biological relevance, examples where monitoring could improve vaccine performance, relevant analytical approaches, and existing resources and guidance for industry on the analysis of glycosylation.

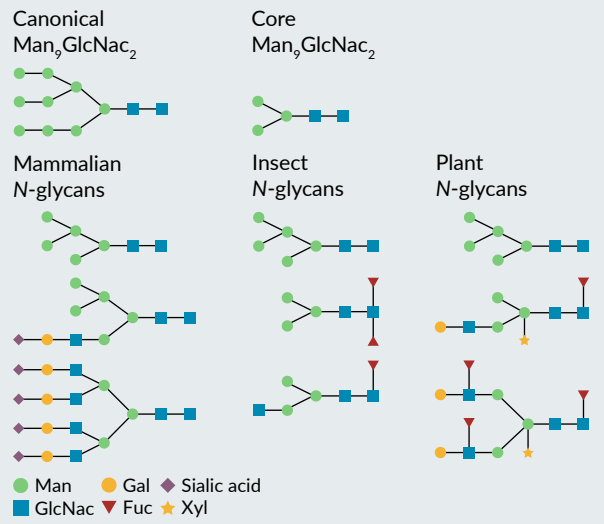
N- AND *O*-GLYCOSYLATION: A BRIEF OVERVIEW

There are two major glycosylation types that occur on eukaryotic proteins, including protein antigens, namely *N*- and *O*-glycosylation [3,4]. Glycosylation occurs most commonly to protein asparagine residues (*N*-glycosylation) and serine/threonine (O-glycosylation), both of which are found on enveloped virus spike proteins [5]. Representative *N*- and *O*-glycans are shown in Figure 1 and Figure 2, respectively. For *N*-glycans, the canonical $Man_9GlcNAc_2$ high-mannose glycan shown in Figure 1 is the *N*-glycan from which all other *N*-glycans are derived. The precursor $Glc3Man_9GlcNAc_2$ is transferred to sites with the generalized peptide sequence Asn-X-Ser/Thr (where X is any amino acid except Pro) within the nascent protein. The Glc residues are trimmed, leaving $Man_9GlcNAc_2$. To generate complex and hybrid glycans in mammalian and plant systems, Man residues of $Man_9GlcNAc_2$ are removed to yield $Man_5GlcNAc_2$. Further trimming of Man may occur in concert with the addition of monosaccharides such as GlcNAc, Gal, and sialic acids, yielding structures such as the representative ones in Figure 1. Also pictured in Figure 1 is $Man_3GlcNAc_2$, a common core to all higher eukaryote *N*-glycans. In plants, the core Man residue adjacent to the $GlcNAc_2$ core residues can be substituted with Xyl, which is associated with allergenic risk [6].

N-glycan processing in insects differs significantly from that of higher eukaryotes, yielding shorter glycans, the majority of which bear few extensions [7]. High-mannose glycans are a significant proportion of *N*-glycans in insect cells. The most common forms are

FIGURE 1

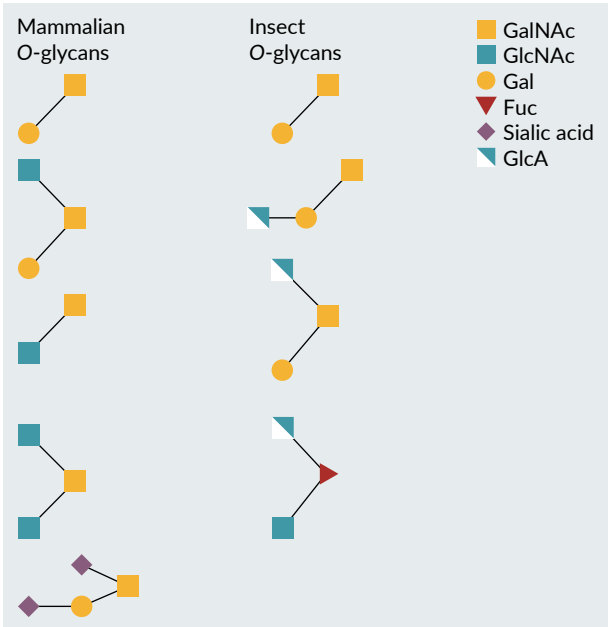
Representative *N*-glycans from mammalian, insect, and plant cell lines.



