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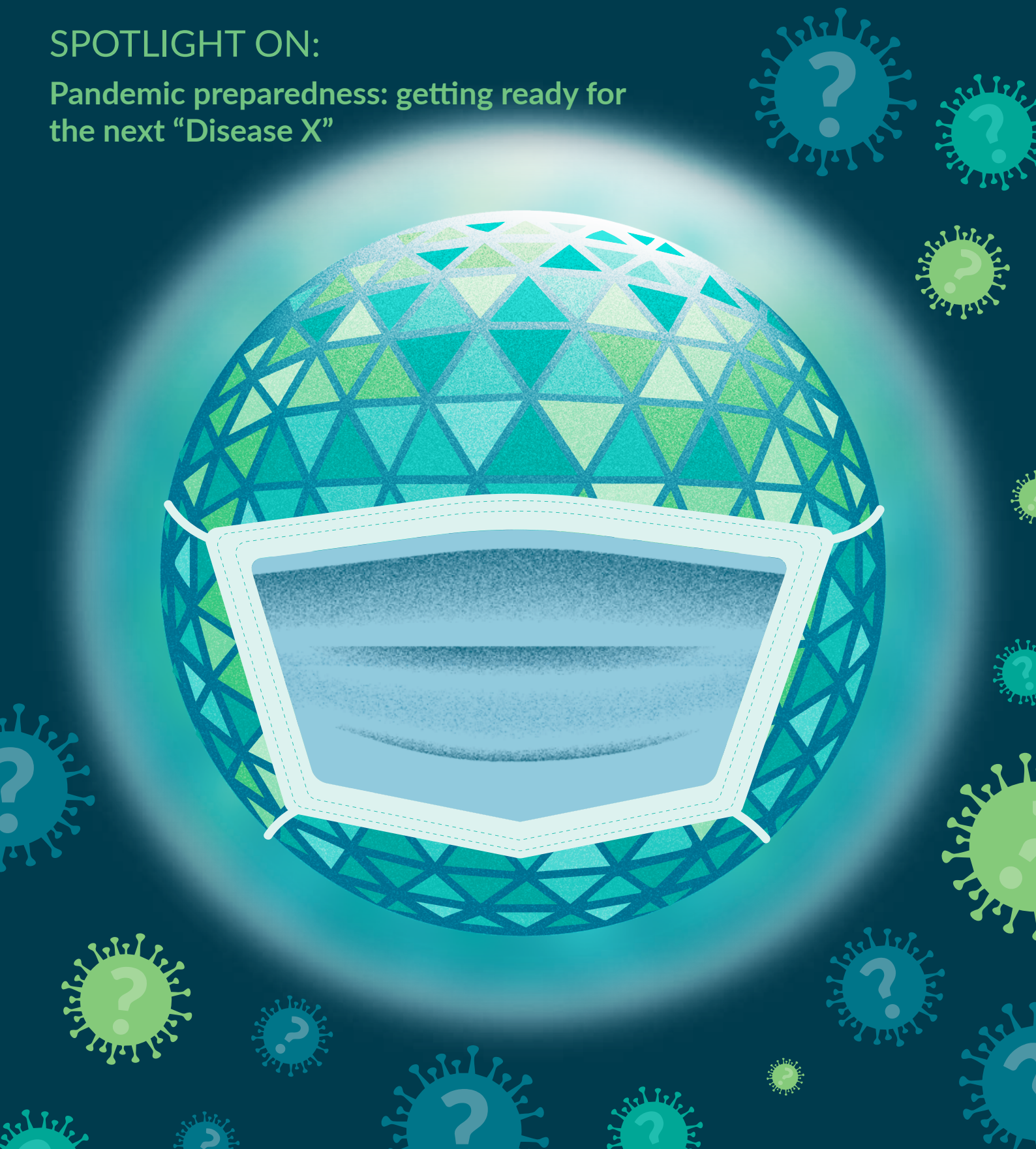
VACCINE INSIGHTS

SPOTLIGHT ON:

Pandemic preparedness: getting ready for the next "Disease X"



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PANDEMIC PREPAREDNESS: GETTING READY FOR THE NEXT 'DISEASE X'

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MVA-vectored universal beta-coronavirus vaccine design & development

Mark J Newman, Mary J Hauser, Arban Domi, Sreenivasa Rao Oruganti, Pratima Kumari & Ashley N Zuniga

Coronaviruses capable of infecting humans have circulated within the population and are well known to the scientific community. These viruses generally cause mild-moderate and recurring respiratory infections but pose minimal serious health risks. However, the more recent emergence of SARS-CoV-1, CoV-2, and MERS clearly demonstrate the risk of new coronavirus 'spillover events' from animal hosts, and this risk can be addressed proactively. A significant level of antigenic variation exists for the Spike protein amongst the coronaviruses that can infect humans and include the evolving variants of SARS-CoV-2. This is a well-recognized hurdle for vaccine development where the focus is on the induction of neutralizing antibody responses. However, a significant level of sequence and antigenic similarity is also known to exist, especially for the nucleocapsid, membrane proteins, and most of the non-structural proteins, and these conserved proteins are targets of the T cell arm of the immune system. Using modern viral vector-based vaccine technologies, it is feasible to design and develop vaccines capable of inducing T cell responses specific to multiple conserved viral proteins, providing a breadth of antiviral function and specificity. Vaccines of this type could serve as the basis for better targeting both SARS-CoV-2 as well as other beta-coronaviruses in a controlled prevention manner. This type of vaccine could be used as a booster to standard-of-care products or specifically for the benefit of unique patient populations where vaccine failure is common. Critically, we could return to a focus on prophylaxis, the prevention of disease through controlled vaccine campaign strategies using products that induce durable immune responses, including immunological memory.

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LIMITATIONS OF FIRST-GENERATION VACCINES FOR SARS-COV-2

The response to the global COVID-19 pandemic by public health entities and the vaccine industry was unprecedented in terms of speed and resulted in the development of multiple vaccines based on different technologies. The primary design focus of the industry was on the use of the SARS-CoV-2 spike (S) protein as the vaccine immunogen with the goal of inducing high levels of neutralizing antibodies [1–6]. By mid-2021, the combination of infection-induced and vaccine-induced antibody-positive individuals (seroprevalence) was reported to exceed 80% [7]. This level was expected to provide a significant level of protection at the population level (herd immunity). Unfortunately, we have observed continued circulation of SARS-CoV-2 variants, making evident several limitations associated with the S-protein-focused approach of first-generation vaccines.

Of immediate concern is the emergence of immune escape variants with changes in the S-gene and -protein sequences that mediate resistance to the neutralizing capacity of vaccine-induced antibodies (Figure 1) [8]. This evolution of SARS-CoV-2 is driven, in part, by the continued circulation of the virus in the immune population wherein vaccine-induced antibodies can support the selection of variants that are resistant to neutralization [9,10]. Variants of concern (VOC) were recognized early in the pandemic, but it was the Delta and Omicron VOC, with almost total resistance to neutralizing antibodies, that highlighted the severity of the problem, driving new waves of infections with serious levels of morbidity [11–18]. To address this issue, bivalent mRNA booster vaccines based on the sequences of both the Wuhan and Omicron (BA5) were developed and received Emergency Use Authorization, accepted primarily on their ability to increase the titer and breadth of neutralizing antibody functions [19–22]. This approach can be effective, as demonstrated in the influenza virus vaccine model, but public acceptance and compliance can be rate-limiting.

A secondary issue is that the kinetics and duration of antibody responses induced by coronavirus infection or vaccination with first-generation products are highly variable and often short-lived, thus limiting the effect of herd immunity. This variation could be a function of the immunogenicity of the S-protein, a limited helper T cell component associated with the use of only a single protein as the immunogen, undefined limitations of the vaccine platforms, an inherent issue with immune responses to coronaviruses, or any combination of these and other factors [23–28]. Antibody response durability is being addressed in the population and experimentally through the use of repeated booster immunizations and heterologous immunization strategies but more needs to be done to better define and address the existing limitations through improved vaccine design [29,30].

A third issue is the often-overlooked patients with special medical limitations or needs. Within the vaccine field, this includes the part of the population that is partially immunocompromised. These individuals often cannot routinely raise nor maintain protective antibody responses following receipt of first-generation mRNA vaccines, contributing to an unacceptable level of variation in vaccine efficacy. This includes patients suffering from and/or being treated for numerous malignancies, autoimmune disorders, transplant patients, dialysis patients, and potentially, even the aging population [31–41]. Approved vaccines were generally as safe in these patients as the general population, allowing for the administration of additional booster doses, thus providing level benefit, but again this may be a limitation that can be better addressed through improved vaccine design [42].

Next-generation vaccines that will increase the magnitude, duration, and functional breadth of immune responses, including the establishment of immunological memory and responses that better protect mucosal tissues, are needed [42]. The desired level of improvement must function to address risk from new

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VOC but also better serve the populations that are under-protected. Ideally, next-generation vaccines will also provide protection from the risk posed by the emergence or spillover of other beta-coronaviruses, analogous to SARS-CoV-2. We believe that this can be best achieved through the design and development of vaccines that optimally engage the cellular, or T cell, arm of the immune system with epitope specificity focused on parts of the virus that are not prone to variation and immune-mediated selection of VOC.

CELLULAR IMMUNITY TO CONSERVED CORONAVIRUS PROTEINS

Coronaviruses that can infect humans are large enveloped, single-stranded positive-sense RNA viruses that share a high level of sequence identity (Figure 1). The major structural proteins are S, nucleocapsid (N), envelope (E), and membrane (M) [43]. Numerous nonstructural proteins (NSP) and open-reading frame proteins (ORF), representing >60% of the genome, include proteins with a diverse range of activities including RNA-dependent RNA polymerase (RDRP), papain-like protease (PLpro), main protease (Mpro), helicase, exo- and endo-ribonucleases and proteins with virulence and immune system downregulation activities [44–47]. Most of the structural proteins and NSP are likely to be immunogenic, based on the prediction of T cell epitopes, and could be considered as additional vaccine targets [48].

The COVID-19 pandemic spurred significant effort into the characterization of T cell response to SARS-CoV-2. As predicted, CD4+ and CD8+ T cell epitopes of structural proteins are well recognized but so are epitopes within known NSP and ORF genes [49–52]. Responses specific to epitopes in the S, M, and N proteins are most common, which correlates with the large size of these proteins and/or their abundance in viral particles. However, responses to epitopes in most of the NSP and ORF proteins have also been detected, indicating breath of response is common in convalescent individuals. CD4+ T cells are prevalent, if not predominant over CD8+ T cells, following both symptomatic and asymptomatic SARS-CoV-2 infections. The majority of predicted or known T cell epitopes (85–95%) are highly conserved amongst VOC, based on amino acid sequences. [48].

The observation that early or pre-existing T cell responses, detected in the absence of prior documented infection and likely induced by previous infections involving coronavirus other than SARS-CoV-2, were significantly associated with lower levels of disease pathogenesis are of particular importance because this supports the belief that a significant level of protection can be mediated by T cells specific for conserved epitopes [49,53–59] This idea is supported by studies completed using murine models for both SARS-CoV-1 and CoV-2, which demonstrated the critical importance of T cells, including memory T cells, for optimal protection, viral clearance and recovery [45,60–62].

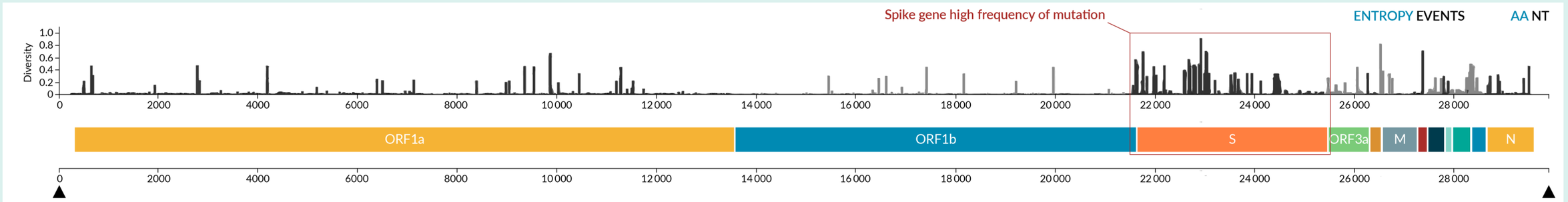
The findings of both animal and human studies support the concept that the induction of T cell responses specific to conserved epitopes represents a logical approach toward the development of vaccines that can better protect against VOC. For example, an experimental mRNA vaccine based on the Wuhan sequences of S and N protected hACE2-transgenic mice against both Wuhan and Omicron virus challenges [63]. Evaluation of S- and N-based vaccine using the Modified Vaccinia Virus Ankara (MVA) platform protected rhesus macaques from infections with Wuhan and Delta variants and hamsters from Omicron infections [64,65]. Thus, the importance of inducing T cell responses specific for multiple viral proteins as a focus for next-generation vaccine design cannot be underestimated.

MAKING A SAFE & EFFECTIVE VACCINE

The production of vaccines designed to induce both CD4 and CD8 T cell responses that are

FIGURE 1

Sequence variation of the SARS-CoV-2 genome.



Source: https://nextstrain.org/ncov/gisaid/global/6m

broadly specific is complicated by the need to deliver multiple immunogenic proteins and to intersect multiple antigen processing and presentation pathways. Genetic vaccines, specifically nucleic acid-based vaccines and viral vectors, are best suited for this role.

Viral-vectored vaccine platforms rely on recombinant viruses engineered to express heterologous antigens to initiate pathogen-specific immune responses [66]. Inherent interactions of the virus vector with the host cell stimulate innate immunity without the need for exogenous adjuvants. Immune responses induced by viral-vectored vaccines are generally characterized by a strong CD8+ T cell response, which is critical to clearance of viral infection. The most commonly used virus vectors are derived from human adenoviruses serotypes 4, 5, 26, and 35, simian adenoviruses, vesicular stomatitis virus, adeno-associated virus, poxviruses like MVA and human cytomegalovirus [67]. Each virus vector harbors a varying degree of virus replication, activation of innate immune pathways, and safety profile.

We focus on the use of the MVA vaccine vector platform. MVA is an attenuated form of vaccinia virus that was developed as a smallpox vaccine. It is a live virus vaccine that readily infects human cells, but it is replication defective and cannot produce a productive infection. It has an established safety record given its approved use as a smallpox and mpox vaccine in immunocompromised individuals [68,69]. Properties of MVA that support its use as a vaccine vector for next-generation SARS-CoV-2 vaccines include:

1. MVA has a large and available genetic coding capacity allowing for the insertion of multiple genes into different sites, supporting the simultaneous expression of multiple immunogenic proteins.

2. MVA preferentially targets antigen-presenting cells *in vivo*, in particular cells of the dendritic cell lineage [70–72]. This is of particular importance for the induction of CD8+ T cells where antigen processing through the proteosome to generate epitopes and direct antigen presentation are required.

3. MVA also presents antigens through the cross-presentation pathway, which is highly effective for the induction of antibody and CD4+ T cell responses [73,74].

4. It lacks critical immune evasion genes present in vaccinia and allows for the induction of innate immune responses which provide an adjuvant effect [75].

5. Pre-existing immunity can impact the utility of viral vectors because antibodies block infection of target cells. This is a concern for many viral vectors, including MVA, because the majority of the world's population born before the early 1970s were vaccinated against smallpox. However, MVA infection of cells and the subsequent expression of encoded genes does not appear to be impacted by pre-existing immunity induced by smallpox vaccination.

6. MVA can be safely and effectively used as a vaccine or a vaccine vector in people of all ages, including immunocompromised individuals [76].

