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Molecular Therapy
Review

Adenovirus-based vaccines—a platform for pandemic preparedness against emerging viral pathogens

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Zoonotic viruses continually pose a pandemic threat. Infection of humans with viruses for which we typically have little or no prior immunity can result in epidemics with high morbidity and mortality. These epidemics can have public health and economic impact and can exacerbate civil unrest or political instability. Changes in human behavior in the past few decades—increased global travel, farming intensification, the exotic animal trade, and the impact of global warming on animal migratory patterns, habitats, and ecosystems—contribute to the increased frequency of cross-species transmission events. Investing in the pre-clinical advancement of vaccine candidates against diverse emerging viral threats is crucial for pandemic preparedness. Replication-defective adenoviral (Ad) vectors have demonstrated their utility as an outbreak-responsive vaccine platform during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Ad vectors are easy to engineer; are amenable to rapid, inexpensive manufacturing; are relatively safe and immunogenic in humans; and, importantly, do not require specialized cold-chain storage, making them an ideal platform for equitable global distribution or stockpiling. In this review, we discuss the progress in applying Ad-based vaccines against emerging viruses and summarize their global safety profile, as reflected by their widespread geographic use during the SARS-CoV-2 pandemic.

INTRODUCTION

The ongoing threat posed by emerging viruses has been highlighted following the introduction of a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), into the human population in late 2019. In addition to coronaviruses, several viruses of concern have been identified by the World Health Organization (WHO) in their "blueprint list of priority diseases" and by the National Institute of Allergy and Infectious Diseases (NIAID) as priority pathogens for biodefense research (Table 1). Many priority pathogens are transmitted by mosquitoes or ticks (i.e., Zika virus), by exposure to infected bats (i.e., Nipah virus), or upon inhalation or ingestion of the urine or feces of infected rodents (i.e., Lassa virus). Viruses with a zoonotic reservoir represent an unpredictable threat for spill-over into the human population. Many of these viruses can cause severe illness with high mortality or fatality, and to date, most lack licensed and approved vaccines or effective pharmaceutical countermeasures. To mitigate the future risks posed by emerging viruses, pandemic preparedness is of the utmost importance. In combination with surveillance programs to establish an accurate geographical distribution of endemic risk areas and investment in diagnostics to determine the true seroprevalence and incidence in humans, advancing the early-stage pre-clinical and translational development of a broad repertoire of candidate vaccines will be crucial.

The target product profile (TPP) for disease-specific vaccines prioritizes characteristics relevant to the nature of the pathogen and the type of risk it poses. Different attributes may be desirable for vaccines aimed at non-emergency prophylactic use versus vaccines designed for use in an emergency outbreak scenario. Preferred characteristics include platforms capable of rapid induction of durable protective immunity, those with an established safety and immunogenicity profile in relevant risk groups, the potential to elicit breadth of protection against variants or viral lineages, and vaccines compatible with thermostability and prolonged shelf life. Replication-defective adenoviral (Ad) vaccines have been a prominent platform in the response to the SARS-CoV-2 pandemic, with vaccines based on three Ad types: HAdV-C5 (Ad5); HAdV-D26 (Ad26); and chimpanzee Y25 (ChAdOx1), receiving emergency use authorization (EUA) across the United States, EU, South America, Asia, and Africa and full approval

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Table 1. WHO and NIAID priority viral diseases for research and development

Disease	Causative agent	Classification (Order, Family)	Zoonotic reservoir/host	Mode of transmission	Case fatality rate in humans	Geographic distribution	PMID
Ebola virus disease (EVD) Marburg virus disease (MVD)	Ebola virus (EBOV) Marburg virus (MARV)	*Mononegavirales Filoviridae* *Mononegavirales Filoviridae*	Fruit bats, family *Pteropodidae* Fruit bats, family *Rousettus*	Exposure to infected animals or humans, body fluids. Hospital or burial ceremonies are high risk.	25%–90% 24%–90%	Central and West Africa Central Africa	29083948 26325242 33309082
Lassa fever	Lassa virus (LASV)	*Bunyavirales Arenaviridae*	*Mastomys* rats	Exposure to rat urine, feces, or fluids	1% or 50%–70% in hospitalized patients. High rates of fetal mortality in third trimester of pregnancy.	West Africa	31479990
Crimean-Congo hemorrhagic fever (CCHF)	Crimean-Congo hemorrhagic fever virus (CCHFV)	*Bunyavirales Nairoviridae*	Viremic livestock, *Hyalomma* ticks	Tick bites, exposure to body fluids of infected livestock or humans (including nosocomial).	4%–40% up to 80% in clinically infected patients	Africa, The Balkans, Middle East, Asia	29083948 28687403
Hantavirus fever renal syndrome (HFRS) Hantavirus pulmonary syndrome (HPS)	Sin Nombre virus (SNV) and Andes virus (ANDV)	*Bunyavirales Hantaviridae*	Deer mice, *Peromyscus maniculatus*	Exposure to rodents and their droppings or body fluids or following inhalation of aerosolized material from rodent urine or feces	0.1%–15% for HFRS 40%–50% for HPS	North/South America (SNV, ANDV) Asia, Europe	19403663 16375712 16375712
Rift Valley fever (RVF)	Rift Valley fever virus (RVFV)	*Bunyavirales Phenuiviridae*	Ruminants, mosquitoes, *Aedes* species	Exposure to infected animals or by mosquito bites during high-density circulation	Up to 35%	Africa and Arabian Peninsula	29083948
Zika fever	Zika virus (ZIKV)	*Amarillovirales Flaviviridae*	Mosquitoes, *Aedes* species	Bite from infected mosquito, vertical transmission, or through sexual contact	Rare, but fetal loss following vertical transmission is 14%, with congenital Zika syndrome in ∼21%.	Africa, Asia, Micronesia, Americas	29083948 31597021

Coronaviruses, including SARS-CoV-1, SARS-CoV-2, and MERS-CoV, have been omitted from this table due to the large amount of published data on these viruses. Emerging viruses from the *Paramyxoviridae* (i.e., Nipah virus) have also been omitted due to space constraints. Table updated from Ewer et al.[1]

in Russia. In this review, we will outline the benefits, risks, and potential future of Ad-based vaccines against emerging viruses, summarize data from pre-clinical and clinical trials using Ad vaccines for "priority diseases," and discuss the safety of Ad vaccines, following their extensive global use during the SARS-CoV-2 pandemic.

BACKGROUND ON VACCINES

Vaccines: Mechanism of action

Most of us know that vaccines protect us from diseases because they teach our immune system to recognize the pathogen and induce an immune response, which prevents us from being infected by a virus. Vaccines prevent the death of >15 million individuals every year. Due to a successful global vaccination program, smallpox was eradicated in 1980 and is no longer the scourge it was for greater than a millennium. Following the introduction of the measles, mumps, and rubella (MMR) vaccine, deaths attributed to these viral infections declined by 96% since 1980 (when global deaths from measles were in the millions).[2,3] Unfortunately, the development of long-lasting, effective vaccines against many viral pathogens can be challenging. Factors that contribute to this include high mutation rates and antigenic diversity, poor immunogenicity of conserved epitopes, technical challenges in engineering structurally authentic immunogens, and a lack of correlates of protection. Therefore, efforts to better understand how different vaccine platforms work, what phenotype of immune response they elicit, and precisely how that response contributes to protection will enable a systematic approach to iterative vaccine design.