Top: canonical $Man_9GlcNAc_2$ is a precursor to all *N*-glycans in each of the three species. $Man_3GlcNAc_2$ is a common core pentasaccharide in all three species and occurs after action by endoplasmic reticulum and Golgi mannosidases. In mammalian species the resulting glycan can be high-mannose (top), hybrid (middle), or complex glycan type (bottom). Hybrid glycans can retain a portion of the original mannose residues of the canonical $Man_9GlcNAc_2$. Insect *N*-glycans may contain up to two Fuc residues linked to the reducing end GlcNAc. Plant *N*-glycans may be substituted with Xyl at the bisected core Man residue. The Symbolic Nomenclature for Glycans has been used [62].

FIGURE 2

Representative *O*-glycans from mammalian and insect cell lines.



Mammalian cores 1–4 are shown to the left (top to bottom). Sialic acid serves as the negative charge group. Insect *O*-glycans are shown to the right. Insect cells do not produce sialyl *O*-glycans and instead produce those containing GlcA, which serves as the negative charge group. Some may also contain zwitterion substitutions such as phosphorylcholine and phosphorylethanolamine, which can be immunomodulatory. Glycan representations are displayed non-reducing (furthest from protein) to reducing end (point at which the glycan is linked to the protein) from left to right. The Symbolic Nomenclature for Glycans has been used [62].

short paucimannose, containing four or fewer Man residues (see representative in Figure 1). The core may contain up to two Fuc residues and some arrangements can present allergenic risk. Some minor extensions with additional monosaccharides can occur but are generally limited to the addition of one or two residues, leading to abbreviated mammalian-like complex and hybrid *N*-glycans.

Eukaryotic cell *O*-glycosylation occurs at specific Ser and Thr residues. While there is no single consensus sequence, *O*-glycosylation tends to occur in regions with stretches of Ser/Thr with interspaced Pro residues. Representative *O*-glycans for mammalian and insect cell lines are shown in Figure 2. The Core-1-like structures are common in higher eukaryotes, but extensions can be complex and branched. Insect *O*-glycans differ from mammalian forms. Some insect *O*-glycans are completely foreign to humans and therefore can be associated with allergenic risk. Less common forms in insect cells can contain zwitterionic substitutions such as phosphorylcholine and phosphorylethanolamine, which can have immunomodulatory activities [8]. Plants do not form similar *O*-glycans, rather, they form xylans and glycosylate hydroxylysine. These would be foreign to humans and present allergenic risk. Plants do not form *O*-glycans that are similar to animal forms. Rather than *O*-glycosylating primarily Ser/Thr, they glycosylate Ser, hydroxylysine (HyL), and hydroxyproline (HyP). They are rarely initiated with GalNAc like mammalian systems and instead initiate *O*-glycan chains with Xyl and Gal [9].

DIFFERENCES IN GLYCOSYLATION ACROSS CELL SUBSTRATES

Vaccines targeting viral antigens have been produced in embryonated chicken eggs but are also produced in MDCK, VERO, MRC5, HEK293, CHO, Sf9, and HIGH5 cell lines, the latter two of which are of insect origin. All of these have been utilized in the production of vaccines approved by the US FDA. Cell substrates used in vaccine production have been reviewed [10]. Collectively, these are termed cell substrates. The glycoprotein antigens may be produced as subunit antigen, as a component of the native or molecularly altered virus, or as component(s) of a virus particle. There have been several experimental vaccines generated in plants or plant cell lines. For instance, tobacco plant cell lines have been used to produce vaccine virus-like particles [11] as well as subunit vaccines [12]. All of these cell substrates have inherent glycosylation properties. Often, the goal in vaccine design is to closely mimic the native form of the antigen, including its glycosylation pattern, as these can be part of antigenic sites or otherwise serve to modulate immune response. As presented in Figure 1 and Figure 2, glycans from mammalian, insect, and plant cell substrates differ in composition and structural details such as linkage and branch configuration, which can impact function. Any *N*- or *O*-glycans foreign to the host may produce allergenic risk [13,14]. Insect *O*-glycan structure is less defined compared to those of mammalian and avian species. Further, both HIGH5 and Sf9 cells have been shown to glycosylate some low-efficiency sequons more frequently than mammalian or egg cell substrates. HIGH5, Sf9, and a mammalianized sf9 cell line, SfSwt-7, all glycosylated Asn 209 in influenza H5N1 hemagglutinin (HA). The sequon centered at Asn209 contained Pro, which is prohibitive to *N*-glycosylation in mammalian and other higher eukaryotic systems. This modification was not seen in the mammalian or hen egg-derived HA [15]. This situation implies that antigenic sites may be differentially modified depending on the cell substrate and thus change antigenic structure if the glycosylation event occurs in such a region.