These combined properties all contribute to the utility of the MVA vaccine vector system to produce next-generation coronavirus vaccines designed to induce broadly specific and functional T cell responses.

The most logical first step in the program was to produce an MVA-vectored vaccine encoding the S protein and a second structural protein. The N protein was selected because of the documented presence of T cell epitopes and positive animal model studies involving SARS-CoV-1 [77–78]. The resulting vaccine, termed COH04S1 (labeled GEO-CM04S1 for clinical development by GeoVax Inc., Atlanta, GA), encodes the S and N proteins based on the Wuhan sequence. COH04S1 was extensively tested in relevant animal models and shown to induce protective immune responses characterized by T cell responses to both S and N [79–82]. The vaccine was successfully tested in a dose escalation safety and immunogenicity Phase 1 clinical trial, which demonstrated it to be highly effective at inducing T cell responses, both CD4 and CD8, at low vaccine doses. Importantly, and as predicted based on the studies of others, the T cell responses were not reduced when measured using Delta and Omicron-specific materials [82]. The COH04S1 vaccine product is the initial step towards a next-generation SARS-CoV-2 vaccine with the ability to increase the breadth and durability of immune responses, and in particular to induce T cell responses to conserved epitopes in both S and N.

Phase 2 clinical trials are being run by GeoVax with a focus on different cancer treatment patients and as a booster in healthy volunteers (ClinicalTrials.gov ID: NCT04977024 and NCT04639466). The use of the vaccine in conjunction with the standard-of-care S-based vaccines, such as the currently approved bivalent mRNA vaccines, in at-risk patient populations is

envisioned as the preferred market. This will include patients suffering from and/or being treated for numerous malignancies, autoimmune disorders, kidney failure and dialysis, and other conditions that compromise the immune system.

POTENTIAL FOR THE DEVELOPMENT OF A VACCINE TO PROTECT BEYOND VOC

As noted, the MVA vaccine vector is characterized by a large genetic coding capacity that can allow the expression of multiple genes of interest and drive the expression of multiple immunogens by a single vaccine. For example, GeoVax previously designed, produced, and clinically tested a single MVA-vectored vaccine for HIV that encoded for gag, protease, reverse transcriptase, env (gp160), tat, vpu, and rev, and the human cytokine GM-CSF [83]. The potential for building on the MVA vaccine vector platform to produce a beta-coronavirus vaccine capable of protecting humans from future SARS-CoV-2 VOC and other circulating coronaviruses appears to be technically feasible and highly conserved NSP may be a suitable focus because of the presence of numerous CD8 T cell epitopes [84,85].

The first step in the design of a beta-coronavirus vaccine based on this approach would be the selection of NSP. There are numerous potential candidates and consideration of multiple factors needs to be included in this effort (Figure 1). We believe the priorities are as follows:

1. The level of amino sequence and T cell epitope conservation amongst different viruses needs to be significant and span across a diverse collection of viruses, beyond SARS-CoV-1 and include MERS and potentially the seasonally circulating viruses associated with the common cold. The net should be cast widely.

2. The evidence supporting the ability of T cells targeting specific proteins to contribute to the control viral replication *in vivo* needs to be evaluated using human epidemiology data and relevant animal models.

3. It is critical that the selected proteins do not pose a toxicity risk to the vaccinee when expressed at higher levels *in vivo* under the control of a vaccine vector. This includes immune system dysfunction.

4. Critical to vaccine production, the selected proteins cannot interfere with the replication of MVA in the avian cells used as the manufacturing substrate.

Based on these factors, we completed an initial analysis and found that many, but not all, of the genes in regions ORF1a and ORF1b are highly conserved and identified NSP3, NSP6, NSP12, NSP13, and NSP14 as logical targets for use as vaccine immunogens. The properties associated with these NSP are summarized in Table 1.

The technical processes for constructing the MVA-vectored vaccines are well established and building on the existing COH04S1 or other prototype research vaccines is feasible. The cytoplasmic expression and the large capacity of MVA to stably accept foreign sequences make it a popular choice for gene delivery, especially for multi-antigen vaccines. However, like most virus-based vectors, the insertion of the foreign genes in MVA using the classic methods is laborious and time-consuming. For a multi-antigen vaccine candidate, the time taken can be multiplied by the number of inserts. Using an approach whereby the MVA genome is cloned into *E. coli* plasmids can significantly reduce the time required to produce new constructs and should support the expansion of MVA-vectored vaccine development [80].

Production of poxviruses has changed little since the 1930s and utilizes primary cells from embryonated chicken eggs. Limitations of this approach become apparent with the need for specific pathogen-free eggs and

TABLE 1

NSP selected as vaccine immunogens

Protein designation	Immunogenicity/antigenicity (Selected literature citations)	Virus function/host cell interactions
NSP3	Grifoni *et al.* [49] Ong *et al.* [86] Quadeer Ahmed McKay [87] Grifoni *et al.* [52]	• Protease • Type 1 interferon antagonist
NSP6	Poland *et al.* [88] Bacher *et al.* [89]	• Facilitates assembly of replicase proteins • Induction of autophagosomes from host endoplasmic reticulum • Limits the expansion of phagosomes
NSP12	Swadling *et al.* [56] Grifoni *et al.* [52]	• RNA-dependent RNA Polymerase (RdRp) • Replication and transcription of the entire SARS-CoV-2 genome is catalyzed by an RdRp
NSP13	Le Bert *et al.* [90] Swadling *et al.* [56] Pan *et al.* [91]	• Zinc binding domain in N terminus • RNA and DNA duplex unwinding with 5' – 3' polarity • Helicase
NSP14	Mateus *et al.* [55] Kared *et al.* [92]	• Translation inhibitory factor • Inhibits host protein synthesis • Inhibits type 1 interferon viral response

continuous introduction of cell substrate. Efforts are underway to replace primary cells with a qualified continuous cell line. Several avian cell lines have been tested that support MVA production including AGE1.CR, DF-1, and EB66 cells [93–95]. Although commercial-scale production has not been undertaken using avian cells, several vaccines were produced utilizing AGE.CR1 or DF-1 cell lines have entered clinical trials.

However, the animal testing process needed to critically evaluate candidate vaccines is complex. Testing will need to address potential adverse pathology risks associated with the NSP directly and with potential deleterious immunopathology associated with vaccine-induced T cell responses. The inclusion of infectious challenge experiments using MERS or other coronaviruses that aren't closely related to SARS-CoV-2 will be needed. Many coronaviruses don't utilize the ACE2 protein as the receptor for infection and this will limit the utility of the hACE2-transgenic mouse model. Reliance on mouse-adapted coronavirus models and an expanded assessment of disease pathogenesis will be required [96–103].

BETTER PREPARATION FOR THE INEVITABLE

The spillover of SARS-CoV-1 and MERS into the human population spurred short-term research interest in vaccines for pathogenic coronaviruses. Luckily, what was known and developed previously could be coupled with cutting-edge vaccine technologies for the development of efficacious first-generation SARS-CoV-2 vaccines with a rapid response mindset. However, we can safely assume future challenges from coronaviruses will evolve and as such, we must consider a more proactive focus with efforts focused on the development of next-generation vaccines. Vaccines capable of inducing durable and protective immune responses to conserved regions, with T cells as a predominant effector mechanism, are needed. The availability of such vaccines would support prophylactic vaccine strategies and campaigns, thus reducing the requirement for approaches focused on rapid response. These vaccines could be produced and distributed in a controlled and equitable manner without the stress and panic endured in the SARS-CoV-2 pandemic.

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AFFILIATIONS

Mark J Newman
GeoVax Inc,
1900 Lake Park Drive, Ste 380
Smyrna, GA 30080

Mary J Hauser
GeoVax Inc,
1900 Lake Park Drive, Ste 380
Smyrna, GA 30080

Arban Domi
GeoVax Inc,
1900 Lake Park Drive, Ste 380
Smyrna, GA 30080

Sreenivasa Rao Oruganti
GeoVax Inc,
1900 Lake Park Drive, Ste 380
Smyrna, GA 30080

Pratima Kumari
GeoVax Inc,
1900 Lake Park Drive, Ste 380
Smyrna, GA 30080

Ashley N Zuniga
GeoVax Inc,
1900 Lake Park Drive, Ste 380
Smyrna, GA 30080

REFERENCES

1. Hsieh CL, Goldsmith JA, Schaub JM, *et al.* Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science* 2020; 369, 1501–1505.
2. Polack FP, Thomas SJ, Kitchin N, *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* 2020; 383, 2603–2615.
3. Ewer KJ, Barrett JR, Belij-Rammerstorfer S, *et al.* T cell and antibody responses induced by a single dose of ChAdOx1 Ncov-19 (AZD1222) vaccine in a phase 1/2 clinical trial. *Nat. Med.* 2021; 27, 270–278.
4. Shinde V, Bhikha S, Hoosain Z, *et al.* Efficacy of NVX-CoV2373 Covid-19 Vaccine Against the B.1.351 Variant. *N. Engl. J. Med.* 2021; 384, 1899–909.
5. Sadoff J, Gray G, Vandebosch A, *et al.* Safety and Efficacy of Single-Dose Ad26. Cov2.S Vaccine Against Covid-19. *N. Engl. J. Med.* 2021; 384, 2187–2201.
6. Voysey M, Clemens SAC, Madhi SA, *et al.* Safety and efficacy of the ChAdOx1 Ncov-19 Vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; 397, 99–111.
7. Jones JM, Stone M, Sulaeman H, *et al.* Estimated US infection- and vaccine-induced SARS-CoV-2 seroprevalence based on blood donations, July 2020-May 2021. *JAMA* 2021; 326, 1400–1409.
8. Phillips N, The coronavirus is here to stay - Here's what that means. *Nature* 2021; 590, 382–384.
9. Pulliam JRC, van Schalkwyk C, Govender N, *et al.* Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. *medRxiv* 2021; preprint.
10. Wang R, Hozumi Y, Zheng YH, Yin C, Wei GW. Host immune response driving SARS-CoV-2 evolution. *Viruses* 2020; 2, 1095.
11. Garcia-Beltran WF, E. Lam EC, *et al.* Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* 2021; 184, 2372–2383.e9.
12. Wang P, Nair MS, Liu L, *et al.* Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *bioRxiv* 2021; preprint.
13. Planas D, Veyer D, Baidaliuk A, *et al.* Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature* 2021; 596, 276–280.
14. McCallum M, Czudnochowski N, Rosen LE, *et al.* Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. *bioRxiv* 2021, preprint.
15. Cao Y, Yisimayi A, Jian F, *et al.* BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by omicron infection. *Nat* 2022; 608, 593–602.
16. Suryawanshi RK, Chen IP, Ma T, *et al.* Limited cross-variant immunity from SARS-CoV-2 omicron without vaccination. *Nature* 2022; 607, 351–355.
17. Tang J, Novak T, Hecker J, *et al.* Cross-reactive immunity against the SARS-CoV-2 omicron variant is low in pediatric patients with prior COVID-19 or MIS-C. *Nat. Comm.* 2022; 13, 2979.

18. Bowen JE, Addetia A, Dang HV, *et al.* Omicron spike function and neutralizing activity elicited by a comprehensive panel of vaccines. *Science* 2022; 377, 890–894.

19. Lin DY, Xu Y, Gu Y, *et al.* Effectiveness of Bivalent Boosters against Severe Omicron Infection. *N. Engl. J. Med.* 2023; 388, 764–766.

20. Chalkias S, Harper C, Vrbicky K, *et al.* A bivalent Omicron-containing booster vaccine against COVID-19. *N. Eng. J. Med.* 2022; 387, 1279–1291.

21. US Food and Drug Administration. Coronavirus (COVID-19) update: FDA authorizes Moderna, Pfizer-BioNTech bivalent COVID-19 vaccines for use as a booster dose (August 31, 2022).

22. Centers for Disease Control and Prevention. Stay up to date with COVID-19 vaccines including boosters. (September 8 2022).

23. Wheeler SE, Shurin GV, Yost M, *et al.* Differential antibody response to mRNA COVID-19 vaccines in healthy subjects. *Microbiol. Spectr.* 2021; 9, e00341-21.

24. Chia WN, Zhu F, Xiang SW, *et al.* Dynamics of SARS-CoV-2 neutralizing antibody responses and duration of immunity: a longitudinal study. *Lancet Microbe* 2021; 2, e240–244.

25. Dupont L, Luke B, Snell LB, *et al.* Neutralizing antibody activity in convalescent sera from infection in humans with SARS-CoV-2 and variants of concern. *Nature Micro.* 2021; 6,1433–1442.

26. Seow J, Graham C, Merrick, *et al.* Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-C0V-2 infection in humans. *Nature Micro.* 2020; 5, 1598–1607.

27. Feng C, Shi J, Fan Q, *et al.* Protective humoral and cellular immune responses to SARS-CoV-2 persist up to 1 year after recovery. *Nat. Comm.* 2021; 4984.

28. Goel RR, Painter MM, Apostolidis SA, *et al.* mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. *Science* 2021; 374(6572), abm0829

29. Munro APS, Janani L, Cornelius V, *et al.* Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre randomised, controlled, phase 2 trial. *Lancet* 2021; 398, 2258–2276.

30. Embi PJ, Levy ME, Naleway AL, *et al.* Effectiveness of 2-dose vaccination with mRNA COVID-19 vaccines against COVID-1-associated hospitalizations among immunocompromise adults—nine states, January-September 2021. *MMWR* 2021; 70,1–7.

31. Rugge M, Zorzi M and Guzzinati S. SARS-CoV-2 infection in the Italian Veneto region: adverse outcomes in patients with cancer. *Nat. Cancer* 2020; 1, 784–788.

32. Assaad S, Avrillon V, Fournier M-L, *et al.* High mortality rate in cancer patients with symptoms of COVID-19 with or without detectable SARS-COV-2 on RT-PCR. *Eur. J. Cancer* 2020; 135, 251–259.

33. Miyashita H, Kuno T. Prognosis of coronavirus disease 2019 (COVID-19) in patients with HIV infection in New York City. *HIV Med.* 2020; 22, e1–2.

34. Dai M, Liu d, Liu M, *et al.* Patients with cancer appear more vulnerable to SARS-COV-2: a multi-center study during the COVID-19 outbreak. *Cancer Discov.* 2020; 10, 783–791.

35. Shree T, Shankar, V, Lohmeyer JK, *et al.* CD-20-target therapy ablates *de novo* antibody response to vaccination but spares preestablished immunity. *Blood Cancer Disc.* 2022; 3, 1–9.

36. Bassi J, Giannini O, Silacci-Freni C, *et al.* Poor neutralization and rapid decay of antibodies to SARS-CoV-2 variants in vaccinated dialysis patients. *PLoS ONE* 2022; 17, e0263328.

37. Morris S, Anjan S, Pallikkuth S, *et al.* Reinfection with SARS-CoV-2 in solid-organ transplant recipients: incidence density and convalescent immunity prior to reinfection. *Transpl. Infect Dis.* 2022; 24(3), e13827.

38. Hause AM, Baggs J, Marquez P, *et al.* Safety monitoring of COVD-19 mRNA vaccine booster doses among parsons aged >12 years with presumed immunocompromised status-United States. *MMWR* 2022; 71, 899–903.

39. Haydu JE, Maron JS, Redd, RA, *et al.* Humoral and cellular immunogenicity of SARS-CoV-2 vaccines in chronic lymphocytic leukemia: a prospective cohort study. *Blood Adv.* 2022; 6, 1671–1683.

40. Cook, LB, O'Dell G, Vourvou E, *et al.* Third primary SARS-CoV-2 mRNA vaccines enhance antibody responses in most patients with hematological malignancies. *Nat. Comm.* 2022; 13, 6922.