Initially, vaccines stimulate the recruitment of effector cells to the site of injection, following an innate immune response. Effective vaccines engage professional antigen-presenting cells (APCs) to present exogenous antigens to naive T cells to initiate B cell and T cell responses (Figure 1). In most cases, we produce T cells that lyse infected cells and neutralizing antibodies (NAbs) that can block viral entry. However, the role of non-neutralizing antibodies (Abs) in mediating protection has become apparent in recent years, and this class of Ab can also contribute to viral clearance. For example, Abs can agglutinate viral particles (vps), which make these large aggregates an easy target for immune cells to phagocytose the complex via Fc receptors (FcRs) and degrade the virus. Alternatively, Abs can also bind to viral glycoproteins expressed on the surface of infected cells and target those cells for destruction via Fc-mediated antibody-dependent cellular cytotoxicity (ADCC)[4–7] or phagocytosis (ADCP).[8] Abs can also activate the complement pathway, which opsonizes and promotes the

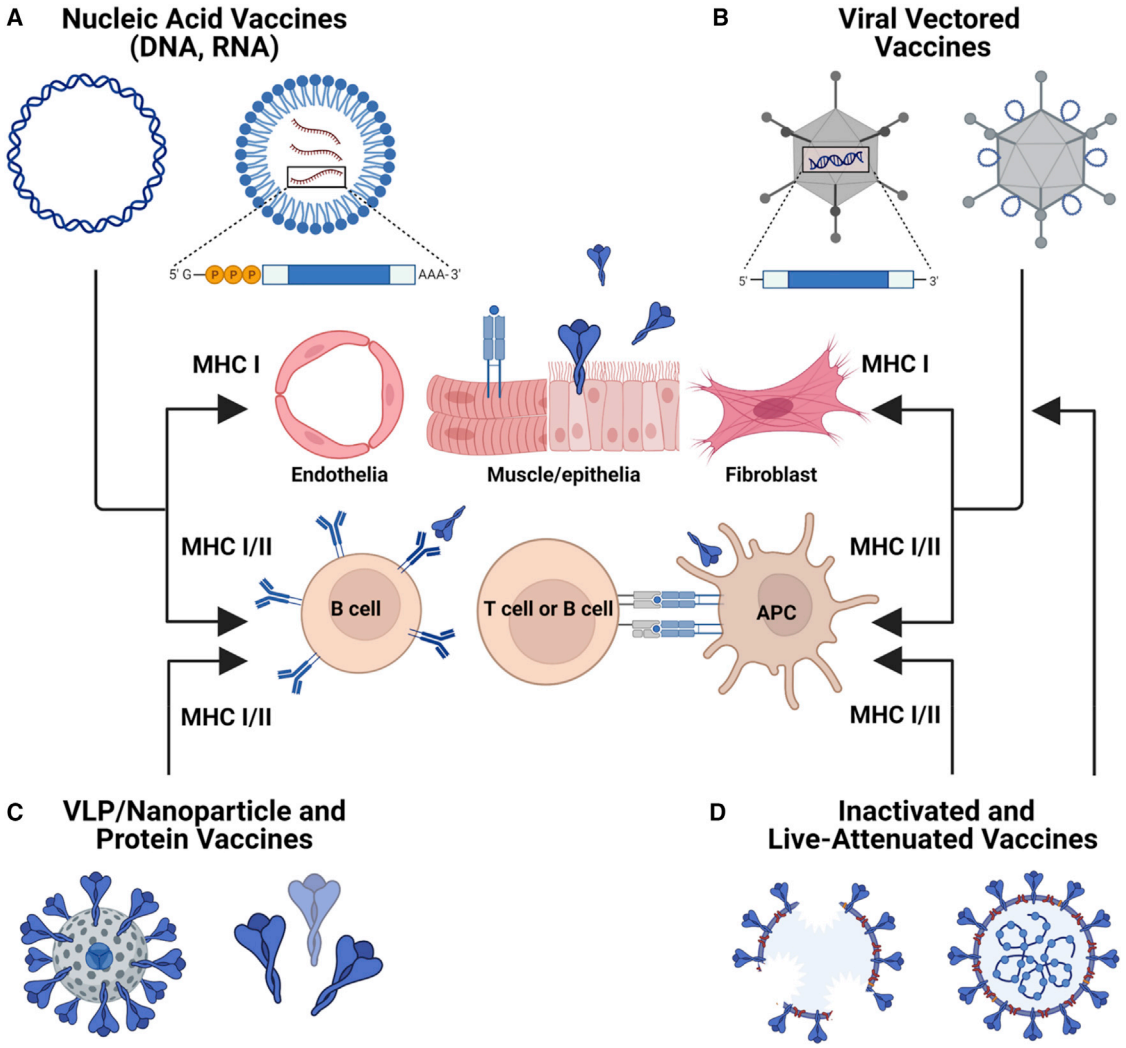

Figure 1. Vaccine platforms for outbreak pathogens

Schematic diagram showing the range of different platforms that can be used for vaccine development. (A) Nucleic-acid-based vaccines (i.e., DNA or mRNA) encode the vaccine antigen target sequence, allowing for transgene expression *in vivo*. These vaccines facilitate both MHC class I antigen presentation from cells at the site of injection and MHC class II antigen presentation by APCs. (B) Similarly, viral-vectored vaccines (i.e., Ads) can also encode the transgene antigen sequence or display peptide antigen on the capsid exterior. These vectors allow for *in vivo* expression and antigen processing via MHC class I and class II. (C) Virus-like-particles (VLPs) or protein-based vaccines are processed in a similar manner to inactivated platforms. (D) Inactivated vaccine platforms are largely scavenged by APCs, resulting in MHC class II presentation, although cross-presentation in dendritic cells (DCs) can facilitate MHC class I presentation. As live attenuated vaccines can infect respiratory epithelia, they can also present antigen via MHC class I. Figure created with BioRender.com.

phagocytosis of viruses and/or damages the envelope (phospholipid bilayer) present on some viruses.

In non-professional APCs, antigen presentation is thought to be limited to major histocompatibility complex class II (MHC class II) presentation and preferentially induces a Th2-skewed response. However, in professional APCs (e.g., dendritic cells [DCs], Langerhans cells, and macrophages), a phenomenon called cross-presentation occurs, where proteins that are taken up from the extracellular environment can be loaded onto MHC class I molecules to promote a Th1 response. There are several pathways toward cross-presentation in APCs, including cytosolic and vacuolar. Following uptake of exogenous antigen by macropinocytosis or phagocytosis, antigen escape from the early endosome, or fusion of the endosome with the endoplasmic reticulum (ER) and subsequent degradation of antigen by the proteasome, facilitates peptide loading onto recycled cell surface MHC class I molecules. It is worth noting that cross-presentation can operate independently of the proteasome and the transporter associated with antigen presentation. In the vacuolar pathway, peptide antigen can be loaded onto MHC class I in the endosome and lysosome. Of note, it has been shown that $CD8^+$ T cell responses elicited by Ad-based vaccines rely on cross-presentation by

subpopulations of DCs.[9] It is considered that cooperation between APCs and non-lymphoid cells likely contributes to the kinetics, maintenance, and phenotype of antigen-specific immune responses elicited by Ad-based vaccines.[10,11] Collectively, the ability to engage multiple coordinated pathways presumably contributes to their capacity to elicit both cellular and humoral immune responses simultaneously.

Vaccines: Platform selection

Understanding what profile of immune response is desirable in mediating protection for a given disease target is an important consideration when selecting an optimal vaccine platform (Figure 1). In the context of vaccine development for emerging viruses, additional considerations may be required, such as the potential need for manufacturing under high containment (i.e., BSL-3) and associated cost and biosafety implications, suitability for stockpiling, cost-per-dose, and stability under cold-chain free conditions.