VACCINE GLYCOPROTEIN ANTIGENS

Some examples of vaccine glycoprotein antigens are HIV GP 120, coronavirus

SARS-CoV-2 spike, influenza HA and neuraminidase, Hepatitis B HBs Ag, Ebola envelope glycoprotein, and Zika envelope and nonstructural proteins. Global aspects of viral glycosylation have been reviewed [16]. Most glycosylation sites are *N*-glycan-type although some contain *O*-glycans as well. For instance, the SARS-CoV-2 spike protein contains *O*-glycosylation sites in key regions near antigenic sites and areas related to function such as those required for conformational change and interaction with the ACE II receptor [17]. Vaccine antigens differ in function compared to therapeutic proteins in that their primary purpose is to generate an immune response. As the target pathogen propagates through the human population, the glycosylation pattern of its glycoprotein antigens tends to change in composition, number of sites, and (through mutation) position, leading to altered structural characteristics. As a result, and coupled with changes in peptide sequence, vaccine antigens must be updated periodically to closely match the circulating target pathogen(s).

GLYCOSYLATION: FUNCTION IN VACCINES

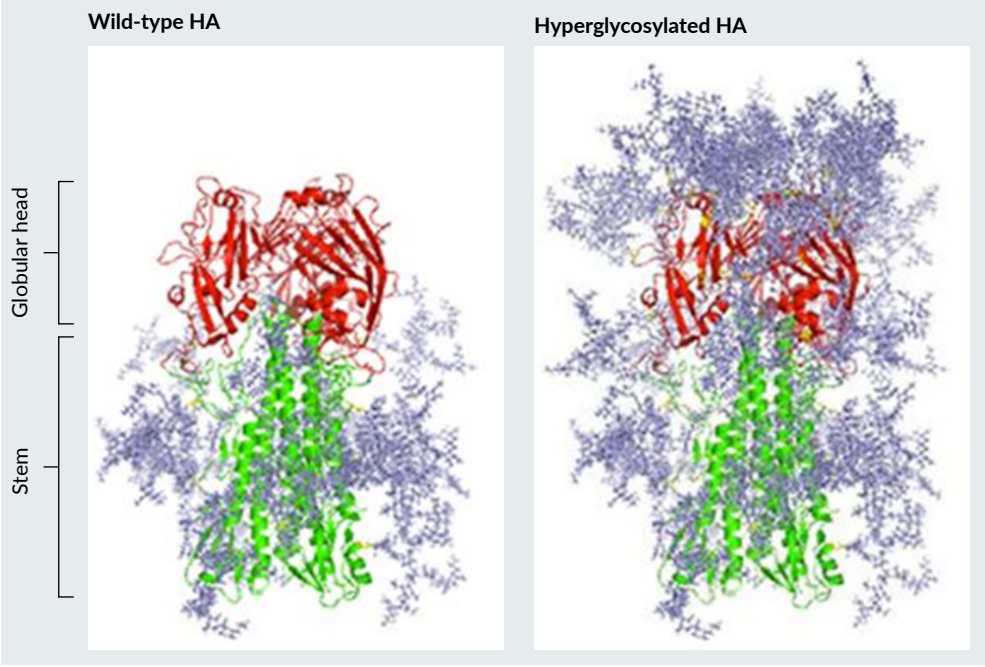
Glycosylation plays a variety of critical roles in vaccine antigen performance such as:

- Antigenic masking,
- Becoming a critical component of an antigenic site, or
- Serving as a target of immune system lectins.