41. Reeg DB, Hogmann M, Neumann-Haefelin C, *et al.* SARS-CoV-2-specific T cell responses in immunocompromised individuals with cancer, HIV or solid organ transplants. *Pathogens* 2023; 12, 244.

42. Morens DM, Taubenberger JK Fauci AS. Rethinking next-generation vaccines for coronaviruses, influenzaviruses and other respiratory viruses. *Cell Host Microbe* 2023; 31146–31157.

43. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat. Rev, Micro.* 2021; 19, 141–154.

DOI: 10.18609/vac.2023.032

44. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS Coronaviruses with the antiviral interferon response. *Adv. Virus Res.* 2016; 96, 219–243.

45. Perlman S, Netland J. Coronaviruses post-SARS: Update on replication and pathogenesis. *Nature Rev. Micro.* 2009; 7, 439–450.

46. Kasuga Y, Zhu B, Jang K-J & Yoo J-S. Innate immune sensing of coronavirus and viral evasion strategies. *Exp. Mol. Med.* 2021; 53, 723–736.

47. Snijder EJ, Decroly E & Ziebuhr J. The nonstructural proteins directing coronavirus RNA synthesis and processing. *Adv. Virus Res.* 2016; 96, 59–126.

48. Grifoni A, Sidney J, Zhang Y, *et al.* A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. *Cell Host Microbe* 2021; 27, 671–680.e2.

49. Grifoni A, Weiskopf D, Ramirez SI, *et al.* Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020; 181, 489–1501. e15.

50. Sette A, Crotty S. Pre-existing immunity to SARS-CoV-2: the knowns and unknowns. *Nat. Rev. Immunol.* 2020; 20, 457–458.

51. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021; 184, 861–880.

52. Grifoni A, Sidney J, Vita R, *et al.* SARS-CoV-2 human T cell epitopes: adaptive immune response against COVID-19. *Cell Host Microbe* 2021; 29, 1076–1092.

53. Sekine T, Perez-Potti A, Rivera-Ballesteros O, *et al.* Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* 2020; 183, 158–168.e14.

54. Rydyznski Moderbacher C, Ramirez SI, Dan JM, *et al.* Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell* 2020; 183, 996–1012.e19.

55. Mateus J, Grifoni A, Tarke A, *et al.* Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* 2020; 370, 89–94.

56. Swadling L, Diniz MO, Schmidt NM, *et al.* Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. *Nat.* 2022; 60, 110–117.

57. Loyal L, Braun J, Henze L, *et al.* Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science* 2021; 8; 374, eabh1823.

58. Kundu R, Narean JS, Wang L, *et al.* Cross-reactive memory T cells associate with protection against SARS-CoV-2 infection in COVID-19 contacts. *Nat. Comm.* 2022; 10; 13, 80.

59. da Silva Antunes R, Pallikkuth S, Williams E, *et al.* Differential T cell reactivity to endemic coronaviruses and SARS-CoV-2 in community and health care workers. *J. Infect. Dis.* 2021; 224, 70–80.

60. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. *Immunol. Res.* 2014; 59, 118–128.

61. Channappanavar R, Fett C, Zhao J, *et al.* Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome in coronavirus infection. *J. Virol.* 2014; 88, 11034–11044.

62. Israelow B, Mao T, Klein J, *et al.* Adaptive immune determinants of viral clearance and protection in mouse models of SARS-CoV-2. *Sci. Immunol.* 2021; 6, eabl4509.

63. Hajnik RL, Plante JA, Liang Y, *et al.* Dual spike and nucleocapsid mRNA vaccination confer protection against SARS-CoV-2 Omicron and Delta variants in preclinical models. *Sci. Transl. Med.* 2022; 14(14), eabq1945.

64. Routhu NK, Gangadhara S, Lai L, *et al.* A modified vaccinia Ankara vaccine expressing spike and nucleocapsid protects rhesus macaques against SARS-CoV-2 Delta infection. *Sci. Immunol.* 2022; 7, eabo0226.

65. Wussow F, Kha M, Kim T, *et al.* Synthetic multiantigen MVA vaccine COH04S1 and variant-specific derivatives protect Syrian hamsters from SARS-CoV-2 Omicron subvariants. *npj Vaccines* 2023; 8, 41.

66. McCann N, O'Connor D, Lambe T, *et al.* Viral vector vaccines. *Curr. Opin. Immunol.* 2022; 77, 102210.

67. Hanks T. New vector and vaccine platforms: mRNA, DNA, viral vectors. *Curr. Opin. HIV AIDS* 2022; 17, 338–344.

68. Pittman PR, Hahn M, Lee HS, *et al.* Phase 3 efficacy trial of modified vaccinia ankara as a vaccine against smallpox. *N. Engl. J. Med.* 2019; 381, 1897–1908.

69. Vollmar J, Arndtz N, Eckl KM, *et al.* Safety and immunogenicity of IMVA-MUNE, a promising candidate as a third generation smallpox vaccine. *Vaccine* 2006; 24, 2065–2070.

70. Altenburg AF, van de Sandt CE, Li BWS, *et al.* Modified vaccinia virus ankara preferentially targets antigen presenting cells *in vitro, ex vivo* and *in vivo*. *Sci. Rep.* 2017; 7.

71. Liu L, Chavan R, Feinberg MB. Dendritic cells are preferentially targeted among hematolymphocytes by modified vaccinia virus ankara and play a key role in the

induction of virus-specific T cell responses *in vivo*. *BMC Immunol.* 2008; 9, 15.

72. Kastenmuller W, Drexler I, Ludwig H, *et al*. Infection of human dendritic cells with recombinant vaccinia virus MVA reveals general persistence of viral early transcription but distinct maturation-dependent cytopathogenicity. *Virol*. 2006; 350, 276–288.

73. Gasteiger G, Kastenmuller W, Ljapoci R, *et al.* Cross-priming of cytotoxic T cells dictates antigen requisites for Modified Vaccinia Virus Ankara vector vaccines. *J. Virol.* 2007; 81, 11925–11936.

74. Shen X, Wong SBJ, Buck CB, Zhang J, Siliciano RF. Direct priming and cross-priming contribute differentially to the induction of CD8+ CTL following exposure to vaccinia virus via different routes. *J. Immunol.* 2002; 169, 4222–4229.

75. Price PJR, Torres-Domínguez LE, Brandmüller C, *et al.* Modified vaccinia virus ankara: innate immune activation and induction of cellular signaling. *Vaccine* 2013; 31, 4231–4234.

76. Radetsky M. Smallpox: a history of its rise and fall. *Ped. Inf. Dis. J.* 1999; 18, 85–93.

77. Peng, H. Yang L-T, Wang L-y, *et al.* Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients. *Virol.* 2006; 351, 466–475.

78. Azizi, A. Aucoin s, Tadesse H, *et al*. A combined nucleocapsid vaccine induces vigorous SARS-CD8+ T cell immune responses. *Genet. Vaccines Ther.* 2005; 3, 7–7.

79. Chiuppesi F, Nguyen VH, Park Y, *et al.* Synthetic multiantigen MVA vaccine COH04S1 protects against SARS-CoV-2 in syrian hamsters and non-human primates. *npj Vaccines* 2022; 7, 7–7.

80. Chiuppesi F, Salazar M d'Alincourt, Contreras H, *et al*. Development of a multi-antigenic SARS-CoV-2 vaccine candidate using a synthetic poxvirus platform. *Nat. Comm.* 2020; 11, 6121–6121.

81. Chiuppesi F, Zaia JA, Frankel PH, *et al.* Safety and immunogenicity of a synthetic multiantigen modified vaccinia virus ankara-based COVID-19 vaccine (COH04S1): an open-label and randomised, phase 1 trial. *Lancet Microbe* 2022; 3(4), e252–e264.

82. Chiuppesi F, Zaia JA, Faircloth K, *et al.* Vaccine-induced spike- and nucleocapsid-specific cellular responses maintain potent cross-reactivity to SARS-CoV-2 Delta and Omicron variants. *iScience* 2022; 25, 104745.

83. Hellerstein M, Xu Y, Marino T, *et al.* Co-expression of HIV-1 virus-like particles and granulocyte-macrophage colony stimulating factor by GEO-D03 DNA vaccine. *Hum. Vacc. Immunother.* 2021; 8, 1654–1658.

84. Kared H, Redd AD, Bloch EM, *et al.* SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. *J. Clin. Invest.* 2021; 131(5), e145476.

85. Ferretti AP, Kula T, Wang Y, *et al.* Unbiased screens show CD8+ T cells of COVID-19 patients recognize shared epitopes in SARS-CoV-2 that largely reside outside the spike protein. *Immunity* 2020; 53, 1095–1107.e3.

86. Ong E, Wong MU, Huffman A, He Y. COVID-19 coronavirus vaccine design using reverse vaccinology and machine learning. *Front. Immunol.* 2020; 11, 1581.

87. Quadeer AA, Ahmed SF, McKay MR. Landscape of epitopes targeted by T cells in 852 individuals recovered from COVID-19: meta-analysis, immunoprevalence, and web platform. *Cell Rep.Med.* 2021; 2, 100312.

88. Poland GA, Ovsyannikova IG, Kennedy RB. SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. *Lancet* 2020; 39, 1595–1606.

89. Bacher P, Rosati E, Esser D, *et al.* Low-avidity CD4+ T cell responses to SARS-CoV-2 in unexposed individuals and humans with severe COVID-19. *Immunity* 2020; 53, 1258–1271.e5.

90. le Bert N, Tan AT, Kunasegaran K, *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* 2020 584; 7821, 457–462.

91. Pan K, Chiu Y, Huang E, *et al*. Mass spectrometric identification of immunogenic SARS-CoV-2 epitopes and cognate TCRs. *Proc. Natl. Acad. Sci. USA* 2021; 118(46), e2111815118.

92. Kared H, Redd AD, Bloch EM, *et al.* SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. *J. Clin. Invest.* 2021; 131(5), e145476.

93. Kallel H, Kamen AA. Large-scale adenovirus and poxvirus-vectored vaccine manufacturing to enable clinical trials. *Biotech. J.* 2015; 10, 641–747.

94. Garber DA, O'Mara LA, Zhao J, *et al.* Expanding the repertoire of modified vaccinia ankara-based vaccine vectors via genetic complementation strategies. *PLOS One* 2009.

95. Jordan I, Lohr V, Genzel Y, *et al.* Elements in the development of a production process for modified vaccinia virus ankara. *Microorganisms* 2013; 1, 100–121.

DOI: 10.18609/vac.2023.032

96. Natekar JP, Pathak H, Stone S, *et al.* Differential pathogenesis of SARS-CoV-2 variants of concern in human ACE2-expressing mice. *Viruses* 2022; 14, 1139.

97. Muñoz-Fontela C, Dowling WE, Funnell SGP, *et al.* Animal models for COVID-19. *Nature* 2020; 586(7830), 509–515.

98. Stone S, Rothan HA, Natekar JP, *et al.* SARS-CoV-2 Variants of concern infect the respiratory tract and induce inflammatory response in wild-type laboratory mice. *Viruses* 2022; 14(1), 27.

99. Roberts A, Deming D, Paddock CD, *et al.* A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathog.* 2007; 3, 0023-0037.

100. Agnihothram S, Yount BL, Donaldson EF, *et al.* A mouse model for Beta-coronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *mBio* 2014; 5(2).

101. Cockrell AS, Yount BL, Scobey T, *et al.* A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. *Nat. Microbiol.* 2016, 2(2).

102. Dinnon KH, Leist SR, Schäfer A, *et al.* A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. *Nature* 2020; 586(7830), 560–566

103. Kumari P, Rothan HA, Natekar JP, *et al.* Neuroinvasion and encephalitis following intranasal inoculation of SARS-CoV-2 in K18-hACE2 Mice. *Viruses* 2021; 13(1), 132

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VACCINEINSIGHTS

PANDEMIC PREPAREDNESS: GETTING READY FOR THE NEXT 'DISEASE X'

SPOTLIGHT

EXPERT INSIGHT

Development of recombinant vesicular stomatitis virus vaccine platform for rapid response to Ebola and COVID-19 outbreaks

Christopher Ton, Michael A Winters, Raymond Ducoat, Douglas D Richardson, Kristine Fuller & Melissa Hughes

Epidemic and pandemic outbreaks can increase mortality, cause upheaval to healthcare systems, and disrupt global economy and security. Given these threats, it is imperative that there are rapid responses to outbreaks to limit human, social, and economic costs of pandemics. The Ebola virus disease (EVD) epidemic and COVID-19 pandemic posed serious threats to global health, affecting millions to billions of people and disrupting public health services worldwide. Although the viruses associated with EVD and COVID-19 have demonstrated strong infectivity, the high fatality rate of EVD has restricted its spread and prevented it from reaching pandemic level. The responses to the Ebola virus and SARS-CoV-2 outbreaks from manufacturers such as Merck & Co., Inc., Rahway, NJ, USA (MSD) have pushed the boundaries for vaccine development in several areas, including accelerated, parallel clinical and commercial development timelines, implementation of single-use technologies in manufacturing, and engagement with partners and regulatory agencies globally. This review describes how MSD 1) applied the recombinant vesicular stomatitis virus (rVSV) vaccine platform to quickly develop a vaccine for Ebola virus and 2) applied both the rVSV platform and prior knowledge gained from development of the Ebola virus vaccine to rapidly respond to the SARS-CoV-2 pandemic.

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PLATFORM PROCESS DEVELOPMENT & MANUFACTURING

Among the various vaccine platforms, live virus vaccines (LVV) are considered the most effective at eliciting life-long cellular and humoral immune responses [1]. However, developing a manufacturing platform for LVV is challenging since different virus families may require different cell substrates, production processes, and purification requirements. LVV can be either attenuated strains or recombinant strains; live recombinant vaccines are replicating viruses that are genetically engineered to carry heterologous antigens. One advantage of live recombinant viruses is that the presentation of heterologous proteins in combination with mimicry of natural infection from live viral vector can generate strong humoral and cellular immune responses without an adjuvant [2]. In the past decade, rVSV has been established as a live recombinant vaccine platform for multiple viral diseases [3].

VSV is a member of the Rhabdoviridae family of negative-stranded RNA viruses and causes non-lethal disease in cattle, horses, and pigs; human VSV infections are rare [4]. rVSV was first developed as a replicating vaccine platform by John Rose and Michael Whitt [5,6]. Many aspects of the rVSV are advantageous for vaccine development:

1. VSV can be propagated to high titers in many cell lines;
2. VSV elicits strong cellular and humoral immunity *in vivo*;
3. The VSV-G protein, the major virulence factor of VSV, can be eliminated, thus attenuating the virus and reducing its reactogenicity [7];
4. There is a low prevalence of immunity to VSV in most of the general population, making it advantageous to use rVSV as a vaccination platform;
5. VSV replicates within the cytoplasm of infected cells and does not integrate into the host genome, reducing the risk of oncogenesis and mutagenesis [8].

Vero cells have been the workhorse for vaccine production over the past 40 years. The cell line was established from cells isolated from a kidney of a normal African green monkey [9]. Vero cells are one of the most common continuous cell lines used for vaccine production; they have been extensively characterized and have gained global acceptance by regulatory authorities [10]. Vero cells do not produce type I interferon in response to viral infections [11], which may explain the susceptibility of these cells to many viruses. This broad susceptibility of Vero cells to many viruses makes them an ideal cell substrate for the development and production of viral vaccines.