Whole viruses: Live attenuated and inactivated vaccines

More than 500 years ago, people in Africa and Asia were using live, unattenuated smallpox to inoculate or "variolate" some members of the community.[12] This approach consisted of lancing a ripe pustule of an infected individual and then inserting the lance subcutaneously into a second, healthy individual. While not without risk, this approach must have saved millions of lives. Europeans caught up in the 18th century and added their twist—which was based on the observation that milkmaids who were infected with cowpox appeared to be protected from the ravages of smallpox. This observation translated into the use of cowpox (Vaccinia) as a vaccine against smallpox (Variola), tirelessly promoted by Edward Jenner.[13]

The advantage of using whole-virus vaccines is the breadth of antigenic targets, as this type of platform can deliver all the proteins in the capsid and possibly internal proteins (which are often highly conserved). Licensed vaccines against influenza virus include live attenuated influenza vaccines (LAIVs) and inactivated influenza vaccines (IIVs): the latter including inactivated whole virion vaccines (WIVs) and split-virion or sub-virion IIV formulations.[14] The MMR vaccine is also based on attenuated measles, mumps, and rubella viruses. A live attenuated vaccine is also used to prevent poliomyelitis, which is caused by poliovirus serotypes 1, 2, and 3. The polio vaccine has reduced disease burden by 99%. Unfortunately, use of live polioviruses can cause "vaccine-derived poliovirus" spread and can be problematic if poliovirus is endemic in regions where vaccination rates are low. Another live virus vaccine used for ~60 years is based on human Ad type 4 and 7 (HAdV-4 and -7) to protect against respiratory illness caused by the same viruses. This oral vaccine has been used almost exclusively in 18- to 30-year-old military recruits since the 1970s.[15–17] The safety profile of HAdV-4 and -7 has been acceptable, and it has saved many lives.[18–20]

There are two principal methods for virus inactivation—heat and chemical crosslinking. The rabies vaccine is a whole virus, inactivated with beta-propiolactone (BPL) (as is CoronaVac, BBIBP-CorV, and Covaxin). In many cases, the crude preparation can retain residual viral nucleic acid, which can facilitate stimulation of innate immune signaling (acting as an adjuvant).[14,21] When considering the development of live attenuated or inactivated vaccines against viral pathogens that have high fatality, the need to grow virus in high containment makes large-scale manufacture impractical.[22] Furthermore, there are additional risks if inactivation procedures are ineffective or suboptimal or if attenuated strains revert to wild type. These concerns have prompted the development of alternative, next-generation vaccine platforms for emerging viral pathogens.[23]

Subunit-based vaccines

Protein-based vaccines are a simple, safe, and scalable platform. Before designing a subunit vaccine, one must know enough about the virus to identify which part of the capsid will be the most effective in mediating protection. Considerations include whether the protein is involved in receptor engagement and whether Abs against it will induce virus neutralization. Ideally, the vaccine will also induce a T cell response to allow infected cells to be lysed. Typically, these platforms include a viral protein that was expressed in cells and purified to near homogeneity before being incorporated into a vaccine formulation. Protein production can be in plant, bacteria, yeast, insect, animal, or human cells. An example of a US Food and Drug Administration (FDA)-approved subunit vaccine is the hepatitis B virus vaccine, where the hepatitis B surface antigen (HBsAg) is produced in yeast.[24] Each production platform has its strengths and drawbacks with respect to post-translational modification of the protein (in particular glycosylation), production potential, upscaling, upstream and downstream processing, and risk of contaminants. A challenge with protein-based vaccines is often their inability to induce an innate immune response, which can have a negative impact on downstream immunogenicity. A formulation step typically stabilizes the protein and incorporates an adjuvant.

Virus-like particles (VLPs) and self-assembling nanoparticles

VLPs are self-forming structures typically composed of a subset of capsid proteins. Due to their size, symmetry, and particulate composition, VLPs are readily taken up by APCs, allowing for receptor-mediated uptake, clustering and activation of pattern-recognition receptors (PRRs),[25] and presentation of particles to lymphocytes so that the immune system will mount an antigen-specific immune response. VLPs can be made from numerous viruses[26,27] and can be engineered to contain or present sequences from other viruses.[28] In some cases, VLPs can be engineered to package nucleic acids,[29] peptides from other pathogens,[30] or molecular adjuvants and immunostimulatory molecules on their surfaces.[31] VLPs can be produced in cells derived from bacteria, yeast, insect and mammals, and in cell-free expression systems and organisms such as silkworm pupae and various plants.

Like subunit vaccines, VLPs benefit from cross-presentation pathways that allow induction of CD8$^+$ T-cell-mediated cytotoxic immune responses and have been shown to utilize the MHC class I receptor recycling pathway of cross-presentation.[32] Several VLP vaccines are commercially available, including Gardasil, a multivalent

human papillomavirus (HPV) vaccine.[33] Although VLPs represent a promising platform for future development, VLPs derived from bacterial and insect cells can be contaminated with endotoxin or baculovirus, they lack mammalian glycosylation, and there are occasionally issues with formulation stability, precipitation and aggregation, or scale-up to meet global demand.

Another innovative vaccine platform is the use of naturally occurring, self-assembling nanoparticles or computationally designed vaccine scaffolds.[34,35] Similar to VLPs, these particles allow for a structurally ordered display of antigen, which, along with their small size, allows them to mimic viruses. Vaccines based on conjugation to bacterial ferritin, which self-assembles into stable nanoparticles allowing for surface presentation of viral glycoprotein ectodomains, have been developed as vaccines against numerous viruses.[36–39] Another bacterial scaffold that has been employed in the development of self-assembling, nanoparticle-based vaccines is lumazine synthase (LS).[40,41]

Nucleic-acid-based vaccines: DNA and mRNA

A primary difference within nucleic-acid-based vaccines, as compared with inactivated, protein-, or nanoparticle-based platforms, is that the antigen is produced from the cell that takes up the vaccine following immunization. Transgene expression of the target antigen from mRNA likely persists for a few days,[42] while DNA vector-based vaccines may provide more sustained antigen presentation.[10,43] Antigens encoded by nucleic-acid vaccines can also be targeted to the cell surface to allow more efficient detection by the developing immune response (Figure 2). DNA-based vaccines (plasmids), which have been explored for greater than 3 decades, are rapidly designed, easily produced, scalable, and thermostable. Clever approaches have also allowed the production of plasmids void of antibiotic resistance genes.[44] DNA-based vaccines also preferentially induce a Th1-biased immune response. Avoiding degradation prior to reaching the nucleus can be a limiting factor for DNA-based vaccines. However, to date, these vaccines have been encouraging in preclinical studies, and significant success in human clinical trials may not be far off.[45–47] Innate responses to DNA and RNA include PRRs that detect uncapped mRNA (TLR7) and unmethylated CpG (TLR9) and those that detect antimicrobial peptides (AMPs) and coagulation factors linked to viral capsid (TLR4).[48–50]

As with plasmid-based vaccines, RNA vaccines have been explored for 3 decades too. The breakthrough that made mRNA viable as a vaccine platform is the modification of their bases, which prevents excessive immunostimulation, allowing evasion of PRR recognition, preventing premature degradation, and therefore enabling increased and sustained transgene antigen expression.[51] In addition, advances in formulation chemistry facilitated the encapsulation of modified mRNA in lipid nanoparticles (LNPs), which display biocompatibility and can accommodate a large mRNA payload,[51] with the capacity to encode more than one antigen for a multi-valent vaccine.[52] Following uptake in target cells at the site of injection, mRNA-based vaccines do not have to enter the nucleus. Therefore, a significant trafficking hurdle is avoided, and mRNA can be very rapidly translated into the targeted protein or antigen in the cytoplasm. The subject of applying mRNA vaccines against infectious diseases is beyond the scope of this review and is covered in detail in comprehensive reviews elsewhere.[23,51]

Pseudotyped, replication-defective, and replication-competent viral vectors

The concept of using viral vectors to deliver gene expression cassettes encoding targeted antigens is also greater than 3 decades old. Of note, some of the first HAdV type 2 and 5 vectors were "vaccines."[53,54] Advantages of viral-vector-based vaccines include the ability of viruses to be taken up efficiently by cells and the potential to engineer replication-defective vectors and capitalize on their inherent immunostimulatory properties (i.e., their symmetry can act as a PAMP). The immunostimulatory effects facilitate activation of innate immunity that can enhance the response to the target antigen.