For example, influenza can enter human populations via a zoonotic leap from avian or swine sources. Generally, at the initiation of such an event, HA, the major antigenic protein of influenza, has few glycans on its globular head, where the majority of the immunodominant antigenic sites reside.

FIGURE 3

H1N1 influenza virus hemagglutinin.



Left: the wild-type virus lacking glycosylation on the head. Right: hyperglycosylated form where glycosylation masks the underlying antigenic sites. Globular head and stem regions are shown at the left. Based on Figure 1 in Eggink *et al.* [32].

Firstly, after zoonotic leap, and as the flu seasons pass, the tendency is to gain glycosylation sites in key antigenic regions, masking the antigenic sites and thus changing their chemical signature [18]. Such changes have been shown to shift immunodominance and neutralizing antibody response away from these antigenic sites [19].

Secondly, while glycans may mask antigenic sites, they can also act as important components of antigenic sites. When studying MDCK, Sf9, and hen egg-derived 2016–2017 H3N2 influenza utilized in seasonal vaccine, Zost *et al.*, found that the egg-derived vaccine HA lost a glycosylation site within antigenic site B due to the mutation of Thr to Lys at residue 160 [20]. The egg-derived vaccine had lower efficacy, which was noted more strongly in younger populations, where prior exposure that would have generated antibodies to this region was less prevalent. Such adaptive mutations occur more often in vaccines derived from hen egg compared to MDCK and other cell substrates [21]. This example emphasizes the differences between cell substrates and the need to monitor glycosylation.

Thirdly, glycans of specific composition and location on a glycoprotein antigen can also be targeted by host innate immune factors. The influenza A HAs of H3N2 and H1N1 strains contain key high-mannose glycans on their globular head domain (see **Figure 3**). These have been shown to interact with two key innate immune system collections—DC-SIGN [22] and lung surfactant SP-D [23,24]. If these high-mannose glyco-sites are missing, the influenza strains are highly pathogenic. These sites are important for immune response, the former for directing the pathogen to the antigen-presenting dendritic cells, and the latter for lung clearance and antigen processing. Influenza vaccine has several presentations including intramuscular injection and nasal delivery. Considering the function of some subsets of glycans as outlined here, knowledge of the glycosylation status of such vaccine antigens could be advantageous in terms of understanding vaccine performance. Monitoring of these key modifications may be warranted since cell culture conditions and the choice of cell substrate can shift glycosylation patterns.

LESSONS FROM COVID-19

The SARS-CoV-2 pandemic produced a massive scientific effort worldwide to rapidly understand this novel new pathogen. Its spike protein, a modified version of which has been used as the vaccine antigen, is heavily glycosylated with 23 *N*-glycans and up to eight *O*-glycans detected, with the majority of sites containing a range of glycans. As part of efforts to better understand spike protein glycosylation patterns, a number of laboratories used a range of liquid chromatography/mass spectrometry approaches to reveal fine structural detail. Spike protein produced as recombinant protein in HEK293, CHO, and sf9 insect cells, as well as in SARS-CoV-2 viral particles, were studied. The reported glycosylation patterns across cell substrate types and in virus-derived versus recombinant forms differed dramatically. HEK293 cell derived recombinant forms were highly glycosylated and contained more large multi-antennary *N*-glycans than other mammalian cell substrates [17,25,26]. Spike protein produced in insect cells contained short *N*-glycans as expected. Insect *O*-glycans detected were short [27]. Other more complex *O*-glycan forms may have escaped detection due to the status of insect *O*-glycan databases available at the time. Those produced in viral particles contained fewer complex and more high-mannose glycans in specific antigenic regions [26]. The Delta strain spike protein, containing the key mutation changing Asp 614 to Gly, differed in glycosylation pattern local to the mutation, likely due to increased local mobility. *O*-glycosylation also differed, especially in regions involved in the conformational change required for receptor binding [28] and antigenic exposure [29]. This suggests that *O*-glycosylation may be key to

presentation of this configuration. Therefore, monitoring of *O*-glycosylation in this case could be important.