EBOLA VACCINE

On August 8, 2014, the WHO declared the EVD outbreak in West Africa a Public Health Emergency of International Concern [12]. Understanding the urgency of developing an effective vaccine for Ebola virus, NewLink Genetics Corp., in partnership with the US FDA, reached out to MSD to develop an rVSV Ebola vaccine candidate. With extensive internal knowledge of developing LVV, working experience with Vero cells, and scaling up viral vaccine production, MSD partnered with NewLink Genetics Corporation to develop the Ebola vaccine manufacturing process. Leveraging data from the Public Health Agency of Canada, NewLink Genetics and contract manufacturer IDT Biologika, existing literature for rVSV, and extensive internal knowledge of scaling up vaccine production with Vero cells, MSD initiated process development of a robust and scalable manufacturing process.

MSD was challenged with scaling up the existing Ebola rVSV process from 90 to 400 roller bottles to meet Pre-Licensed Patient Access (PLPA) needs. Understanding that the process had to be scaled up quickly, Vero

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cell expansion experience from the RotaTeq® vaccine was leveraged to develop the Ebola vaccine process. There were two main areas of focus:

1. Infection and harvest parameters;
2. Scalable downstream unit operations.

To accomplish this, the development team performed repeated cell expansions to generate material to initiate harvest/infection and downstream experiments. Specifically, increased filter surface area, a new tangential flow filter scheme, decreased lumen diameter to maintain shear, and reduced circulation rate was implemented.

Providing significant starting material to these teams was imperative to allow the creation of multiple side-arm experiments to test various process changes simultaneously in parallel experimental arms. This methodology also provided opportunities to complete non-GMP full-scale runs on the upstream process in the pilot plant facility, allowing electronic notebook documentation to later be adapted to production batch records. Experiments were led by a pilot plant operations team, leveraging experience from team members who had previously worked in biologic and vaccine process development areas. The multitude of small-scale purification runs provided hands-on experience to the team that would later be tasked with scale-up for GMP production. Co-locating process development and GMP clinical manufacturing in the same organization with the same scientists eliminated the need for tech transfer.

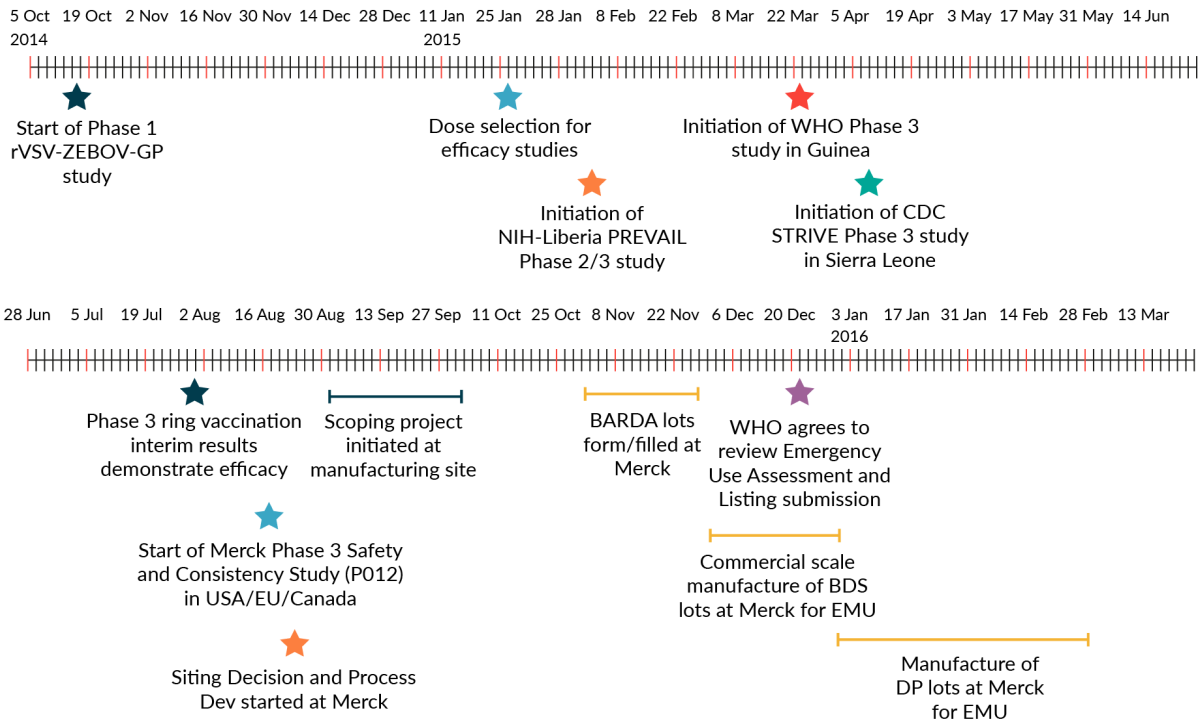
To accelerate the manufacture of drug substance for Ebola vaccine, the development timeline was drastically compressed (Figure 1). The time from initiation of process development activities at MSD to completion of manufacture for the first batch of GMP PLPA drug substance was 7 months. MSD was able to shorten the development timeline for rapid transfer to manufacturing by executing development and manufacturing scale-up activities in parallel and by implementing single-use technologies. The 400-roller bottle manufacturing process, while not state of the art, was completely disposable end-to-end. Single-use systems provided agility and scalability in a manufacturing facility. Different single-use systems at different scales were installed, commissioned, or removed quickly to meet production requirements. Furthermore, the use of single-use systems reduced manufacturing timelines via the elimination of cleaning validation, clean-in-place, and sterilization-in-place.

Prior to the partnership with MSD, IDT Biologika had initiated a Phase 1 clinical study utilizing material from their existing roller bottle process. In order to use the data from this ongoing study for lot consistency, MSD could not deviate from the roller bottle process to deliver PLPA material. Only changes that supported an increased manufacturing scale were evaluated. For upstream, the process was scaled from 90 to 400 roller bottles to produce the necessary drug substance volume sought for PLPA use. The optimal multiplicity of infection and time of harvest were also determined for the scaled-up process. For downstream, loading studies were performed on the clarification filter to minimize surface area and properly size the filter area needed at larger scale. Range-finding experiments were conducted on the enzyme treatment step in an attempt to reduce the amount of Benzonase® endonuclease used in the process, thereby reducing cost. Temperature studies were conducted to evaluate if simpler, room-temperature manufacturing operations could be utilized. A constant volume ultrafiltration/diafiltration process was implemented to keep process volumes low and reduce manufacturing times. Multiple ultrafiltration filters were evaluated to replace the existing filter, which was not available at the increased scale.

Parallel process development, scale-up, and process transfer to the clinical manufacturing facility also enabled rapid Ebola vaccine development. As each process step was defined, GMP batch records were created by the same engineers, leveraging their experimental knowledge and experience. This brought speed and accuracy to the authoring process.

FIGURE 1

ERVEBO® development timeline.



BARDA: Biomedical Advanced Research and Development Authority; BDS: Bulk drug substance; CDC: Centers for Disease Control and Prevention; DP: Drug product; EMU: European Medicines Agency; NIH: National Institute of Health; WHO: World Health Organization.

Large-scale roller bottle processes, particularly at the scale demonstrated here, were manual in nature and required intensive hands-on training for execution. The pilot plant operations staff quickly recruited and upskilled new contract staff members to support clinical GMP manufacturing operations. After completing training, these staff members were assigned to help complete experimental work, later transferring these important skills to GMP production.

MSD initiated a Phase 1 clinical trial in fourth quarter 2014, and a Phase 2/3 and consistency lot studies were initiated in February and April 2016. Clinical efficacy data was obtained in June 2016, and ERVEBO® was licensed by the FDA in 2019 [13]. Prior to licensure, the VSV Ebola vaccine was deployed in Guinea in 2015 during the West African Ebola epidemic and the 2018-2020 Democratic Republic of Congo outbreak using the PLPA process, demonstrating 100 and 97.5% efficacy respectively after a single dose [14,15]. The success of ERVEBO demonstrated the effectiveness of the rVSV vaccine platform in pandemic settings.

COVID-19 VACCINE

In response to the COVID-19 outbreak, MSD and the International AIDS Vaccine Initiative (IAVI) applied the rVSV vaccine platform to develop V590, a vaccine candidate for SARS-CoV-2 (Figure 2) [16]. Early integration and real-time data sharing between discovery and process development teams at MSD enabled clone selection for optimal antigenicity and manufacturability. The use of the ERVEBO vaccine production platform also reduced the time required for V590 process development prior to the production of Phase 1 clinical supplies. For example, the ERVEBO upstream roller bottle process, with minor modifications, was leveraged for the production of V590 Phase 1 clinical

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supplies. The number of roller bottles was increased from 400 to 600 to ensure a sufficient supply of drug substance for Phase 1 clinical trials, and the infection time was reduced by roughly 12–24 hours compared to the ERVEBO process. While several downstream purification process steps were adopted directly from the ERVEBO process, differences between the VSVΔG-ZEBOV-GP and VSVΔG-SARS-CoV-2 viruses required the removal of the ERVEBO protease incubation step with TrypLE™ from the V590 process. This ultimately led to the inclusion of an aseptic, flow-through chromatography step using gamma-irradiated, sterilized Capto™Core 700 resin (Cytiva) to increase clearance of residual host cell proteins. A change to the final drug substance buffer was also incorporated to allow for improved V590 drug product shelf-life.

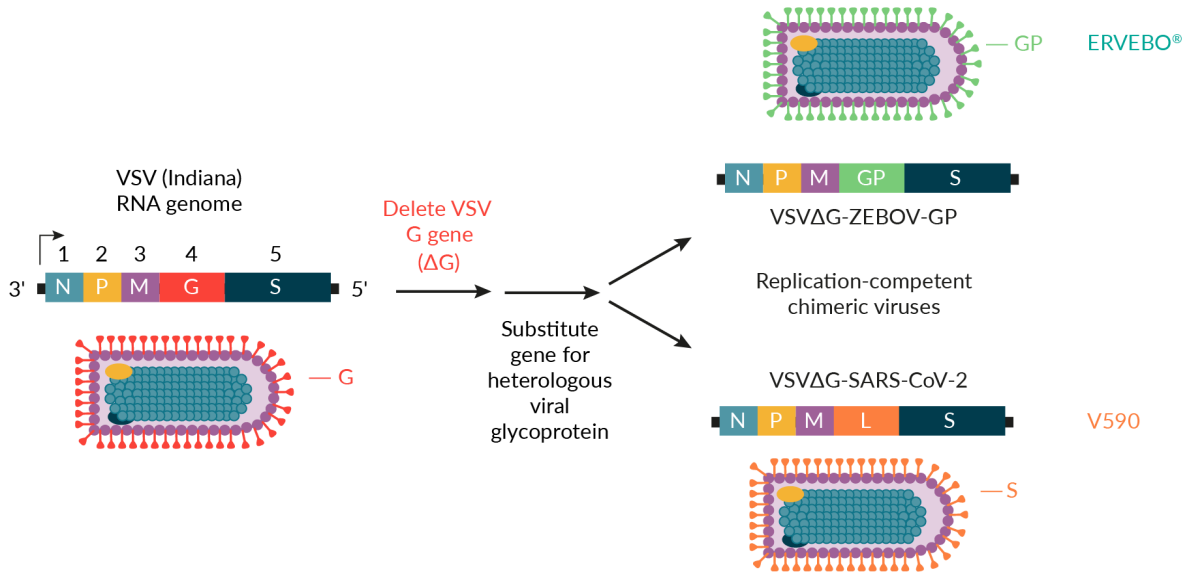
Though it was recognized that a roller bottle process with aseptic downstream processing would not be used for commercial-scale production due to the large number of anticipated doses for a COVID-19 vaccine, this fit-for-purpose approach allowed for rapid production of Phase 1 clinical supplies. Phase 1 clinical supplies were generated approximately 2 months after V590 clone selection (Figure 3). This is in contrast to traditional preclinical development of vaccines, which usually takes 1–2 years [17].

Other factors besides leveraging the ERVEBO vaccine production platform also enabled V590 process development and production of Phase 1 clinical supplies. The development of multiple Simple Western™ assays allowed for rapid (<1 day) turnaround of analytical results to measure viral and host cell protein levels across downstream processing steps [18]. On-demand potency with rapid plaque and microplaque assays was also quickly established, providing virus infectivity results in less than 48 hours. Project teams were also highly coordinated to align objectives and experimental plans across several workstreams (upstream, downstream, formulation, analytics).

The development time for the COVID-19 vaccine was also shortened by running development and manufacturing activities in parallel. While Phase 1 clinical materials were being manufactured, development and scale-up activities for the commercial-scale

FIGURE 2

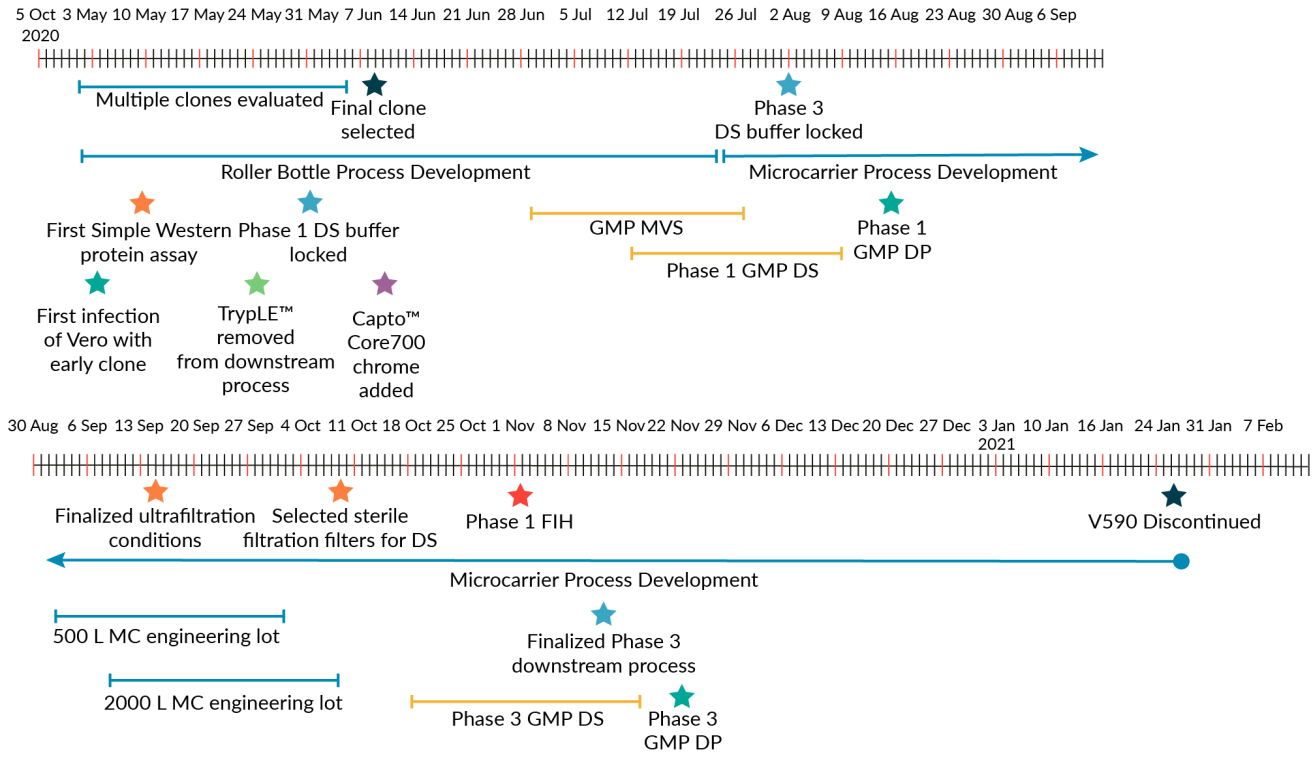
rVSV vaccine platform for the development of ERVEBO® and V590.



VSV: Vesicular stomatitis virus.