Among the most studied viral vectors for vaccine applications are Ads,[55–58] poxvirus: modified vaccinia Ankara (MVA),[56,59] adeno-associated virus (AAV),[60] rhabdovirus:[61] vesicular stomatitis virus (VSV),[62,63] paramyxovirus: Newcastle disease virus (NDV),[64,65] human parainfluenza virus,[66] and Sendai virus.[67] An expression cassette encoding one or more antigens can be incorporated into the genome of the vector (i.e., Ad, MVA, and AAV), which is then expressed in cells that take up the vaccine (Figure 2). Typically, vectors are injected intramuscularly (i.m.). Advantages include robust and inexpensive production, high safety profiles, and a tendency to generate Th1-skewed or balanced Th1 and Th2 responses. Drawbacks include pre-existing immunity against the vector, which can reduce vaccine efficacy and may preferentially amplify a pre-existing response (versus generate a robust *de novo* response against the encoded transgene[s]). Alternatively, viral vectors can be pseudotyped and genetically engineered to display heterologous glycoprotein antigens (i.e., VSV and NDV), vectors can be (re-)targeted to specific cell types via modification of the receptor-binding domains, or (sero)types with a preferential tropism (e.g., APCs) can be selected. Moreover, some vectors can be engineered for a single replication cycle to boost efficacy[68] or capsid proteins can be modified to include antigenic epitopes from a target pathogen.[69,70] The latter approach allows antigen to be processed and presented by MHC class II during vaccine uptake, and depending on the platform, simultaneous production of genome-encoded antigen can allow for MHC class I presentation.

Specifically, replication-defective Ad vaccines have several characteristics that enhance their potential as an adaptable plug-and-play platform technology well suited to pandemic preparedness initiatives.[1] They have a stable double-stranded DNA (dsDNA) genome that can be engineered to encode one or more vaccine antigens;[59,71–73] their broad geographic use during the SARS-CoV-2 pandemic highlights their suitability for rapid manufacturing to meet global demand; they are safe and immunogenic in healthy adults,[55,57,74–77] infants as young as 1 week of age,[78,79] the elderly,[55] and immunocompromised;[80–82] and they are compatible with thermostabilization and lyophilization procedures,[83–85] allowing them to be stockpiled or

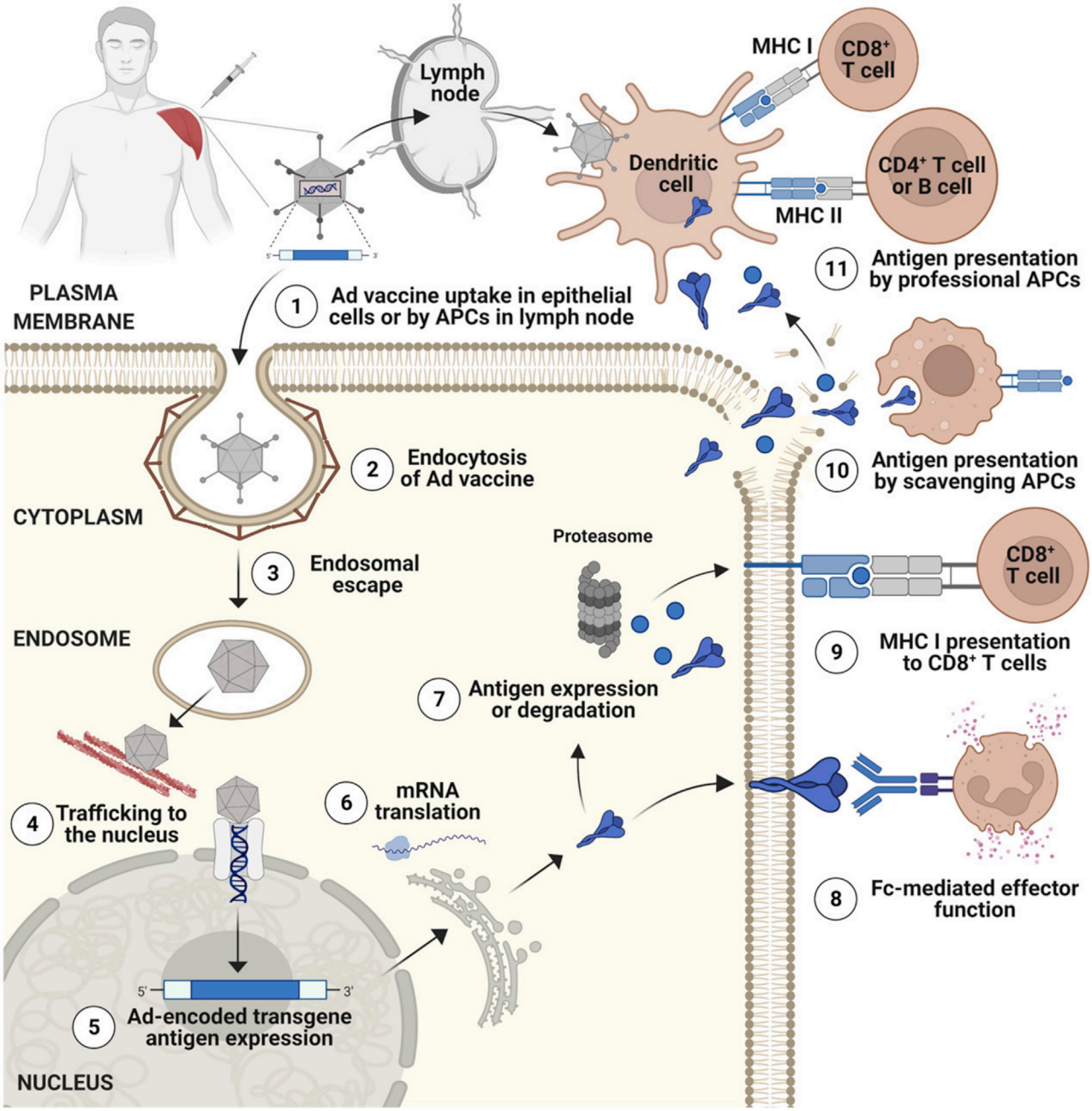

Figure 2. Schematic diagram outlining the antigen-presentation mechanisms used by Ad-based vaccines

(1) Ad-vaccine is taken up by muscle cells or antigen-presenting cells (APCs) at the site of injection or following trafficking to draining lymph nodes (dLNs). (2) In parenchymal cells (i.e., muscle), uptake can be mediated by endocytosis. (3) Ad vaccine escapes from the endosome. (4) Partially disassembled Ad capsids traffic to the nucleus using the microtubule network. (5) Once in the nucleus, the encoded vaccine transgene antigen is transcribed. (6) mRNA corresponding to the encoded transgene antigen is exported to the cytoplasm and is translated into protein. (7) Antigen is expressed, and some antigen is degraded by the proteasome. (8) Depending on the antigen design, glycoproteins that normally traffic to the plasma membrane will follow this path and can potentially be recognized by Abs, including those capable of Fc-mediated effector function. (9) Degraded peptide antigen can be loaded onto MHC class I for direct presentation to $CD8^+$ T cells. (10) Secreted antigens can be released into the extracellular space or apoptosis of transgene-expressing cells can also facilitate antigen release. Extracellular (exogenous) antigen can be scavenged by macrophages or other APCs at the site of injection. (11) Antigen fragments arriving in the dLN are phagocytosed by professional APCs and peptides processed and presented to T cells via appropriate MHC molecules. Figure created with BioRender.com.

distributed without the need for specialized ultra-cold storage. Finally, they are substantially cheaper than mRNA platforms, potentially allowing for a more equitable global vaccine distribution. These factors are all important considerations when developing vaccines against outbreak pathogens, which may be geographically endemic in low- and middle-income countries (LMICs).