ROLE OF GLYCOSYLATION IN VACCINE DESIGN

Engineering of glycosylation has been used as an immune-focusing tool to target immune responses away from or toward specific target regions. These modification strategies have three purposes: to mask antigenic sites and drive immune response to less antigenic regions, to remove glycosylation sites to increase immune response to antigenic regions, and to modify antigenic regions to avoid pathological immune response such as antibody-dependent enhancement (ADE) [30].

Changing the peptide backbone exposure by adding or removing glycosylation sites can alter regional antigenicity [2]. This approach has been used in efforts to produce more universal vaccine candidates for influenza and other viral pathogens [31]. A hyperglycosylated H1N1 influenza virus has been produced to drive the immune response in this way. To do this, seven glycosylation sites were added to the head region of HA, masking the key antigenic sites Ca1, Ca2, Cb, Sa, and Sb [32,33] (see **Figure 3** for head and stem regions). Furthermore, in another example, removing stem glycans from influenza HA, thus exposing the underlying peptide backbone, elicited more potent neutralizing antibodies [34]. The result was generation of neutralizing antibodies to the stem region, an area that is not normally strongly antigenic compared to the head region.

In another example, deletion of 3 or 4 glycosylation sites from HIV GP120 Env trimer near the CD4 receptor binding domain enabled more accessibility for B-cell receptors to the receptor binding region [35]. Guinea pigs immunized with these mutant pseudo-viruses had significantly higher neutralizing antibody titers against the CD4 receptor epitope compared to controls. The authors noted correlation of the increase in neutralizing titer with increased surface area exposure of the epitope due to glycan removal.

Glycosylation can also impact ADE, a phenomenon whereby non-neutralizing or poorly neutralizing antibodies can facilitate the entry of a virus into host cells via the Fcg receptor in monocytes [36]. Essentially, the virus uses these antibodies like a Trojan horse to enter and destroy key immune cells, thus enhancing disease. This phenomenon is a concern for flaviviruses such as Zika, Dengue, and related pathogens in this taxon. For example, in the case of Zika, Tai *et al.*, engineered a glycosylation site at residue 375 within a domain that contains dominant epitopes and tends to generate non-neutralizing antibodies linked to ADE [37]. The result was prevention of non-neutralizing antibodies to this region as measured using sera from mice immunized with both the glycosylated and non-glycosylated versions. ADE-avoiding vaccines are currently in development using such approaches [38].

ASSESSMENT OF GLYCOSYLATION IN VACCINES

Because glycosylation pattern, composition, position, and structure can affect vaccine quality, thorough characterization of glycans is needed. While the analytical methods utilized to monitor glycans are essentially the same as for other glycoproteins, some optimization may be needed to accommodate the drug substance or drug product presentation, such as the matrix and excipients.

A wide range of techniques are available for glycoprotein analysis and the selection of the suite of methods used will be dictated by the chemical information sought. These include *N*-and/or *O*-glycosylation information, glycan composition, fine structure such as anomeric configuration and branch structure, glyco-site occupancy, and glyco-site heterogeneity. Decision trees are available that lead to appropriate methods for processing, release, and analysis to procure the desired

information [39–41]. Reference standards should be used to establish system suitability and to support accurate glycan identification and quantitation.

Glycosylation analysis can be performed at four different levels, as outlined in USP General Chapter <1084> Glycoprotein and Glycan Analysis–General Considerations [41]:

- Glycans released from the protein,
- Monosaccharide content,
- The intact protein, and
- Glycopeptides generated by enzymatic digestion.

A variety of analytical methods can be applied for released glycan analyses to reveal monosaccharide composition, anomeric configuration, branch structure, and the abundance of each glycan [42–44]. Intact glycoprotein analysis can provide information concerning overall glycan content and composition. Analysis at the glycopeptide level can reveal site occupancy, site heterogeneity, and site composition, with some fine structural detail. Monosaccharide analysis is commonly used to assess the presence and quantity of specific glycan features, such as sialic acid.