FIGURE 3

V590 development timeline.



DP: Drug product; DS: Drug substance; FIH: First in human; MC: Microcarrier; MVS: Master virus seed.

production process were executed at the same time, thus reducing cycle times by approximately 18 months. Cross-training was also implemented to ensure efficient process transfer to clinical production, and manufacturing staff were trained on each process step prior to GMP manufacture. This training provided opportunities for the manufacturing team to develop a strong technical knowledge of the production platform. Both Ebola and COVID-19 vaccine production leveraged existing MSD manufacturing facilities with standard unit operations, which enabled rapid process transfer to the clinical manufacture area. In addition, leveraging the existing rVSV vaccine production platform facilitated rapid scale-up and manufacture by utilizing the available validated equipment and GMP-quality raw materials for manufacturing.

To maintain an accelerated timeline, there was significant pre-investment into the development of the commercial-scale production process prior to the availability of Phase 1 clinical results. For commercial-scale production of V590, it was not possible to leverage the Phase 1 roller bottle process for large-scale manufacture. To achieve the number of vaccine doses required to support the pandemic scale, the commercial manufacture process would need approximately 10,000 roller bottles per batch. Thus, we developed a scalable, microcarrier-based bioreactor (2000 L) production process to generate the number of vaccine doses needed for pandemic scale. The 2000 L bioreactor achieved a peak virus titer of ~1.0e+7 plaque forming unit (PFU)/mL [19]. The introduction of the bioreactor process also removed an aseptic control risk inherently associated with roller bottle cultures. To this end, the incorporation of terminal sterile filtration was also considered critical to eliminate aseptic processing and reduce the risk of non-sterile product. The use of a microcarrier bioreactor process and the inclusion of a terminal sterile filtration step for the

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commercial scale V590 production required substantial process development. Several factors enabled commercial-scale process development to be completed quickly:

- The Vero cell expansion process, which is currently used for MSD's commercial vaccines and part of its LVV microcarrier process, was leveraged for the V590 commercial scale process so that only the microcarrier N-1 cell expansion step had to be developed to supply a sufficient number of cells for 2000 L bioreactor inoculation;
- Experience from the LVV platform process also enabled a consistent and high-quality supply of Vero cells for infection with VSVΔG-SARS-CoV-2 on a regular schedule. This provided material for downstream process development and production of drug substance for assay and formulation development;
- The utilization of single-use technology, existing equipment, consumables, and raw materials enabled process development experiments to start quickly, increased process flexibility, and allowed for rapid implementation of process changes and demonstration of process iterations [19];
- Processing buffers/media were identified early in development, and the number of buffers used was minimized to reduce the workload required for qualification testing;
- The same type of filters for the clarification (Sartoclean® CA, Satorius) and hollow fiber tangential flow filtration (ReadyToFilter Hollow Fiber Cartridge, 750 kilodaltons nominal molecular weight cutoff membrane, Cytiva) steps that were used for the of manufacture Phase 1 supplies were used in the Phase 3 process. Volumetric loadings were optimized to minimize filter surface area requirements.

While LVV development usually takes approximately 18–24 months from clone selection to implementation of a Phase 3 clinical manufacturing process, the factors above enabled MSD to develop a Phase 3 GMP-compliant 2000 L single-use bioreactor process for V590 in approximately 5 months from clone selection, with Phase 3 clinical supply produced in less than 6 months.

REGULATORY INTERACTIONS

Before ERVEBO approval, emergency use doses were provided to Africa using the IND under Expanded Access protocols. Because this vaccine targeted an unmet medical need, ERVEBO was granted Breakthrough Therapy designation by the FDA and PRIority MEdicines (PRIME) designation by the European Medicines Agency (EMA) [20]. This enabled increased interactions with the regulators (~23 interactions between the EMA and FDA) throughout Biologics License Application and Marketing Authorization

TABLE 1

Overview of the expected and actual regulatory agency review periods.

	Standard review period	Accelerated review period	ERVEBO® Experience
FDA	6–10 months	6 months (Priority Designation)	~3 months
EMA	210 days (12–14 months to obtain MA)	150 days (8 months to obtain MA)	~8 months to obtain Conditional MA
WHO prequalification	Median consistently 200 days following reference NRA approval	Shortly following reference NRA approval	1 day following reference NRA approval
Participating NRAs (individual countries participating)	Varies: typically 2–4 years following reference NRA approval	Maximum 90 days following reference NRA approval (per roadmap)	Ongoing: earliest obtained 39 days following reference NRA approval

EMA: European Medicines Agency; FDA: Food & Drug Administration; MA: Marketing Application; NRA: National Regulatory Authority; WHO: World Health Organization.

Application submission and approval. The applications were submitted using a rolling submission strategy agreed upon with the regulators and also leveraged a collaborative review process with WHO, African VAccine REgulatory Forum (AVAREF), and multiple African countries to ensure approvals were obtained expeditiously where the vaccine was needed most. An overview of expected and actual review periods is shown in Table 1.

For the COVID-19 vaccine candidate, MSD was able to leverage the ERVEBO roller bottle platform production process to waive preliminary nonclinical studies. Due to the urgency caused by the pandemic, MSD was also able to engage early with agencies to discuss options to accelerate the path to first-in-human. These early engagements included a pre-IND meeting, multiple informal meetings with the FDA and EMA, and Type C written interactions all enabling rapid response and a collaborative sponsor-regulator experience. The early interactions with the FDA enabled the use of a Type V Drug Master File to submit available CMC sections for review earlier than the complete Phase 1 IND package. This allowed the Phase 1 review process to proceed to first-in-human much faster than the normal timeline. After Phase 1, Type C written feedback was also rapidly obtained by submitting written background packages to the IND instead of holding Type C meetings. MSD's V590 was found to be safe in a Phase 1 clinical trial but was discontinued due to low antibody responses [21].

EXPANDED ACCESS

Prior to ERVEBO approval, the rVSV Ebola investigational vaccine was used to help to contain the outbreaks in the Democratic Republic of the Congo and surrounding countries. Hundreds of thousands of labeled stockpile vaccine doses were deployed through a pre-license access pathway. Pre-license access aims to provide life-saving investigational drugs or vaccines prior to the approval of the drug or vaccine by a regulatory authority. MSD partnered closely with the WHO to align relevant health authority requirements for the use and export/import of rVSV Ebola vaccine into outbreak countries. MSD's quality control systems were responsible for assessing and approving the WHO's pre-license access requests and subsequently releasing investigational vaccine lots for use in designated countries. Following release, the MSD logistics organization closely collaborated with specialized pharmaceutical couriers, airlines, and WHO country representatives to seamlessly and routinely deliver vaccine supplies under -70°C dry ice shipment conditions. Extensive pre-license access experience was gained in providing rVSV Ebola vaccine. Lessons learned over time have been employed forward to streamline pre-license access processes and better prepare MSD to respond to future expanded access needs.

CONCLUSION

MSD's responses to the Ebola epidemic and SARS-CoV-2 pandemic have demonstrated the benefit of leveraging the rVSV vaccine platform for the rapid development of vaccines. Despite the unprecedented speed of developing these vaccines, opportunities exist for further acceleration of development timelines. As the human population continues to grow and there is habitat destruction, urban development, and increased global travel, the Ebola virus epidemic and SARS-CoV-2 pandemic will not be the last infectious disease outbreaks impacting global human health. Stopping the next emerging pandemic will require utilizing vaccine production platforms and technologies to speed process development and manufacturing scale-up. To ensure a high probability of success for a vaccine candidate in a pandemic, establishing multiple vaccine platforms will be key in developing an effective vaccine quickly. Improvements in regulatory policies and communications to enhance their flexibility without compromising vaccine safety and efficacy are also

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critical for pandemic preparedness. Long-term strategies for investment into developing new vaccine platforms and application of new technologies for manufacturing infrastructure must be implemented to prepare for accelerated response to future pandemics. Taking these steps for proper preparation will facilitate rapid vaccine development and production to protect society from future public health emergencies.

REFERENCES

1. Al-Jighefee HT, Najjar H, Ahmed MN, Qush A, Awwad S, Kamareddine L. COVID-19 Vaccine Platforms: Challenges and Safety Contemplations. *Vaccines* 2021; 9(10), 1196.
2. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments [published correction appears in *Nat. Rev. Immunol.* 2021 Jan 5]. *Nat. Rev. Immunol.* 2021; 21(2), 83–100.
3. Scher G, Schnell MJ. Rhabdoviruses as vectors for vaccines and therapeutics. *Curr. Opin. Virol.* 2020; 44, 169–182.
4. Rose JK, Schubert M. Rhabdovirus genomes and their products. In: *The Rhabdoviruses* (Editor: Wagner RR). 1987; 129–166, Plenum Publishing Corporation.
5. Lawson ND, Stillman EA, Whitt MA, Rose JK. (1995). Recombinant vesicular stomatitis viruses from DNA. *Proc. Nat. Acad. Sci. USA* 1995; 92(10), 4477–4481.
6. Whitt MA. Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. *J. Virol. Methods* 2010; 169(2), 365–374.
7. Yahalom-Ronen Y, Tamir H, Melamed S *et al.* A single dose of recombinant VSV-ΔG-spike vaccine provides protection against SARS-CoV-2 challenge. *Nat. Commun.* 2020; 11(1), 6402.
8. Monath TP, Fast PE, Modjarrad K *et al.*; Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG). rVSVΔG-ZEBOV-GP (also designated V920) recombinant vesicular stomatitis virus pseudotyped with Ebola Zaire Glycoprotein: Standardized template with key considerations for a risk/benefit assessment. *Vaccine* 2019; 1, 100009.
9. Yasumura Y, Kawakita Y. Studies on SV40 in tissue culture-preliminary step for cancer research in vitro. *Nihon Rinsho.* 1963; 21(21), 1201–1215.
10. Barrett PN, Mundt W, Kistner O, Howard MK. Vero cell platform in vaccine production: moving towards cell culture-based viral vaccines. *Exp. Rev. Vaccines* 2009; 8(5), 607–618.
11. Desmyter J, Melnick JL, Rawls WE. Defectiveness of interferon production and of rubella virus interference in a line of African green monkey kidney cells (Vero). *J. Virol.* 1968; 2(10), 955–961.
12. World Health Organization (WHO), WHO Statement on the Meeting of the International Health Regulations Emergency Committee Regarding the 2014 Ebola Outbreak in West Africa, 2014.
13. Wolf J, Jannat R, Dubey S *et al.* Development of Pandemic Vaccines: ERVEBO Case Study. *Vaccines (Basel)* 2021; 9(3), 190.
14. Henao-Restrepo AM, Longini IM, Egger M *et al.* Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet* 2015; 386(9996), 857–866.
15. Henao-Restrepo AM, Camacho A, Longini IM *et al.* Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!) [published correction appears in *Lancet.* 2017 Feb 4;389(10068):504] *Lancet* 2017; 389(10068), 505–518.
16. Saville M, Cramer JP, Downham M *et al.* Delivering pandemic vaccines in 100 days – what will it take? *N. Engl. J. Med.* 2022; 387, e3.
17. Espeseth AS, Yuan M, Citron M *et al.* Preclinical immunogenicity and efficacy of a candidate COVID-19 vaccine based on a vesicular stomatitis virus-SARS-CoV-2 chimera. *EBioMedicine* 2022; 82, 104203.
18. Gillespie PF, Wang Y, Hofmann C *et al.* Understanding the Spike Protein in COVID-19 Vaccine in Recombinant Vesicular Stomatitis Virus (rVSV) Using Automated Capillary Western Blots. *ACS Omega* 2023; 8(3), 3319–3328.
19. Ton C, Stabile V, Carey E *et al.* Development and scale-up of rVSV-SARS-CoV-2 vaccine process using single use bioreactor. *Biotechnol. Rep. (Amsterdam, Netherlands)* 2023; 37, e00782.
20. Wolf J, Bruno S, Eichberg M *et al.* Applying lessons from the Ebola vaccine experience for SARS-CoV-2 and other epidemic pathogens. *NPJ Vaccines* 2020; 5(1), 51.
21. Robbins JA, Tait D, Huang Q *et al.* Safety and immunogenicity of intramuscular, single-dose V590 (rVSV-SARS-CoV-2 Vaccine) in healthy adults: Results from a phase 1 randomised, double-blind,

placebo-controlled, dose-ranging trial. *EBioMedicine* 2022; 82, 104138.

AFFILIATIONS

Christopher Ton
Process Research & Development, Merck & Co., Inc., Rahway, NJ, USA

Michael A Winters
Process Research & Development, Merck & Co., Inc., Rahway, NJ, USA

Raymond Ducoat
Process Research & Development, Merck & Co., Inc., Rahway, NJ, USA

Douglas D Richardson
Analytical Research & Development, Merck & Co., Inc., Rahway, NJ, USA

Kristine Fuller
Chemistry, Manufacturing, and Controls, Merck & Co., Inc., Rahway, NJ, USA

Melissa Hughes
Global Clinical Supply, Merck & Co., Inc., Rahway, NJ, USA

AUTHORSHIP & CONFLICT OF INTEREST

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INTERVIEW

OPENCORONA: lessons learned from a pandemic vaccine consortium

Early in the COVID-19 pandemic, the EU-funded OPENCORONA project brought together academics, manufacturers, and technology providers in the quest to develop a vaccine. Now, the resulting DNA vaccine is in clinical trials and its developers believe that the ability to induce broad T cell immunity will make it a valuable addition to the current vaccine lineup. **Charlotte Barker,** Editor, *Vaccine Insights*, speaks to two of the consortium leaders, **Matti Sällberg,** Professor, Karolinska Institutet, and **Eva-Karin Gidlund,** Head of Alliance & Business Development, NorthX Biologics to find out more.

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Q What is the OPENCORONA project?

MS: The OPENCORONA project started in the early days of the pandemic. When we realized in January 2020 that COVID-19 was likely to become a pandemic, my lab

at Karolinska Institutet started looking at vaccine design. We knew from the start it would be a DNA vaccine because we had a lot of resources already in place for this platform. When the EU put out a call for vaccine development proposals, we were able to form a consortium of seven partner organizations that had the capacity to take a vaccine all the way from the research discovery phase into the clinic. Now, 2 years and 10 months on, the first subject in the Phase I clinical trial has been vaccinated.

EKG: It's a Horizon 2020 Pan-European project with a budget of €3 million and we are proud to have brought a vaccine to clinical trial in under 3 years on that comparatively low budget. Other vaccines made it onto the market quicker, but because we took a different approach to the design of our vaccine, we believe it has some important advantages over existing vaccines and will be valuable as a booster.

Q Who are the partners in the consortium?

MS: The consortium consists of:

- Karolinska Institutet: responsible for project coordination, vaccine design, early testing, and selection in animal models;
- Folkhälsomyndigheten (Public Health Agency of Sweden): provided access to BSL-4 and BSL-3 animal facilities and mouse and ferret models for challenge studies;
- Justus Liebig University Giessen: carried out testing to ensure the vaccine candidates did not over-activate the immune system and cause cytokine storms;
- IGEA SPA: designed and developed a CE-marked delivery device for *in vivo* electroporation of DNA vaccines;
- North X Biologics: produced HQ plasmid to be used in toxicological studies and GMP plasmid for the phase 1 clinical study;
- Adlego (now Scantox): performed toxicological testing according to GLP;
- Karolinska University Hospital: currently running the phase 1 clinical trial.

Q How did you approach the initial vaccine design?

MS: We are used to working with viruses like hepatitis C, which are extremely genetically variable, so our approach is to include as much as possible of the virus, including conserved elements. For COVID-19, we did not want to focus only on spike (S) protein epitopes because while the S protein can induce neutralizing antibodies it also has a high mutation rate, and it is the T cell responses that protect us from severe disease and death in the long run.