TARGETS FOR OUTBREAK PATHOGEN VACCINE DEVELOPMENT

Beyond vaccines for SARS-CoV-2, which are outlined in detail in a recent review,[86] some of the most advanced Ad-based platforms have been developed against Ebola virus (EBOV), a member of the family *Filoviridae*. Vaccines based on Ad5, Ad26, and chimpanzee vector ChAd3 have undergone clinical evaluation as standalone or heterologous prime:boost regimens with MVA. The Ad26.ZEBOV + MVA-BN-Filo regimen received regulatory approval on July 1, 2020 by the European Medicines Agency (EMA). To date, Ad-based vaccines for Ebola virus have been shown to be safe and immunogenic in children,[87,88] healthy adults,[56,89–92] and HIV-infected individuals (Table 2).[81,82] Phase I clinical trials have also been initiated to evaluate Ad vaccines against members of the *Flaviviridae* (i.e., Zika virus),[58] *Togaviridae* (i.e., Chikungunya virus),[93] or *Orthomyxoviridae* (i.e., H5N1 avian influenza)[75,94] families (Table 3). Viral hemorrhagic fevers, including viruses from the *Arenaviridae*, *Nairoviridae*, *Hantaviridae*, and *Phenuiviridae* (all order *Bunyavirales*), in addition to emerging viruses from the *Paramyxoviridae* family (i.e., Nipah virus), are also important targets for vaccine development due to their potential for high mortality, their zoonotic life cycle (resulting in unpredictable outbreaks), and the lack of licensed prophylactic countermeasures. The antigen targets for vaccines against several outbreak pathogens are highlighted in Figures 3A–3D.

Filoviridae; Marburg virus (MARV) and EBOV

MARV was identified as the causative agent for Marburg virus disease (MVD) following an outbreak in Germany in 1967, and the EBOV was first identified in 1976 in the Democratic Republic of Congo (formerly Zaire). Both viruses are members of the family *Filoviridae*, in the order *Mononegavirales*, and possess a viral envelope with a single-stranded, negative-sense RNA genome (Figure 3A). Infection with MARV or EBOV can result in severe viral hemorrhagic fever with fatality rates of 25%–90%.[95,96] Survivors of EBOV infection can suffer with long-term sequelae.[97,98] Fruit bats of the *Pteropodidae or Rousettus* families are believed to be natural hosts,[99] and initial infection with EBOV or MARV may be through exposure to animals, with epidemics arising following subsequent human-human transmission through direct contact or exposure to infectious body fluids. Filoviruses represent a serious threat due to their high case fatality; the potential for unpredictable, rapidly expanding epidemics; and the risk for bioterrorism (Table 1). The Ebola virus outbreak in 2013–2016 in Africa was responsible for 11,000 deaths.[95] This epidemic prompted widespread collaborative efforts to develop vaccines, which led to the regulatory approval of two vaccine regimens: one based on a pseudotyped VSV bearing the EBOV glycoprotein (GP) and a second based on a heterologous prime:boost regimen with an Ad26 prime and boost with MVA, encoding one or more filovirus GPs. Additional Ad-based platforms, chimpanzee Ad vector ChAd3 and Ad5, have also undergone extensive pre-clinical and clinical testing as candidate vaccines against EBOV (Table 2).

Correlates of protection for EBOV have not been conclusively defined.[100] Ab responses directed toward the viral GP have been associated with protection in animal models.[101] However, a role for GP-specific $CD8^+$ T cells in mediating protection has also been demonstrated in non-human primates (NHPs),[102] where passive transfer of high titer sera did not confer protection, but selective depletion of $CD8^+$ T cells abrogated protection in 80% of animals.[103] Pioneering studies in the early 2000s tested Ad5-based vaccines encoding the EBOV GP or nucleoprotein (NP) at doses of 1×10^{10} vps in mice or 2×10^{12} vps in NHPs in a single-shot versus homologous prime:boost regimen administered i.m.[101,104] As reported for Ad-based vaccines,[105] induction of antigen-specific immune responses was rapid (<3 weeks). However, Ab responses to GP were not boosted by the second homologous Ad5-GP immunization, likely due to anti-vector immunity. When a single-shot regimen containing an equal mixture of Ad5-GP/Ad5-NP was tested in NHPs, it conferred complete protection from infection within 1 month of immunization,[104] even with a high challenge dose. Building upon these promising findings, the Nabel laboratory subsequently evaluated strategies to modify the encoded GP antigen to eliminate its inherent cytopathic effects while maintaining protective efficacy or used approaches to improve GP-specific immune responses by enhancing transgene expression by codon optimization.[106,107]

Considering the more advanced pre-clinical state of Ad-based vaccines against Ebola virus relative to other outbreak pathogens, a broader range of studies exist. These include testing mucosal delivery, the use of diverse human and non-human Ad vector platforms, heterologous prime:boost regimens, or the construction of multi-valent vaccine candidates. Ad5 vaccines encoding GP from *Zaire ebolavirus*, administered intranasally (i.n.) to several animal species (i.e., mice, guinea pigs, and NHPs), have been shown to provide complete protection from lethal challenge, comparable to i.m. immunization,[108–111] and can bypass pre-existing immunity to the Ad5 vector carrier.[108,110] Alternative strategies to overcome pre-existing immunity to common Ad serotypes include the use of Ad vectors derived from rare or non-human Ads.[10,14,112] Yang and colleagues reported the construction of two chimpanzee Ad vectors, AdC7 and AdC68, expressing the Ebola virus GP from the 2014 outbreak. A single i.m. immunization with each vector at 2×10^{10} vps elicited GP-specific Ab responses, although only AdC68 elicited detectable antigen-specific interferon (IFN)-γ enzyme-linked immune absorbent spot (ELISpot) responses.[113] This observation again emphasizes that distinct Ad platforms elicit a range of immunological phenotypes,[10] and vaccines will thereby require customization for specific disease targets. When tested in a heterologous prime:boost regimen, AdC7-AdC68 was found to be optimal, inducing GP-binding Abs, pseudovirus NAbs, and GP-specific T cells. ChAd3- and ChAd63-based vaccines have also been tested in NHPs, with the ChAd3 platform being identified as superior in eliciting protection from lethal challenge, a factor associated with a higher magnitude of