N-glycans can be released enzymatically or chemically and can be analyzed in native or derivatized forms, primarily as reducing end derivatives or as permethylated forms. Reducing end derivatives have the advantage of linking a UV/fluorescent tag for the facilitation of chromatographic analysis such as described in USP Chapter <212> Oligosaccharide Analysis [45]. The latter is superior in terms of revealing linkage and branch structure but lacks the UV/fluorescent tag. Glycan abundance and composition can be determined utilizing high-performance liquid chromatography (HPLC) methods including, but not limited to, hydrophilic interaction chromatography, high pH anion exchange chromatography, reverse phase, porous graphitized carbon, and other column matrixes. Capillary electrophoretic methods are also used. All of these have been hyphenated to mass spectrometer detectors, adding mass and some structural information to such analyses. For these techniques and further information concerning methods see [45–48]. Separation methods used for released *N*-glycans can be adapted for *O*-glycan analysis. Contrary to *N*-glycans, *O*-glycan release methods are most often performed chemically by beta-elimination [49]. Some chemical methods allow for incorporation of a UV/fluorescent reducing end tag to *O*-glycans, and facilitate detection in chromatographic methods [50]. *O*-glycans are also amenable to permethylation procedures. For monosaccharide analysis, HPLC and gas chromatography/mass spectrometry methods are typically used such as described in USP Chapter <210> Monosaccharide Analysis [51].

Analysis of intact glycoprotein is generally limited to those glycoproteins with few post-translational modifications (PTM). High-resolution mass spectrometry-based methods are used in such analyses, but the spectrum becomes more difficult to interpret as the number of PTMs increases. However, bioinformatics approaches are improving, and high-resolution instrumentation should expand the capability for intact protein analysis over time.

Glycopeptide analysis is often highly informative since it enables analysis of glycan abundance and compositional heterogeneity at individual glycosylation sites. Standard methods utilize high-resolution mass spectrometry coupled to HPLC or capillary electrophoresis [52–55]. However, fine structural detail such as anomeric configuration (alpha or beta) and specific diastereomer information (i.e. Man, Gal, Glc) typically cannot be determined. Often branch structure and the particular identities of each monosaccharide component can be implied based on an understanding of the cell substrate but caution must be used in such interpretation strategies. Concerning branch position,

intramolecular migration can occur, thus confounding accurate assignments [56]. A range of fragmentation modalities are also available that can be selected for their advantages in spectral information content for glycopeptide assignment [52]. A range of informatics software has been developed to aid in the interpretation and assignment of both free glycans and glycopeptides [57].

New analytical approaches such as the multi-attribute method (MAM) leverage the specificity of mass spectrometry to monitor multiple attributes, including both *N*- and *O*-glycosylation, in a single assay [58–60]. USP Chapter <1060> Mass Spectrometry—Based Multi—Attribute Method for Therapeutic Proteins provides guidance for these approaches [61]. The MAM approach is designed to simultaneously monitor multiple product quality attributes. This approach is similar to glycopeptide analysis in the sense that proteins are digested into peptides and the peptides analyzed by mass spectrometry to identify and quantitate product quality attributes. However, it can be applied to many other PTMs, such as oxidation, deamidation, glycation, truncations, fragmentation or clips, and *N*- and *C*-terminal modifications. MAM typically includes a characterization phase, which is used to identify specific attributes that impact product quality, and a monitoring phase, which uses an automated workflow to monitor defined product quality attributes. While to date MAM has been primarily applied to monoclonal antibodies, such approaches are being adopted for other therapeutic proteins as well.