Most COVID vaccines used the 2003 SARS outbreak as a blueprint, taking the S protein, modifying it with the known stabilization mutations, and producing it as an RNA, DNA,

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"We included the receptor binding domain (RBD) of the S protein, which binds to ACE-2 receptors. The RBD is genetically variable, so we included RBDs from three different variants—Wuhan, Alpha, and Beta."

– **Matti Sällberg**

protein, or adenoviral vector. We decided from the beginning to go a different way, even if it took longer.

We included the receptor binding domain (RBD) of the S protein, which binds to ACE-2 receptors. The RBD is genetically variable, so we included RBDs from three different variants—Wuhan, Alpha, and Beta. Fortuitously, the Beta variant shares many mutations with the Omicron variants that later swept the globe.

We combined these with the membrane (M) protein and the nucleocapsid (N) protein. Both have a very high homology between the current circulating strains in humans and those present in bats and other animals. We wanted to protect against different types of SARS-CoV.

We believe this combination of antigens makes our vaccine well-suited to use as a booster dose, as it adds new responses to complement the responses induced by the Spike-based vaccines.

Q What was different about working on a pandemic vaccine versus previous projects you've been involved with?

EKG: For me, the way we adapted the project as a result of the constantly emerging new data that appeared during the pandemic was totally new. For example, once people began receiving COVID-19 vaccines, we re-designed the clinical trial to allow for the fact that most people would have been vaccinated by the time we finished recruiting.

MS: That is true. There were two major changes in direction. After 9 months, different variants of the virus started showing up, and we realized we had to redesign to include some of these variants in the vaccine. Unfortunately for North X, that meant redoing the HQ batch production! Of course, that caused a delay, but I think it's made the final vaccine more timely, offering protection against a wider range of variants.

As Eva said, we also changed the clinical trial design from being a first-line vaccine to a booster vaccine.

Q What steps did you take to allow you to move as quickly as possible?

EKG: Those who have experienced writing an EU funding application will know that it typically takes months. Matti called us about this proposal and said, 'Can you have it ready by next week?' One of the things that sped up the project and made it possible was that lengthy decision-making was put aside. In a pandemic, everybody has to be on their toes and take decisions fast, even when we don't have all the facts. The call for proposals from the EU was only open for a few weeks and the review process was dramatically streamlined.

MS: One of the most essential things was that we already had the contacts we needed. We had partnerships in place for manufacturing and delivery technology. And we worked together very effectively, with each member of the consortium actively preparing for their step, so there was no time lost.

EKG: The fact that Matti's group had data from previous DNA vaccines they had worked on also helped and made it much easier to take decisions.

MS: We already knew that DNA vaccines work in humans, and how to design, test, and deliver them. As it turned out, RNA vaccines were the frontrunners, but in January 2020, RNA vaccines had never been used outside small clinical trials. If you had said in 2019 that we would be making RNA vaccines for a pandemic, no one would have believed you.

Q What stage is the project at now?

MS: We recently initiated a randomized, double-blind, placebo-controlled, dose-escalation phase I clinical trial in healthy adult volunteers who have previously received three RNA vaccine doses, and we will follow them for 3 months to track neutralizing antibodies and T cell responses. We are also having them take a rapid antigen test every week during the trial to get an idea of reinfection rates.

We see our vaccine as a complement to existing vaccines. Preclinical studies showed strong and broad T cell activation with our vaccine so we believe it might particularly benefit those with an inability to produce antibodies or an altered immune system, like dialysis patients.

Q What are the key factors for a successful consortium?

EKG: Collaborating in the middle of a pandemic is not easy, but our consortium of seven partners has worked very well together throughout. Indeed, I have never experienced such a successful consortium, despite not being able to meet in person for many months.

When Matti mapped out this consortium, he was careful to include different areas of expertise, with limited crossover. Of course, we communicate and collaborate, but each entity has a distinct role.

Our grant coordinator and project manager worked hard to keep track of all seven partners and make sure that we all delivered on time. Planning became especially important because

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> "One of my big lessons from the pandemic is to never be surprised that you're surprised. Again and again, the pandemic has shown us that we still have a lot to learn. One must be humbled by the learning process!"
>
> – **Eva-Karin Gidlun**

many resources became scarce during the pandemic. Items that may have been delivered within 4 weeks pre-pandemic are now taking 3 or even 12 months. Planning is everything.

Openness and straightforward communication are also key. You need to be able to say early on when you will not be able to deliver your timelines and ask for help when you need it.

MS: For a scientist, a big challenge is knowing when to stop experimenting. Every day you come up with a better idea, a different way of doing things, but you cannot keep doing that in clinical development. When you find a candidate, you need to say 'stop' or "go."

That is hard for a scientist to live with because the same day you commit to a candidate drug, you may come up with a better idea. You must realize that this 'better idea' will take another 2 years to reach the same point. It's tough to adapt to that way of thinking but it was essential for everyone to understand that we have a defined goal—to do a clinical trial. My experience of large consortia is that projects are too often talked to death or changed to death! With a small group, this risk is minimized.

Q What lessons have you learned from this project and from the pandemic more generally?

MS: One of my big lessons from the pandemic is to never be surprised that you're surprised. Again and again, the pandemic has shown us that we still have a lot to learn. One must be humbled by the learning process!

EKG: For me, it emphasized that cash is king. The vaccines that made it into the clinic within a year had many more zeros in their funding allocation than ours! If you can, ask for more money than you think you need. We learned so much during this project, and this pandemic, that we all have things we would like to explore further.

MS: Yes, with double the money, things certainly would have moved faster!

EKG: Another lesson to take from the project is that consortia like this are a good opportunity for a private company to engage with early-phase research. I would encourage companies like ours to be bolder in collaborating and building ongoing relationships with academics.

MS: It is good for academia too. By taking part in clinical development, academics understand much more clearly how a product is made.

EKG: All of us have had the opportunity to learn from different sectors and fields, and follow the vaccine from idea to development, manufacturing, release, and now clinical trials. It's been a real journey!

BIOGRAPHIES

MATTI SÄLLBERG got his DDS and PhD from Karolinska Institutet in 1992, a post doc from Scripps Research Institute, and was appointed professor in biomedical analysis at Karolinska Institutet in 2000. He was co-founder of SVF Vaccines AB in 2015. His major research interests are viral immunology, immunotherapies, and vaccines

EVA-KARIN GIDLUND is the Head of Alliance and Business Development at NorthX Biologics (NorthX). Eva-karin has a PhD in Medicine from the Karolinska Institutet in Stockholm. Her main focus as a scientist for the last decade have been genetic and epigenetic changes and modifications in healthy humans and in patients. She is a former TEDxSpeaker, Bünsow Business speaker, author and international presenter that has competed in the Science Grand Prix . In 2019 after a postdoc, she became Collaborations Development Manager for a large CDMO within the Cell- and Gene Therapy filed. In 2021 she was appointed the Head of Alliance and Innovation at NorthX. In October 2021, the Swedish government recognised NorthX as a national and international innovation hub and now a new era and shift from a CDMO towards an innovation centre is emerging. Her main goal is to bridge the gap between innovation, academia, SMEs and the pharma industry to supply innovative medicines to patients and to help early phase innovative companies to reach success.

AFFILIATIONS

Matti Sällberg, PhD
Professor,
Karolinska Institutet

Eva-Karin Gidlund, PhD
Head of Alliance & Business Development,
NorthX

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VACCINEINSIGHTS

PANDEMIC PREPAREDNESS: GETTING READY FOR THE NEXT 'DISEASE X'

SPOTLIGHT

Reflections on the global mpox outbreak

Rosamund Lewis
World Health Organization

"...it is critical to continue to build on all we have learned and sustain investment in continuing research for vaccines against orthopoxviruses, which still have surprises in store for us."

VIEWPOINT

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Charlotte Barker, Editor, Vaccine Insights spoke to Rosamund Lewis, Technical Lead for mpox, WHO, on March 15, 2023, about the lessons learned from the global response to mpox. This article has been written based on that interview.

In May 2022, there was a sudden and unexpected outbreak of mpox worldwide, with cases appearing in many countries that had not seen mpox before. Mpox is caused by monkeypox virus, part of the orthopoxvirus genus that also includes the causative agent of smallpox, variola virus. On July 23, 2022, WHO Director-General Tedros Adhanom Ghebreyesus declared the mpox outbreak to be a Public Health Emergency of International Concern [1].

After a year of a global response to this new health emergency, many actions were taken by health authorities and affected communities around the world, and the outbreak is being tamed. On May 11, 2023, just 2 days short of a year after the first case in the global outbreak was reported, WHO lifted the emergency declaration. However, more than 20 countries across the world are still reporting cases and work continues to monitor the situation, respond to outbreaks, and improve access to diagnostic tests, vaccines, and treatment [2].

EPIDEMIOLOGY

The core of any outbreak response is epidemiology—knowing who is being affected and where. One of the primary reasons we were well prepared was that we were able to build on years of work on this previously little-known disease. When cases of mpox started being reported in previously unaffected countries, WHO was able to very quickly assemble an emergency response team, including an epidemiology team, which was immediately able to elicit support and receive data from regions and countries, as is the mandate of WHO.

From the start of the outbreak, we were able to provide diagnostic support very quickly with polymerase chain reaction (PCR) test kits, protocols, and sharing of information. WHO created a data platform to integrate all the information being received from countries on cases, including age, sex, sexual orientation, possible modes of exposure, symptoms, and other features of cases being reported. Due to the ongoing COVID-19 pandemic, countries and commercial testing companies had developed PCR and genomic sequencing capacities they didn't have before, so the technical and human resources were already in place.

These epidemiological data allowed us to rapidly ascertain that in countries reporting new cases, the disease was affecting mainly men who have sex with men with multiple casual partners and was not moving into the wider population over time. This in turn allowed health agencies at all levels to work directly with the most affected communities to quell the outbreak and to coordinate the offer of health services such as diagnostics and vaccines for those most at risk.

COMMUNICATION

Another critical element that WHO was able to put into motion quickly and effectively was communication. This included information for the general public as well as risk communication through community engagement with the most affected communities [3]. The WHO Health Emergencies Programme risk communication experts worked hand-in-hand from the beginning with the WHO human immunodeficiency virus (HIV), hepatitis, and sexually transmitted diseases (STI) Department. Our colleagues in this team run existing programs related to HIV and STIs, so were well placed to reach affected communities and work with them to develop messaging that was acceptable to them. The result is a vast array of products, infographics, Q&As, and audiovisual material describing how people who are at risk can protect themselves. Materials were made widely available in several languages through WHO regional and country offices, as well as directly through community organizations that could adapt them to their needs. We also had existing training materials regarding mpox in endemic regions that we could adapt quickly to create guidance early in the outbreak.

VACCINES & ANTIVIRAL DRUGS

WHO has an institutional memory of smallpox eradication, and an ongoing program of smallpox preparedness, so we were able to provide guidance on vaccines and immunization.

Second- or third-generation vaccinia vaccines have an excellent safety profile, and it

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was thought they would be effective against other infections caused by orthopoxviruses. However, there had been no studies specifically demonstrating the effectiveness of these vaccines for use during an outbreak of mpox. Without that data, the vaccines were not authorized for emergency use for mpox outbreaks and were not prequalified. Therefore, WHO was able to issue guidance based on available information, but not directly procure vaccines, leaving the responsibility for procurement with national public health authorities. Some countries in Latin America have chosen not to vaccinate at all, while some regions, such as Montreal, Canada, ramped up quickly from a model of post-exposure vaccination for contacts to a primary prevention model of vaccination for those at risk.

The final piece is antiviral agents, such as tecovirimat, which are also a product of the smallpox preparedness research program and have been demonstrated to be effective *in vitro* and *in vivo*, including against mpox in non-human primates and prairie dogs, which is the model used to study monkeypox virus pathogenicity and treatment. Again, these drugs did not have emergency-use authorization or prequalification, and they lack the 200-year history of smallpox vaccines. Therefore, WHO guidance on antiviral agents stressed the need for more evidence, and a research meeting was convened to discuss further research and develop a protocol to serve as a template for entities that want to do their own trials. There are now several trials underway for use of antiviral therapeutics for mpox and WHO did make some treatment courses available for compassionate use.

LOOKING AHEAD

We have learned some important lessons from COVID-19 and mpox. However, we need to move away from the rinse-and-repeat cycle of funding for pandemic and epidemic preparedness. Often, randomized controlled trials for essential countermeasures are only put in place after a major outbreak, leading to situations like that for mpox, where a lack of data means WHO is unable to assess and prequalify available vaccines and therapeutics. Ideally, clinical trial protocols should be in place before an outbreak, ready to be deployed as soon as they are needed.

Sustaining funding and human resources beyond the initial crisis is a challenge. In the case of mpox, while there was huge global interest, funding did not easily follow. A combination of human nature, media scrutiny, and limited resources mean that attention (and funding) quickly drop off once an outbreak is no longer seen as a major threat. However, we need sustained investment across a broad strategic research agenda if we are to apply lessons learned and build on new knowledge to handle future outbreaks more effectively.

In mpox, there is still a lot we need to do to maintain and strengthen surveillance and prevention and control programs. In terms of geography and case numbers, the disease has not yet returned to where it was before this outbreak and travel-related cases and community transmission continue to occur. Even after the rapid decline in cases in 2023, we are now seeing new outbreaks in many locations across the world, including in Japan, Taiwan, China, Central and South America, and the USA, with some outbreaks leading to sustained community transmission.

The goal now is to eliminate human-to-human transmission through sustained effort. This will involve the integration of mpox detection, prevention, and care with other programs such as those for HIV and STIs. We also want to strengthen the integration of mpox prevention with other programs, which allows us to reach vulnerable and marginalized populations. For example, in the US, reaching more marginalized groups who face greater stigma, such as Black and

brown men, with tests and vaccines was less effective early in the response, so new efforts are being made to reach these groups.

While mpox is now undoubtedly an infectious disease that spreads between people, this outbreak again highlights the importance of One Health and of better understanding zoonotic diseases before they begin to spread efficiently in humans. We need to learn more about what happens at the animal–human interface. WHO's Strategic Advisory Group on the Origin of Novel Pathogens (SAGO) recently laid out the research agenda for the investigation of the origins of outbreaks, using mpox as an example [4].

Finally, we need to pull together findings from across the broader research agenda to develop a comprehensive strategy and countermeasures for future outbreaks—not just for mpox, but all pathogens with pandemic potential. We need rapid and accurate epidemiological data to inform communication, public health measures, and medical countermeasures. In all measures, emphasizing a destigmatizing and ethical approach is critically important. Despite the lifting of the emergency phase for this outbreak, it is critical to continue to build on all we have learned and sustain investment in continuing research for vaccines, diagnostics and therapeutics against orthopoxviruses, which still have surprises in store for us.

BIOGRAPHY

DR ROSAMUND LEWIS is a leading public health physician and medical epidemiologist. She is the World Health Organization Technical Lead for the global mpox response and heads the Smallpox Secretariat of the WHO Health Emergencies Programme in Geneva. She has expertise in emergency preparedness, health security, immunization, disease surveillance and outbreak response, and risk communication. Dr Lewis has worked at global, national, and municipal levels, supporting a wide range of disease control programmes, including for the global covid-19 response. Dr Lewis holds degrees in Science, Medicine, and Epidemiology & biostatistics, Fellowships in Family Medicine and in Public Health and Preventive Medicine, and a Master of management in Health Leadership. She has published widely and holds an adjunct professorship at the University of Ottawa.