Table 2. Adenoviral vaccine clinical trials for Ebola

Disease target (antigen)	Vector (source)	Group (phase)	Dose/route	Regimen (type) and interval (time)	T cell response	Antibody response (Post-Ad immunization versus pre-immunization or placebo)	Clinical Trials.gov	PMID (year)
Ebola ZEBOV GP (*Zaire ebolavirus* glycoprotein)	Ad26 (Johnson & Johnson)	Healthy adults ≥18–50 >50–70 (phase 2)	5×10^{10} vps (i.m.)	+Boost MVA/28 days MVA/56 days MVA/84 days (1×10^8 IUs)	IFN-γ ELISpot and flow cytometry but only measured post-MVA boost	GMC (95% CI), EU/mL 332 versus <40 (D29 versus D1) 361 versus <40 (D57 versus D1) 242 versus <40 (D85 versus D1)	NCT02564523	34714820 (2021)
		HIV^+ adults ≥18–50 (phase 2)		+Boost MVA/28 or 56 days (1×10^8 IUs)		GMC (95% CI), EU/mL 368 versus <40 (D29 versus D1) 291 versus <40 (D57 versus D1)		
Ebola ZEBOV GP (*Zaire ebolavirus* glycoprotein)	Ad26 (Johnson & Johnson)	Healthy adults ≥18 years (phase 1, 2)	5×10^{10} vps (i.m.)	+Boost MVA/56 days (1×10^8 IUs)	Not reported in this study	GMC (95% CI), EU/mL 236 versus 69 (D57 versus D1)	NCT02509494	34529963 (2021)
				MVA/56 days (1×10^8 IUs) +Ad26.ZEBOV at 2 years		GMC (95% CI), EU/mL 269 versus 60 (D57 versus D1) 30,411 versus 279 (D741 versus D720)		
Ebola ZEBOV GP (*Zaire ebolavirus* glycoprotein)	Ad26 (Johnson & Johnson)	Healthy children 1–17 years (phase 2)	5×10^{10} vps (i.m.)	+Boost MVA/56 days (1×10^8 IUs)	Not reported in this study	GMC (95% CI), EU/mL D57 versus D1 12–17 years: 314 versus ∼40 4–11 years: 390 versus ∼40 1–3 years: 693 versus ∼40	NCT02509494	34529962 (2021)
Ebola ZEBOV GP (*Zaire ebolavirus* glycoprotein)	Ad26 (Johnson & Johnson)	Healthy adults ≥18–50 years (phase 1)	5×10^{10} vps (i.m.)	+Boost MVA/28 days MVA/56 days	IFN-γ ELISpot SFUs/10^6 (FC of median) G1: 4.1 G2: 2.3	GMC (95% CI), EU/mL G1: 532.9 versus 18.3 (D29 versus D1) G2: 581.1 versus 22.0 (D29 versus D1) 854.3 versus 22.0 (D57 versus D1)	NCT02313077	27092831 (2016)
Ebola EBO-Z GP (*Zaire ebolavirus* glycoprotein)	ChAd3 (GSK)	Healthy children 1–17 years (phase 2)	1×10^{11} vps (i.m.)	No boost, single-shot regimen	Geo mean FC (95% CI) $CD4^+$ D30 versus D0 13–17 years: 2.1 6–12 years: 2.3 1–5 years: 2.6	GMC (95% CI), EU/mL D30 versus D0 13–17 years: 1,564 versus 30 6–12 years: 1,395 versus 23 1–5 years: 2,406 versus 22	NCT02548078	32199492 (2020)
					$CD8^+$ D30 versus D0 13–17 years: 1.7 6–12 years: 2.0 1–5 years: 2.4	D365 versus D0 13–17 years: 716 versus 30 6–12 years: 752 versus 23 1–5 years: 1,424 versus 22		

(*Continued on next page*)

Table 2. *Continued*

Disease target (antigen)	Vector (source)	Group (phase)	Dose/route	Regimen (type) and interval (time)	T cell response	Antibody response (Post-Ad immunization versus pre-immunization or placebo)	Clinical Trials.gov	PMID (year)
Ebola BIVALENT EBO GP (*Zaire and Sudan ebolavirus* glycoprotein)	ChAd3 (GSK)	Healthy adults ≥18–50 (phase 1)	G1: 2.0×10^{10} vps G2: 2.0×10^{11} vps (i.m.)	Single dose	Flow cytometry $CD4^+$ Zaire D28 versus D0 G1: ~0.1% versus <0.05% G2: ~0.2% versus <0.05% $CD8^+$ Zaire D14 versus D0 G1: ~0.01% versus <0.1% G2: ~0.4% versus <0.1%	GMT (95% CI), EC_{90} Zaire GP: D28 G1: 331 versus baseline G2: 2,037 versus baseline	NCT02231866	25426834 (2017)
					$CD4^+$ Sudan D28 versus D0 G1: ~0.1% versus <0.01% G2: ~0.2% versus <0.01% $CD8^+$ Sudan D14 versus D0 G1: ~0.01% versus <0.01% G2: ~0.2% versus <0.01%	Sudan GP: D28 G1: 279 versus baseline G2: 936 versus baseline		
Ebola EBO-Z GP (*Zaire ebolavirus* glycoprotein)	ChAd3 (GSK)	Healthy adults ≥18–50 (phase 1)	1.0×10^{10} vps 2.5×10^{10} vps 5.0×10^{10} vps (i.m.)	+Boost MVA/3–10 weeks (1.5×10^8 PFUs 3×10^8 PFUs)	IFN-γ ELISpot SFU/10^6 D14 versus D0 633 versus <50 Flow cytometry D14 versus D0 $CD4^+$: 0.2% versus 0.13% $CD8^+$: 0.004% versus ?	GMT (95% CI) D28 versus rVSV-ZEBOV ChAd3 prime: 752.4 rVSV-ZEBOV: 920.7	NCT02240875	25629663 (2016)
Ebola (GP)	Ad5 (CanSino Biologic)	Healthy adults ≥18–60 years (phase 1)	4×10^{10} vps 1.6×10^{11} vps (i.m.)	Single dose or homologous prime:boost	IFN-γ ELISpot SFU/10^6	GMT (95% CI), ELISA EC_{90} Prime D28 versus D0: reported in PMID: 25817373 Prime:boost D196 versus D168: Low dose: 6,110 versus 197.9 High dose: 11,825 versus 575.5	NCT02326194 NCT02533791	28017642 (2017)
Ebola (GP)	Ad5 (CanSino Biologic)	Healthy adults ≥18–60 years (phase 1)	4×10^{10} vps 1.6×10^{11} vps (i.m.)	Single dose	IFN-γ ELISpot SFU/10^6 (median, D14) Low dose: 465 High dose: 765 Flow ICS $CD4^+/CD8^+$ IFN-γ, TNF, IL-2 increased	GMT (95% CI), ELISA EC_{90} Prime D28 versus placebo: Low dose: 682.7 versus 5 High dose: 1,305.7 versus 5	NCT02326194	25817373 (2015)

FC, fold change; GMC, geometric mean concentration; GMT, geometric mean titer; ICS, intracellular cytokine staining; IL, interleukin; IUs, infectious units; SFUs, spot forming units; vps, viral particles.