As observed with influenza and COVID-19, the glycosylation patterns of proteins in the pathogen change over time due to mutations in the sequence and evolutionary pressures, resulting in the need to update vaccine antigens to the new variant. Glycosylation of vaccine antigens is heavily influenced by the cell line used during manufacturing, which can result in differences in glycosylation site occupancy, glycan compositions, heterogeneities at the glyco-site level, and other structural characteristics. Glycan patterns in vaccines are also frequently engineered to modulate antigenicity or to reduce the potential for adverse events, such as ADE. Therefore, a thorough understanding and analysis of glycosylation of vaccine antigens is often needed to ensure a safe and effective product. While there are several different methods that can be used to analyze glycosylation, conventional methods rely on release of glycans from the protein backbone, which can help identify and quantify, but also find the location of the glycan within the protein sequence. More modern methods such as high-resolution mass spectrometry and MAM can provide more detailed information on the location of the glycan, as well as structural details to better inform vaccine design and support consistent quality, safety, and efficacy.

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VACCINE INSIGHTS

VIEWPOINT

Shaping immunity against infectious diseases with multivalent DNA vaccines

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"...multivalent DNA vaccine development holds tremendous promise to expand vaccine effectiveness and delivery..."

VIEWPOINT

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Immunization has dramatically transformed human and animal health. Since its earliest days, vaccination has served as a fundamental strategy for infectious disease prevention, providing population-level coverage for childhood diseases and seasonal infections, and serving as a rapid response to pandemic pathogens. Yet, there is continued circulation of endemic, emerging, and reemerging pathogens for which there are no licensed prophylactic measures. The successes of nucleic acid technologies during the COVID-19 pandemic, exemplified in the first two licensed mRNA vaccines [1] and the first DNA vaccine receiving emergency

use authorization for human use [2], are reinvigorating vaccine development to tackle this urgent unmet need.

The inherent stability of DNA offers advantageous features such as thermostability and extended shelf life. These characteristics are pivotal for transport and storage in resource-constrained environments, like low and middle-income countries. Furthermore, the ability to encode large transgenes and well-established modular assembly pipelines are key attributes of DNA-based platforms. This versatility extends to combination strategies of individual DNA vaccines as a multivalent drug product. Multivalent synthetic DNA vaccines are therefore emerging as part of the exciting nucleic acid-based vaccine landscape as a strategy to induce robust and durable immunity in diverse global populations.

MULTIVALENT VACCINES & CHALLENGES

Many of the most successful vaccines are multivalent, including the MMR, DTaP/Tdap, seasonal influenza, pneumococcal conjugate, HPV, and several recommended vaccines, including COVID-19 vaccines and recently licensed respiratory syncytial virus vaccines [3]. Such vaccines provide single formulation coverage against multiple serotypes, strains, and, in some cases, related and unrelated pathogens. Co-administration of multivalent vaccines and combination vaccines can reduce the number of total vaccines and overall injections, improving the likelihood of uptake [4,5].

However, along with the many traditional vaccine development challenges, multivalent vaccines face additional barriers, including achieving desired broad protective immunity at the preclinical and early Phase 1 clinical research stages and demonstrating sufficient non-inferiority data to support licensure [6,7]. Particular care must be taken to minimize interference between vaccine components, including competing immunodominance profiles that would result in a significant reduction in effectiveness compared to the individual licensed products.

From both efficacy and immunology perspectives, the strengths of multivalent vaccines lie in targeting complex pathogens, where single-antigen targeting may not provide sufficient protection. Multivalent vaccines can induce broadly protective immunity and mitigate the rapid evolution that leads to pathogen escape. Here, synthetic DNA vaccines have the potential to rise to the many challenges faced by multivalent vaccine development.

MULTIVALENT DNA VACCINES: A STRATEGY FOR INDUCING BROAD PROTECTIVE IMMUNITY

Synthetic multivalent DNA vaccines are rapidly progressing through preclinical studies, and early to late-stage clinical trials as both preventative and therapeutic vaccines targeting infectious diseases including HPV, HIV-1, chikungunya, dengue, influenza, Ebola, SARS-CoV-2, hantaan/puumala virus, cytomegalovirus and others [8–10]. Synthetic DNA vaccines, typically given via intradermal or intramuscular injection, historically struggled with poor immunogenicity in humans due to challenges with DNA delivery into the cell nucleus. Recent advancements in delivery methods like needle-free jet injection and electroporation have improved nuclear delivery. These methods, paired with synthetic gene design enhancements such as codon optimization for better mammalian cell expression, RNA structure analysis, and structural engineering, have resulted in improved *in vivo* DNA expression and enhanced immune responses (reviewed in [8,11]). Synthetic DNA vaccines stimulate both humoral and cellular immunity. Combining them with gene-encoded adjuvants broadens antibody responses, activates $CD4^+$ and $CD8^+$ T cell subsets, and establishes memory immune responses.