AFFILIATION

Rosamund Lewis, MD
Technical lead for mpox,
Health Emergencies Programme,
World Health Organization

REFERENCES

1. World Health Organization. WHO Director-General's statement at the press conference following IHR Emergency Committee regarding the multi-country outbreak of monkeypox - 23 July 2022.
2. World Health Organization. 2022-23 Mpox (Monkeypox) Outbreak:Global Trends. (Jun 6 2023).
3. World Health Organisation. Public Health.
4. WHO Scientific Advisory Group for the Origins of Novel Pathogens (SAGO). Recommendations to better understand the origins of and factors for the emergence and re-emergence of mpox. (December 22 2022)(Accessed May 2023).

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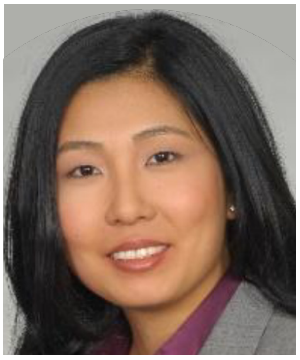
SPOTLIGHT

INTERVIEW

Pandemic preparedness: the vaccine manufacturer's perspective



Charlotte Barker, Editor, *Vaccine Insights*, speaks to **Jane True,** VP, mRNA Commercial Strategy & Innovation and Global Pandemic Security Lead, Pfizer, to get her thoughts on building pandemic preparedness and the complexities of vaccine equity.

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Q Your background wasn't originally in science—what brought you to the pharmaceutical industry?

JT: I have undergraduate and master's degrees in music, but after I graduated, I knew that I did not want a career in music. After going to a local recruitment company, I was placed at a generic pharmaceutical company where I worked on international sales and marketing with various local distributors and completed a couple of business development evaluations.

After getting my MBA, I joined a consulting firm focused on life sciences. Early on, I worked on a project in the vaccines space and found it completely fascinating. Vaccines are

different enough from traditional pharmaceutical products to be particularly interesting, but similar enough to apply the knowledge I'd gained. In 2008, I started working on flu vaccine, which is a completely different animal compared to other vaccines.

> "Another quality of mRNA that could prove very useful in potential future pandemics is the fact that it operates as a platform."

Q What does your current role entail and how has it been influenced by the COVID-19 pandemic?

JT: I am responsible for mRNA Commercial Strategy and Innovation at Pfizer. I manage mRNA pipeline products and work with my R&D colleagues on identifying some of the earlier companies in the field.

Without the pandemic, we would probably still be 5–7 years away from the first mRNA product. We have now proven that mRNA works in vaccines; in other spaces, it is still very early in the process, but given the huge investments and interest in the space, I believe that there is going to be rapid acceleration. If you invest in it, if you fund it, it will happen.

Q What are the key lessons we can learn from the COVID pandemic?

JT: Firstly, we still need to continue to invest in innovation. Manufacturers of flu vaccines had the technology, manufacturing capacity, and even some policy initiatives prepared for a flu pandemic, but these proved unsuitable for COVID-19. There were some major vaccine players that could not produce a vaccine fast enough or at all. We need to develop better vaccine technologies and obtain early information on novel pathogens.

Another lesson learned is diversification. Operation Warp Speed was successful in part because there was investment in multiple vaccine manufacturers and multiple vaccine technologies, increasing the odds that at least one vaccine would prove successful. Diversification in public health tactics is also very important. With COVID-19, we saw social distancing, lockdowns, mask use, and then vaccines—each of those measures played its part.

Companies like Pfizer also worked on antivirals, which some questioned given the success of COVID-19 vaccines. However, for future pandemic preparedness having a broadly protective antiviral is extremely important. Although we can make vaccines quickly now, especially with mRNA technology, we need the means to treat people at risk in the early days of an outbreak. Pandemic preparedness requires a holistic approach.

Q How can we leverage the potential of mRNA to guard against future pandemics?

JT: One of the benefits of mRNA technology is that it is very fast to manufacture. At the beginning of the pandemic, there was no infrastructure for manufacturing or delivering

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mRNA. Everybody started from zero, and Pfizer was able to build up that scale to make billions of doses available. Right now, there are not many other vaccine technologies that can come close to mRNA in terms of manufacturing speed.

Another quality of mRNA that could prove very useful in potential future pandemics is the fact that it operates as a platform. Immunogen and antigen research will still be needed to create the necessary RNA sequence, but we have built up a solid safety database for the encapsulation piece. This should allow future mRNA vaccines to move through regulatory pathways faster.

Given the unprecedented speed at which vaccine development is now moving, regulators rightly want to be sure that the vaccines produced are still safe and effective for the whole population. Hence, it may take some time for regulators to move toward treating mRNA as a platform, but I think we can get there. After all, mRNA COVID vaccines now have some of the largest safety databases on the planet.

Q What are your thoughts on the draft WHO pandemic accord [1] and on vaccine equity more generally?

JT: I appreciate the WHO taking concrete action in this space. However, part of what made us successful in this pandemic was being unencumbered by some of the things that the WHO is proposing (with the best intentions) to implement going forward. Restrictions on how research is carried out and IP protections could get in the way of a quick vaccine rollout and make manufacturers question the viability of developing an innovative vaccine. In my view, anything that would give pause or hesitation in future pandemics risks being detrimental.

Another thing that's come up in discussions of vaccine equity is having more manufacturing in low- and middle-income countries, but it's important to consider the sustainability of those manufacturing facilities. During inter-pandemic periods, there is currently not enough vaccine demand to sustain those facilities. We take our capital expenditure decisions very seriously and do not want to build a facility that may have to be shut down in 5–7 years because there is not enough demand. We are seeing examples of this already with some of the plants that have been or will be built in Africa.

There is no one right answer to vaccine equity. The takeaway for me is that anything that we can do to make pandemic preparedness sustainable is what we should be doing. For example, Pfizer has pledged to provide innovative medicines, including vaccines, at a not-for-profit price to 45 lower-income countries.

Q How should we move forward?

JT: Given that COVID-19 was not only a public health crisis but an economic one, I worry about the potential for an H5N1 pandemic. How much money are governments now going to have to combat a new pandemic?

The entire world has been able to learn from the COVID-19 pandemic, and it is important to keep in mind what we've learned to prepare for the next one. We need to continue to be vigilant. Now that the emergency is no longer staring us right in the face, I fear that we

will start to become apathetic. I want to make sure that there are continued, collaborative, global discussions on future-readiness efforts. It would be a real shame if, when we face the next pandemic threat, we had to build everything up from scratch once more.

BIOGRAPHY

JANE TRUE is the Vice President for Pfizer mRNA Commercial Strategy & Innovation and Global Pandemic Security Lead. She leads a team responsible for mRNA platform, pipeline and product strategy. Jane's team has also led the charge on Pandemic Preparedness, preparing the global community for future pandemics and achieving pandemic security. Jane has 20 years of experience in pharmaceuticals. She began work in the pandemic preparedness and medical countermeasures space in 2008, prior to the 2009 H1N1 pandemic. Prior to joining Pfizer, Jane was VP of Commercial Development at Seqirus, Inc. (part of CSL) where she was responsible for portfolio and pipeline strategy including mRNA and commercial strategy. In her last role, she was also responsible for global marketing, global market access (pricing, HEOR, access and reimbursement), competitive intelligence and commercial analytics and co-chaired the Portfolio Governance Committee. Prior to Seqirus/CSL, Jane spent most of her time in strategy and operations consulting as an independent consultant, and also with PwC and Capgemini Consulting. Jane holds a BA and Master of Music degree from Binghamton University (State University of New York) and received her MBA from New York University Stern School of Business.

AFFILIATION

Jane True
VP
mRNA Commercial Strategy & Innovation and Global Pandemic Security Lead,
Pfizer

REFERENCE

1. WHO. Zero draft of the WHO CA+ for the consideration of the Intergovernmental Negotiating Body at its fourth meeting. (February 1 2023).

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VACCINE INSIGHTS

LATEST ARTICLES:

EXPERT ROUNDTABLE

Enabling rapid vaccine development through manufacturing innovation & process efficiency

Charlotte Barker, Editor, *Vaccine Insights,* speaks to (pictured left to right) **Cleo Kontoravdi,** Professor of Biological Systems Engineering, Imperial College London, **Murali Muralidhara,** Chief Manufacturing Officer RVAC Medicines, **Sirat Sikka,** Field Applications Scientist, Nucleic Acid Therapeutics, Thermo Fisher Scientific & **Hao Chen,** AVP, Vaccines Process R&D, Merck




The global effort to fight COVID saw the fastest vaccine rollout of all time, saving millions of lives. With the ongoing demand for COVID vaccines and the threat of future pandemics, maintaining and building global vaccine manufacturing capacity has never been more important.
In this article, a panel of vaccine manufacturing experts discusses how they are meeting the need for rapid, flexible, and affordable vaccine production—and what we can learn from the accelerated development and manufacture of SARS-CoV-2 vaccines.

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Q How has the COVID pandemic—and the rise of mRNA vaccines—changed the vaccine manufacturing space and what lessons have been learned?

MM: mRNA is a new and challenging modality for the industry. When we approached regulators with an mRNA therapeutic for the first time, back in my AstraZeneca days, we were not sure whether filing would fall under new drug application (NDA) or biologics license application (BLA). The process is semi-synthetic and semi-biologic, involving an *in vitro* translation process followed by lipid nanoparticle encapsulation as a delivery system.

The benefit of mRNA is that it provides a tremendous opportunity for speed compared to traditional modalities, such as viral vaccines, inactivated viral vaccines, or subunit-based adjuvanted vaccines. On the other hand, the most important challenge still to address is the stability and shelf life of these products, to allow global distribution.

HC: Absolutely, COVID has brought about huge change for the vaccine industry and other industries, and there are a few major lessons learned.

One is to have a systematic pandemic response plan, and always be ready. We had those things to some extent in the past but now it is time for us to make greater efforts to build a comprehensive and collaborative plan across industries. Second, it is important to review manufacturing capacity, to be ready as part of the response plan.

Lastly, it is important for the industry that the public is much more aware of vaccines. People are more aware of the benefits of vaccines but there are controversies as well, so we need to look at how we can resolve that down the road.

CK: Beyond manufacturing, quality control (QC) is a major issue. One of the things we have learned is that delays in vaccine supply may often be due to QC rather than manufacturing capacity.

To address this, we need more tools that can fit in with new technologies and we need them fast. If we look at nucleic acids, in particular, the necessary analytical technologies are in the hands of very few companies. Having the insight and knowhow to QC products and release them is going to be critical.

SS: Everyone in the industry has learned a lot from this pandemic—perhaps most importantly that if we all come together and work towards addressing a situation, we can make it through and help humanity globally.

We should continue to collaborate and exchange knowledge and information to help eliminate unnecessary process steps and accelerate the approval of critical vaccines. We also need to address how to get those vaccines to certain locations globally. I hope we will be able to take those lessons forward to other vaccines that we develop in the future.

"A lot of raw materials are common to many bioprocesses and procurement of those has been a real bottleneck during the pandemic. Having distributed manufacturing and planning ahead would definitely be beneficial."

– Cleo Kontoravdi

Q What are some of the greatest bottlenecks in rapid scale-up and production of vaccines and what steps are vaccine makers taking to address these?

CK: In a pandemic situation like COVID, we need to accelerate scale-up. And of course, we're always dealing with unprecedented situations. So having access to equipment, infrastructure, and expertise is key.

Plus, we need to do all that without impacting the normal supply chains for other medicines that are required at the same time as these emergency-use vaccines or medications, which has been very challenging. A lot of raw materials are common to many bioprocesses and procurement of those has been a real bottleneck during the pandemic.

Having distributed manufacturing and planning ahead would definitely be beneficial. However, we cannot mitigate these risks entirely, because some future events are unknown.

SS: Definitely having everything planned in advance not only helps with eliminating those bottlenecks in scale-up but also reduces time and cost. The end goal is to get vaccines to the patient quickly enough to prevent diseases from spreading and do so cost-effectively. The industry is working towards addressing many of the bottlenecks, such as better analytics to accelerate scale-up.

To Cleo's point about raw materials, open interaction with your raw material or solution provider will help them to plan in advance and mitigate bottlenecks that would impact your timeline for manufacturing.

HC: The fundamental issue is that vaccines are complicated to develop and manufacture. That comes down to a lack of understanding of the biology involved, which is a very difficult field. As a company, we are trying to address that through a better scientific and mechanistic understanding of the fundamental biology, but also trying to build more efficient and robust manufacturing platforms.

MM: Although vaccines need tiny quantities per dose compared to therapeutics, the volume required is enormous and that is what poses a challenge. Centralizing vaccine manufacturing is very difficult, as is global distribution of vaccines across different

> "One thing we would like to see is more expedited approvals from regulators based on the key critical quality attributes. Currently, 80% of the manufacturing time is devoted to quality checks and documentation."
>
> – Murali Muralidhara

territories, where no cold chain may exist. The pandemic pressure tested every system possible, including the supply chain.

Q What would you like to see from regulators to allow faster approvals?

MM: One thing we would like to see is more expedited approvals from regulators based on the key critical quality attributes (CQAs). Currently, 80% of the manufacturing time is devoted to quality checks and documentation. As we gain more knowledge about the platform, I would like to see some kind of Drug Master File approach so that if we can hit key CQAs, we can expedite the release of manufactured products and trigger the supply chain logistics prior to the full release of the product.

CK: I agree now is a good time to talk about accelerated protocols for manufacturing and even platform prequalification. The RNA production platform is almost agnostic to the virus, proved by how fast a process could be developed and vaccines produced for SARS-CoV-2. It is a similar situation for some viral vector and baculovirus platforms. Prequalification is difficult when these platforms are cell-based but could be more easily achieved in the case of the RNA platform. To allow this, we need to work together in terms of approved assays, improved materials, and making these materials available IP-free (a major bottleneck).

The next step would be formal regulatory approval for some of these assays to be used, not as a single product but as a platform. Then we can move towards more distributed manufacturing.

Vaccine manufacturing is currently very much a privilege of high-income countries, especially in pandemic-response situations. However, there is local infrastructure in a lot of countries worldwide, and manufacturers that have received World Health Organization (WHO) authorization to produce other types of vaccines. We should consider sharing protocols, assays, and materials with those local manufacturers, so they are pandemic-ready.

HC: I think faster approval, especially in a pandemic setting, is important, but the basic quality must be maintained. For me, the key point is not necessarily making the process faster per se but more collaboration across borders, and for different regulatory agencies

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globally to have some alignment. Better regulatory alignment would accelerate product development across the board.

SS: We must always have a case-by-case analysis of the vaccine, certainly. Whether it is mRNA-based or any other modality, it is necessary to assess it individually in terms of potency, safety, and efficacy. The analytical piece of it that eventually plays a role in understanding all of the CQAs has to be in line with the regulatory agencies. It is important to have regulators, solutions providers, drug developers, and manufacturers sharing their know-how.

I think with solution providers and regulatory agencies working closely together we could accelerate drug development and manufacturing by having documents in place for all of the raw materials that go into manufacturing these vaccines well in advance, so that the drug developers have these documents to hand when filing. We would have that already assessed for us to share with them for filing, such as Drug Master Files, regulatory plans, regulatory support packages, and any other quality documents.

Q How can we reduce cost and improve the accessibility of vaccines—particularly newer platforms like RNA?

HC: Cost is first and foremost in many parts of vaccine development and manufacturing strategy for global access. We should start with the end in mind—even if we choose to launch in high-income countries, we need to keep in mind that vaccines are for people everywhere.

I also think we need a different mindset in the vaccine industry—we should encourage more scientific collaboration, publications, presentations, and interactions in this field. We can learn from small molecules and biologics too.