Table 3. Adenoviral vaccine clinical trials for emerging viruses

Disease target (antigen)	Vector (source)	Group (phase)	Dose/route	Regimen (type) and interval (time)	T cell response (IFN-γ ELISpot. SFU/10^6 PBMCs)	Antibody response (Post-Ad immunization versus pre-immunization or placebo)	Clinical Trials.gov ID	PMID (Year)
Zika (M + Env)	Ad26 (Johnson & Johnson)	Healthy adults ≥18–50 years (phase 1)	G1: 5×10^{10} vps G2: 1.0×10^{11} vps (i.m.)	Single dose	D15 versus D1 (Env) G1: ∼600 versus ∼83 G1: 250 versus ∼83	GMT (95% CI), MN_{50} G1: ∼40 versus <10 (D57 versus D1) G2: 103.4 versus <10 (D57 versus D1)	NCT03356561	33587687
				Homologous prime:boost	D71 versus D1 (Env) G1: ∼1,100 versus ∼83 G1: 400 versus ∼83	GMT (95% CI), MN_{50} G1: 1,065.6 versus <10 (D71 versus D1) G2: 956.6 versus <10 (D71 versus D1)		
Chikungunya (Capsid, E3, E2, 6k, E1)	ChAdOx1 (University of Oxford)	Healthy adults ≥18–50 years (phase 1)	G1: 5×10^9 vps G2: 2.5×10^{10} vps G3: 5×10^{10} vps (i.m.)	Single dose	D14 versus D0 D14: 1,031 versus 180.1 D28: 541.1 versus 180.1 D56: 398.2 versus 180.1 D182: 352.8 versus 180.1 All groups combined	GMT (95% CI), $PRNT_{50}$ G1: ∼32–256 versus <6 (D28 versus D0) G2: ∼64–384 versus <6 (D28 versus D0) G3: ∼64–384 versus <6 (D28 versus D0) Against ×4 CHIKV lineages	NCT03590392	34330906
						GMT (95% CI), ELISA units G1: 80.99 versus 4.74 (D182 versus D1) G2: 205.9 versus 4.72 (D182 versus D1) G3: 169.7 versus 3.00 (D182 versus D1) Against E2 protein		
Avian influenza (H5 HA)	Replicating Ad4 (PaxVax)	Healthy adults ≥18–40 years (phase 1)	G1: 1×10^{10} vps (oral, enteric) G2: 1×10^3 vps–1×10^8 vps (tonsillar) G3: 1×10^3vps–1×10^8 vps (i.n.)	Single dose	Flow cytometry increases in IFN-γ^+ $CD69^+$ $CD4^+$/$CD8^+$ in tonsillar and i.n. groups	Pseudovirus IC_{50} (median) G1: ∼170 versus ∼35 (W8 versus W0) G2: ∼800 versus ∼35 (W8 versus W0) G3: ∼320 versus ∼35 (W8 versus W0)	NCT01806909 NCT01443936	33529172
Avian influenza (H5 HA)	Replicating Ad4 (PaxVax)	Healthy adults ≥18–49 years (phase 1)	G1: 1×10^{10} vps (oral, enteric) G2: 1×10^3 vps–1×10^8 vps (tonsillar) G3: 1×10^3vps–1×10^8 vps (i.n.)	Single dose	Not reported in this study	Pseudovirus IC_{50} (median) G1: ∼210 versus ∼? (W8 versus W0) G2: ∼836 versus ∼? (W8 versus W0) G3: ∼352 versus ∼? (W8 versus W0)	NCT01443936	31004012

?, values not provided; HA, hemagglutinin; IC50, half-maximal inhibitory concentration; MN_{50}, microneutralization titer-50; $PRNT_{50}$, plaque reduction neutralization test-50.

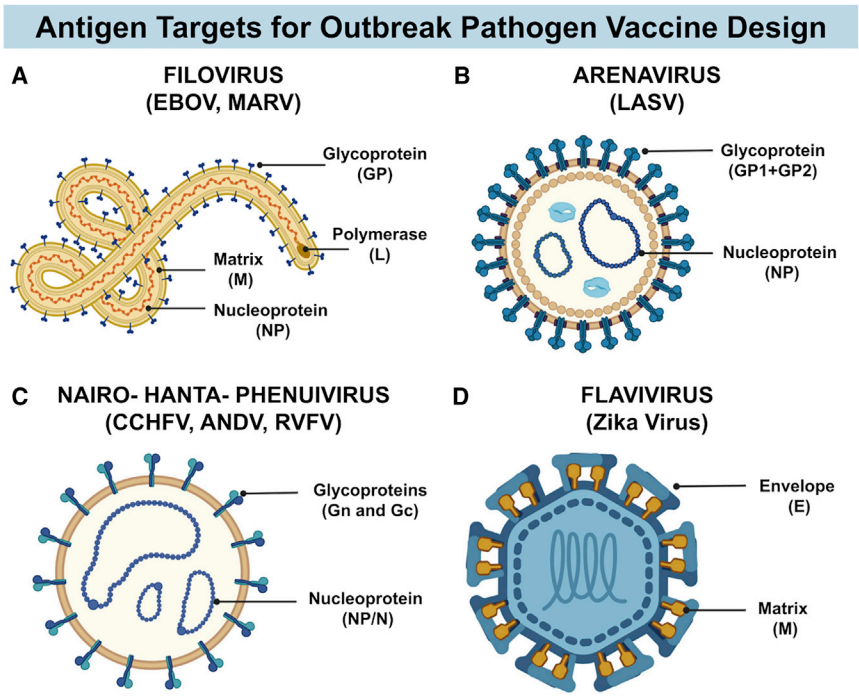

Figure 3. Schematic diagrams of the structure of several emerging viruses identified as priority pathogens by the WHO

(A) A general structure of the *Filoviridae* family, highlighting antigen targets that have been employed in vaccine design. (B) Structure of the *Arenaviridae* family, showing antigen targets for vaccine development. (C) A schematic structure for viruses from the families *Nairoviridae*, *Hantaviridae*, or *Phenuiviridae*, order *Bunyavirales*, again showing vaccine target antigens. (D) Diagram showing the general structure of Zika virus, a member of the *Flaviviridae* family, and major targets for vaccine design. Figure created with BioRender.com.

both cellular and humoral immune responses.[102] However, protective immunity waned 10 months post-immunization with a single shot of ChAd3, which may limit its use beyond emergency, reactive-use applications. However, this effect could be overcome by use of a heterologous MVA boost at week 8, which facilitated the maintenance of a high frequency of tumor necrosis factor (TNF) and IFN-γ^+ co-producing $CD8^+$ T cells at the 10-month challenge time point, which were associated with increased protection. The ChAd3 platform, as well as its use in a heterologous prime:boost regimen with MVA,[56] has now been evaluated in human clinical trials and has been found to be safe and immunogenic in children[88] and healthy adults[89,92] (Table 2).

The WHO TPP preferred criteria for Ebola virus vaccines considers platforms that confer greater than 80% efficacy in preventing disease, are suitable for use in all age groups, and can rapidly elicit immunity as well as platforms capable of targeting multiple filovirus species with a single vaccine. With these criteria in mind, Ad-based vaccines are suitable. Both Ad26 and ChAd3 platforms have been safely tested in clinical trials in children[87,88] and adults.[56,90–92] Species D Ad26 and species B Ad35 vaccines encoding GPs of diverse filoviruses have been shown to elicit cross-reactive B and T cell responses,[114] suggesting that combining GP antigens in a single vaccine could elicit broad protection. In support of this, a bivalent formulation for ChAd3 in which both the GP from EBOV and the Sudan strain (SUDV) were encoded[102] did not negatively affect protection of macaques from challenge with a lethal dose of EBOV. This vaccine candidate subsequently advanced to phase I clinical testing (Table 2).[92] More recently, a study by Sebastian and colleagues described the construction of ChAdOx1 encoding the GP from *Zaire ebolavirus*, *Sudan ebolavirus*, as well as *Marburg virus* and tested its immunogenicity in mice and efficacy following a single-shot regimen in guinea pigs.[115] GP-specific Ab responses were elicited against each distinct virus, and guinea pigs were completely protected from lethal challenge with EBOV (although protection against other strains was not confirmed). Recently, innovative technologies in computational antigen design facilitated encoding of conserved T cell epitopes, or "pan-filovirus epitopes," from NP, matrix (M), and polymerase (L) in ChAdOx1 or MVA vectors (Figure 3A).[116] In this approach, the GP was not included as an antigen and no filovirus-specific NAbs were induced. Despite this, a heterologous Ad prime:MVA boost conferred complete protection from challenge with EBOV and MARV in mice, demonstrating the breadth of protection that can be elicited toward highly conserved T cell epitopes.

Arenaviridae: Lassa virus (LASV)

LASV is an enveloped single-stranded, bisegmented, ambisense RNA virus that is a member of the order *Bunyavirales* (Figure 3B). The virus was first identified in Nigeria in 1969 as the causative agent of an acute viral hemorrhagic fever. Infection is caused by exposure to the urine or feces of infected *Mastomys* rats, and LASV infects 100,000–500,000 people annually.[95] Infection can be asymptomatic-mild in endemic areas, and as such, the true incidence is unclear. However, high mortality can be observed in hospitalized patients (15%–70%) and during the third trimester of pregnancy, where fetal loss is common and mortality can reach 90% (Table 1).[117] Long-term health effects in survivors are common, including chronic neurological complications and hearing loss. As a result of its high case fatality rate, documented reports of human-to-human transmission, the potential to cause nosocomial infections, and a history of imported cases in countries outside of West Africa, the development of an effective vaccine suitable for use in high-risk populations is a priority for global health security.