Synthetic DNA vaccine platforms continue to develop as strategies to respond to emerging and re-emerging human pathogens,

zoonoses, and potential pandemic diseases. They offer flexibility in engineering, allowing single DNA vaccines to express multiple antigens, and combine multiple DNAs into one formulation. It is possible to incorporate multiple surface proteins to induce antibody responses and internal proteins to shape cellular immunity, offering potential approaches to address pathogen escape and redundancy mechanisms using rational design approaches. Synthetic DNA candidates can be designed to dissect immunological mechanisms related to individual vaccine components, including titration of vaccine antigens and to study induction of broad immunity against similar and divergent pathogens.

FUTURE PROSPECTS FOR MULTIVALENT DNA VACCINES

The future of vaccine development hinges on cross-disciplinary science. As metrics for vaccine-associated protection against infectious diseases evolve, fostering collaborative research among teams with diverse biological, manufacturing, and clinical expertise is essential to advancing DNA vaccines and other platforms. Rather than solely pursuing sterilizing immunity, vaccine development should encompass a range of infection control strategies, including reducing pathogenesis, limiting transmission, and aiming to prevent hospitalization while minimizing morbidity and mortality.

The landscape of synthetic DNA vaccines is evolving. Different synthetic DNA forms including antibiotic-free plasmid systems, minicircles, and closed linear DNA forms continue to advance, and there is renewed excitement about rapid amplification technologies. Although cGMP plasmid DNA manufacturing pipelines are established, additional process development and regulatory pathways are necessary for clinical evaluation of different DNA forms. Improvements in formulation and delivery have the potential to further enhance DNA vaccine immunogenicity. Innovative approaches like highly engineered synthetic DNA nanomedicines, epitope strings, and gene-encoded adjuvants show promise, particularly for expansion of germinal centers, focusing of cytotoxic $CD8^+$ T cell responses, and establishment of durable memory. Further research to understand correlates of protection associated with DNA vaccines and multivalent combinations will be important.

In addition to their utility for global vaccines, multivalent DNA vaccines have the potential to be expanded as personalized medicine strategies against chronic infections and for control of antimicrobial resistant pathogens in diseases like cystic fibrosis. Similar strategies are being evaluated preclinically and in human trials as cancer immunotherapy (reviewed in [12]). Furthermore, while DNA vaccines have previously been approved for use in animals, multivalent combinations have potential to further reduce cost and improve broad protective immunity. In conclusion, multivalent DNA vaccine development holds tremendous promise to expand vaccine effectiveness and delivery and to complement One Health strategies.

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AMI PATEL is an Assistant Professor in The Wistar Institute Vaccine & Immunotherapy Center in Philadelphia, Pennsylvania, USA. She holds a BSc in Microbiology and Immunology from McGill University, an MSc in Medical Microbiology from the London School of Hygiene & Tropical Medicine, and a PhD in Medical Microbiology from the University of Manitoba. She received postdoctoral training at the San Raffaele Telethon Institute for Gene Therapy, Milan, Italy, the University of Pennsylvania, and The Wistar Institute. Patel is developing gene-encoded biologics, specifically non-viral DNA vectors, for vaccine and immunotherapy against emerging infectious diseases, with the goal of understanding cellular and immune mechanisms that contribute to protection versus pathogenesis. Patel's most recent studies include DNA vaccine development against ebolavirus, MERS-CoV, SARS-CoV-2, and DNA-encoded antibodies against multiple viral and bacterial pathogens. Her scientific advances in these areas include: 1) short and long-term study of DNA vaccine candidates in mice and macaques; and 2) sequence engineering and *in vivo* delivery developments to the rapidly advancing field of nucleic acid gene-encoded antibodies, specifically DNA-encoded antibodies.

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