CK: It is true that mRNA vaccines are expensive, currently. Our analysis to date shows that it is mainly the raw materials that are impacting the overall drug cost. It is a cell-free process, so it is not costly to set up, and because it is so productive it does not need to be scaled up as much as cell-based processes.

The overall costs come down to the raw materials, some of which are just expensive to make, and others come from single suppliers. As we move forward to consider RNA vaccines more broadly, not just for pandemic situations but for infectious diseases or cancers caused by viruses for example, then we think about having a multitude of raw materials suppliers, which will also de-risk the overall supply chain for the future.

We can also consider providing IP-free materials, which is where the academic and ancillary community can come into play. How can we provide IP-free materials, especially to manufacturers in lower- and middle-income countries (LMICs)?

Analytics is another potential cost, especially when we look at establishing manufacturing where there is no pre-existing knowhow. As Sirat was saying, by sharing knowhow, tools, and operating protocols, then we can have a much faster start to the development process, which is where the majority of the cost comes in.

"Optimizing your process and understanding all the factors that play a role in it could also impact your costs. The sooner you understand your process and the more control you have over it, the lower your chance of problems down the line."

– Sirat Sikka

We also need to rethink the whole clinical trial landscape and how to support distributed manufacturing of already approved vaccines such that we can have equity in access, which we didn't have during the COVID pandemic.

MM: Vaccines are still expensive for the majority of the world, so the most important element is how to bring down the costs of production to make them affordable and accessible. There is much you can do in the supply chain and distribution hubs, but if you can reduce the cost of goods in the production process, it will be more affordable for countries that cannot afford the price tag today. As Cleo says, if you can manage the raw material supply chain really well, that is where I see the biggest opportunity for us to reduce the cost of the vaccines.

SS: For mRNA vaccines, we are still trying to work towards understanding how the cost of raw materials could be reduced. One avenue is increasing the yield; for example, engineering enzymes to increase the productivity in the *in vitro* transcription reaction.

Optimizing your process and understanding all the factors that play a role in it could also impact your costs. The sooner you understand your process and the more control you have over it, the lower your chance of problems down the line, which then require more time, more resources, and more investment.

The earlier we focus on reducing costs, starting from raw materials straight into process development, the easier it is going to become for the industry to divert those resources to other projects, and not just invest in a single program.

Q How can pharmaceutical companies, solutions providers, and others work together to make vaccine production more efficient and bring down costs?

SS: When we think about the acceleration of drug or vaccine development, we often only look towards the developers, manufacturers, or regulatory agencies. But suppliers and solutions providers can play a very important role in expediting aspects of the process.

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As I mentioned earlier, having documents in place ahead of regulatory filing is a key aspect. That way we have a clearer picture of what is needed in advance.

There was a report by WHO in 2011 stating that there is a gap in expertise or resources for technology transfer to allow vaccine manufacturing investments and capabilities in LMICs. Having suppliers work together with drug developers could be very important in this respect—we know a lot that is going on in the industry and have our own experts in-house.

Another point we touched on earlier is that drug developers and manufacturers should have open interactions about their timelines so that suppliers or solutions providers can be prepared to support them.

CK: In biologics, I see so much partnership between drug companies and equipment providers. The equipment providers have become much more than that; for example, they are embedding software platforms for analytics. They provide end-to-end solutions that allow manufacturers to move towards online monitoring and reduce batch failure. It all adds up—if we can reduce batch failure, that will reduce the overall costs per dose.

If we can learn from the more traditionally expensive products, such as antibodies, and transfer learnings from those platforms to vaccine technologies, I think we reduce costs.

MM: It takes a whole orchestra playing in harmony to develop a vaccine all the way to human arms. Everybody is a key player in this space.

Discovery can develop your vaccines in a matter of 2 months, but if it takes 12 months to get into human arms, the overall system is not very efficient. Developers, manufacturers, suppliers, regulators, other solutions providers, governments, and insurers all have a part to play. That was the thinking behind Operation Warp Speed, to bring all sectors together and work as a unit to deliver COVID vaccines.

HC: We need to break down certain boundaries through mechanisms like industry forums, publications, and pre-competitive collaboration. These specific mechanisms are important, so we don't stay at a conceptual level.

Q Why is it important that more vaccines are produced in LMICs and what needs to happen to enable this?

CK: I think there are two main reasons. One is because the rate of routine vaccination tends to be lower in LMICs, and we want to support improvements in quality of life and life expectancy. Unfortunately, there is no real financial incentive for global pharma to go into this space. There are some companies of course that are front runners and understand their social responsibility. But, for the most part, vaccine access needs to be built up from the ground up in LMICs.

The second reason is that many important viruses and variants (for example, Ebola and Lassa fever) actually emerged in LMICs. Scientists in these countries were able to isolate the viruses pretty quickly. But once they had that sequence, they could only send it off and hope for the best. Imagine enabling institutes in those countries to go all the way to vaccine design and manufacture—that could be very powerful and allow an almost real-time response to

"Driving down the cost is important, but so is cross-industry collaboration with non-governmental organization and local governments, and lots of creativity to make a real impact."

– Hao Chen

new viral threats. With new accelerated platforms such as mRNA, we could give these countries the chance to vaccinate first responders and medical personnel and stop viruses in their tracks. We could even prevent the next pandemic.

SS: It absolutely makes sense to enable every location to be able to address any diseases that emerge and prevent their spread. Another benefit would be tapping into local knowledge and understanding of what is needed for their population. Local scientists can best understand local storage conditions, supply chains, and medical systems and develop the most suitable formulations, etc.

HC: First and foremost, it is the right thing to do. Diseases do not recognize borders or income. At Merck, we try never to forget that medicine is for the people, not for the profits. As I said earlier, we should definitely begin with the end in mind. A product launch is just the start—we must consider global access.

There are challenges with the very complicated and disrupted supply chain these days, so we need to make deliberate efforts to make this happen. Driving down the cost is important, but so is cross-industry collaboration with non-governmental organization and local governments, and lots of creativity to make a real impact.

MM: Humans are humans, whatever part of the planet they live on. Everyone deserves better healthcare and better medicines. So how do we make it affordable to those countries? Of course, there are non-profit organizations like the Bill and Melinda Gates Foundation, GAVI, CEPI, and WHO—they are doing a phenomenal job. But it is also important for pharmaceutical companies to subsidize vaccine prices, carry out technology transfers, and set up local manufacturing hubs to supply vaccines in an expedited way.

Q Global supply chain delays and shortages are an issue for all drug manufacturers—how has this issue affected vaccines specifically, and what is your advice for managing disruption and minimizing risk?

HC: Supply chain disruptions affect us from the commercial manufacturing side but even more so from the clinical and development side. The availability of simple things like consumables can be a big problem.

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Beyond building a resilient supply chain and shifting to more resilient types of manufacturing, it is important to build multi-sourcing into both development and supply chains. Of course, that is easy to say but not very straightforward to implement!

For me, leading a process development organization, we also try to modernize pathways. There are a lot of old technologies in this industry and there is a big opportunity for us to upgrade production platforms to make a more universal fit for different modalities, and more portable processes so that we can easily transfer them to different locations, to build in supply agility.

CK: Planning ahead and having sufficient stocks is very important. However, we cannot expect huge amounts of facility space, consumables, and raw materials to be ready just in case. All of these will have a huge cost and an expiry date.

There is an in-between situation with reduced risk, but it would require some investment, and my question is whether that investment should only be for suppliers and manufacturers or whether the public health benefits of vaccines warrant national or international facilities with spare capacity to respond to these situations. Perhaps we cannot fully rely on the private sector for all of these interventions. A lot of countries, including the UK, have been making investments in facilities that can drive innovation.

As Sirat said earlier, innovations in formulation are going to be key going forward. In particular, stability in ambient temperatures is going to unlock many doors and global distribution chains for RNA vaccines.

SS: Again, this comes back to understanding what is needed right in the beginning. There still could be things you learn as you go along the process of development. But we can draw from other experiences and other modalities like mAbs and implement that knowledge as early as we can in the process. Then it is a case of having that open conversation with suppliers that we mentioned earlier.

MM: Any supply chain is going to be impacted by a number of elements, including transportation, geopolitics, climate conditions, infrastructure, and artificial intelligence. Orchestrating the supply chain is sometimes more art than science and connecting those dots together is very tricky, especially in the current global circumstances.

During the COVID pandemic, shutdowns in certain parts of the world affected key raw materials for the electronic or vaccine industries, such as microchips or lipids for lipid nanoparticles, which directly impacted our ability to make vaccines.

Amazon has been very successful in moving products from Point A to Point B in an expedited way, with AI-based supply chains and inventory management systems. I hope pharmaceutical companies will achieve that kind of speed and efficiency one day. It is very important for us to realize that putting a vaccine into human arms is just as critical as discovering and developing them.

Q What technology innovations in bioprocessing are having the greatest impact on process efficiency in vaccine manufacturing—especially in the downstream processing and analytics area?

SS: When we think about process efficiency, we are thinking about two things: shorter production time and higher productivity, in order to reduce costs for affordable treatments. To maximize process efficiency, you can eliminate steps from the process, optimize it to increase productivity, or both.

Thinking about downstream processing, specifically, affinity chromatography can play an important role. Vaccines are interesting for affinity chromatography, because the molecules in different vaccines are so different, depending on the disease and how we are trying to target it.

Taking mRNA as an example, affinity chromatography works very well. A lot of companies are looking into it because it enables the elimination of certain steps, and also provides a higher-quality product by reducing process- and product-related impurities. By adding affinity chromatography as a step, you reduce not only the number of steps but also the development time and resources that go into optimizing those steps. Plus, less buffer is used and there is less waste generated.

For other novel vaccines there is the possibility of developing a custom resin, or just optimizing the use of standard resins such as anion exchangers and hydrophobic interaction chromatography (HIC) by better understanding the critical process parameters that play a role in process efficiency.

In terms of analytics, we have not seen the complete adoption of process analytical technology (PAT) in vaccine manufacturing. However, it could be game-changing for process efficiency to have in-line, real-time monitoring of your process.

CK: There are a lot of challenges with PAT, and I know that many manufacturers have already made infrastructure investments but are perhaps not reaping the benefits yet. It is still a novel technology from a regulatory point of view. However, in-line sensing will give us actionable feedback that eventually could be translated into higher confidence in the quality of our products, and the robustness of our processes. Collecting this data over time will definitely increase our confidence in our manufacturing processes; for example, by identifying better manufacturing conditions. Only the integration with analytics that PAT offers can give us that.

Solutions providers are now going all the way to providing software that will connect to the analytics and equipment that can manipulate our process. The tools exist. Perhaps we lack the confidence that we need to employ them in real-time, in-line, but we need to be working towards that. Then we will learn a lot and be able to move toward platform prequalification. Once we have the data we can go to regulatory authorities with a higher degree of confidence. In the long term, I hope this will also increase our confidence in releasing batches in real-time.

MM: Everyone is talking about modular manufacturing. You can draw an analogy with the Gigafactories that Tesla has built, which are essentially clones of one another. Modular manufacturing is a similar concept, although with a smaller footprint to allow companies to 'lift and shift', and clone these facilities across the globe very quickly.

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In downstream processing, as others have mentioned, the bottleneck has been the analytical testing and QC. As others have mentioned, there is a lot of effort going into PAT. It may not be possible to achieve a complete characterization of the process, but if you can monitor key quality attributes at certain steps of the process without human interference, that brings enormous efficiency into the downstream process. The biopharma industry has already achieved that for standalone monoclonal antibody or recombinant protein production.

Another area of innovation that we are actively looking into is continuous processing. For example, how we can make mRNA manufacturing a continuous process from plasmid DNA to *in vitro* translation to mRNA and then lipid encapsulation. Of course, it cannot be a completely autonomous and continuous process, but even if we can do that for some parts, that gives us huge efficiency gains.

HC: Specifically for vaccine manufacturing, digital twin initiatives are starting to gain momentum. It is not reaping the rewards it could be right now, but I think we are driving in the correct direction.

Certainly, the notion of continuous manufacturing, both upstream and downstream, is of interest. How much we can apply that to the vaccine industry is case-by-case but I think there is potentially a huge opportunity there, especially for continuous chromatography in certain scenarios in downstream processing.

BIOGRAPHIES

CLEO KONTORAVDI is a Professor of Biological Systems Engineering at Imperial College London. Her research involves the development of comprehensive platforms that combine modeling with experimentation for bioprocess understanding, design, and optimization. She collaborates extensively with industrial partners including GSK, AstraZeneca, Amgen, and vaccine manufacturers from around the world.

MURALI MURALIDHARA is the Chief Manufacturing Officer at RVAC Medicines, where he oversees CMC, development, manufacturing, and supply chain of mRNA-based vaccines and therapeutic products. He has over 22 years of technical and leadership experience, including roles at Pfizer Biotherapeutics, AstraZeneca, and GSK Vaccines.

SIRAT SIKKA is a Field Applications Scientist in the Nucleic Acids Therapeutics group at ThermoFisher Scientific. She started her career as a downstream process development scientist at GSK Vaccines, working on early-phase development and optimization for recombinant proteins and nucleic acid vaccines, before joining ThermoFisher Scientific in 2019.

HAO CHEN is Associate Vice President of Vaccine Process R&D at Merck, Sharp & Dohme, where he leads process development and GMP manufacturing for vaccine drug substance and drug product intermediates, with a broad portfolio of vaccine modalities from RNA and recombinant proteins to polysaccharide conjugates and live viral vaccines.

AFFILIATIONS

Cleo Kontoravdi
Professor of Biological Systems Engineering,
Imperial College London

Murali Muralidhara
Chief Manufacturing Officer,
RVAC Medicines

Sirat Sikka
Field Applications Scientist, Nucleic Acid Therapeutics,
Thermo Fisher Scientific

Hao Chen
AVP, Vaccines Process R&D,
Merck

AUTHORSHIP & CONFLICT OF INTEREST

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- Simplified workflow helps to maximize efficiency and reduce the complexity of subsequent polish steps
- Excellent scalability, allowing purification from benchtop to commercial manufacturing

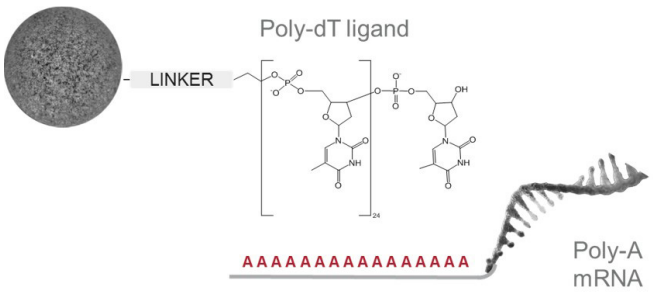

Figure 1. Mechanism of action of POROS Oligo dT(25) affinity resin. The poly-dT ligand allows binding with poly-A tailed mRNA molecules through AT base pairing.

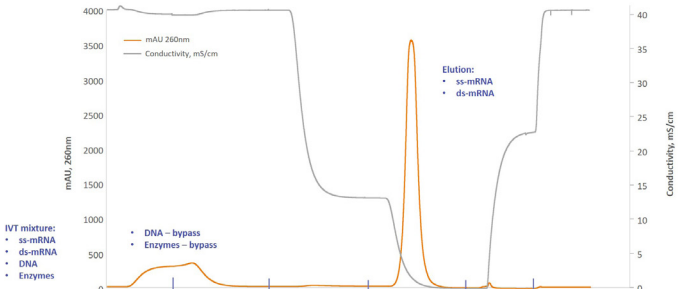

Figure 2. Chromatogram showing efficient separation of a 2000nt mRNA from an IVT mixture at a load concentration of 2 mg/mL. Elution was performed using H_20 and yielded >95% recovery.

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