A correlate of protection for Lassa fever has not been conclusively identified. A role for cellular immunity in protection has been inferred from pre-clinical models and human survivors, where the development of NAbs has been found to be delayed or weak.[95] In

contrast, T cell activation has been associated with control of infection in NHPs.[118] Ad-based vaccines are well established to elicit potent cellular immune responses, suggesting they may be a suitable platform for protecting against Lassa fever. To date, vaccines based on Ad5 and chimpanzee Ad vector ChAdOx1 have been tested pre-clinically. Maruyama and colleagues constructed two replication-defective, Ad5-based vaccines against Lassa virus encoding the viral NP or precursor GP complex (GPC) (GP1 + GP2)—the surface GP of arenaviruses and a potential target for Abs.[119] Using a homologous prime:boost regimen, the authors sequentially immunized Hartley guinea pigs i.m. with 1×10^{10} infectious units (IUs) of Ad5-NP followed by Ad5-GPC 16 days later and challenged animals with 8×10^4 plaque-forming units (PFUs) of Lassa strain LF2384 at D40 post-immunization. Serum Abs capable of binding both NP and GP were detected in vaccinated animals, but NAbs prior to challenge were low (plaque reduction neutralization test-50 [$PRNT_{50}$]: 1:10) and were only observed in three out of eight animals. Despite this, all Ad-immunized animals completely survived the challenge and LASV was not detected in the brain, lung, liver, spleen, kidney, or serum, whereas animals immunized with a control Ad succumbed to disease. The authors hypothesized that non-neutralizing anti-NP or anti-GPC Abs capable of engaging Fc-mediated effector functions might contribute to protection, as this mechanism was proposed as a novel correlate of protection in another study.[6] However, the latter role was not formally investigated in the Maruyama study, nor were antigen-specific T cell responses.

A more recent study described the construction of a ChAdOx1 vaccine against Lassa fever.[120] Again, the LASV GPC antigen was selected and immunogenicity was evaluated in a single-shot or homologous prime:-boost regimen in mice, followed by efficacy testing in Hartley guinea pigs using 1×10^5 median tissue culture infectious dose ($TCID_{50}$) of a guinea-pig-adapted Josiah strain LASV challenge virus. Mice were immunized i.m. with 1×10^8 IUs, and when a boost was administered, the same dose was used with a 28-day interval (D28). T cell responses to both the encoded lineage IV GPC (Josiah strain), as well as cross-reactive responses toward three heterologous strains from lineages I–III, were measured by ELISpot and flow cytometry, with predominantly $CD8^+$>$CD4^+$ responses detected. Similarly, immunization with ChAdOx1-GPC resulted in breadth of reactivity against lineage I–III GPs by ELISA. Interestingly, no benefit of homologous boosting was observed in mice, with comparable levels of T cells or Abs following the single-shot or prime:boost regimen. In contrast, an increase in GP-specific Abs was detected in guinea pigs that received the homologous prime:boost. In support of prior evidence that suggested that NAbs are not required for protection against Lassa fever, the ChAdOx-GPC vaccine did not elicit NAb responses, but guinea pigs were 100% protected from clinical disease. Although complete sterilizing protection was not achieved, only very low levels of LASV RNA were detected in the tissues of vaccinated animals.

The WHO TTP for a vaccine against Lassa virus prioritizes non-emergency preventative use, which could be used in endemic regions and would be suitable for use in healthcare workers and pregnant people. Ideally, this vaccine should provide >90% efficacy in preventing infection or disease, be a single-shot vaccine, elicit breadth against four Lassa virus lineages (I–IV), and confer long-lasting, durable protection. An additional consideration is the possibility to co-administer this vaccine with other vaccines licensed for the same age and population groups without any negative impact on immunogenicity or safety, particularly in the context of co-infection with malaria, Ebola, or HIV.[119] The general properties of Ad vaccines fulfill many of these criteria: there are reports of a single-shot immunization in animal models conferring protection[121] and Ad-based vaccines can elicit immune responses with substantial breadth[121] (with evidence of prolonged somatic hypermutation),[122] they can stimulate long-lived immunity in animals[123] and humans,[124,125] and they have already been safely co-administered with routine Expanded Program on Immunization (EPI) vaccines without affecting their immunogenicity.[79] The Coalition for Epidemic Preparedness Innovations (CEPI) (https://cepi.net) is currently supporting the development of a ChAdOx1-based vaccine against Lassa fever in partnership with University of Oxford and Janssen Vaccines & Prevention.[126] It is likely that this vaccine will enter phase I clinical trials in the near future.

Nairoviridae: Crimean-Congo hemorrhagic fever virus (CCHFV)

Crimean-Congo hemorrhagic fever (CCHF) is an acute viral infection caused by CCHFV and transmitted by Ixodid ticks, primarily of the *Hyalomma* genus. The virus belongs to the order *Bunyavirales* and is enveloped with a tri-segmented, negative-sense RNA viral genome (Figure 3C). There is growing concern regarding increasing reports of imported cases, expanding endemic regions, and broadening geographic distribution of the tick vector due to climate change, habitat disruption, or bird migration.[127,128] The pathogen has a wide host range, and humans can become exposed through tick bites or by exposure to body fluids from viremic livestock or humans. Outbreaks in hospital settings have also been reported.[129] The high mortality (4%–40%) and a lack of licensed vaccine or treatment highlights the urgency for vaccine development (Table 1). However, to date, this has been hampered by limited availability of immunocompetent models to fully evaluate vaccine efficacy and a lack of information regarding correlates of protection. Furthermore, differences in the ability of distinct vaccine platforms delivering CCHFV antigens to confer protection have been reported,[130–132] suggesting that a specific phenotype of immunity may be preferential (i.e., particular immunoglobulin G [IgG] subclass or phenotype of antigen-specific T cell) or that an effective design approach should consider targeting multiple antigens simultaneously.

Pre-clinical studies with diverse vaccine platforms have indicated that the surface glycoproteins (Gn and Gc) or the nucleocapsid (N) are attractive targets for CCHFV vaccine design. In particular, N is highly conserved between CCHFV strains[133] and it is reported to be immunogenic during infection, and vaccines encoding N developed against other members of the order *Bunyavirales* have been protective.[132,134] As such, Zivcec and colleagues evaluated the immunogenicity and efficacy of an Ad5-based vaccine encoding N in $IFNAR^{-/-}$ mice, administered as a single-shot or homologous prime:boost regimen.

Mice were immunized i.m. with 1.25×10^7 IUs Ad5-N and boosted on D28 with 1×10^8 IUs of the same construct administered i.n., in an effort to bypass anti-vector immunity. Four weeks later, mice were challenged with 1,000 lethal dose 50 (LD_{50}) CCHFV administered subcutaneously. Although anti-N IgG responses were detected in immunized animals, the single-shot regimen only provided partial protection from lethal challenge (33%), and the prime:boost regimen resulted in 78% survival. However, viremia was substantially reduced, and viral load and infectious titer in the liver and spleen were decreased in the prime:boost regimen. Considering that these experiments were performed in an immunocompromised IFN-signaling-deficient $IFNAR^{-/-}$ mouse model, the partial protection observed in this study supports the rationale for inclusion of N in a vaccine candidate for CCHFV. Recently, structural insights into the Gc trimer[135] (the only known target for NAbs)[136] or the secreted glycoprotein GP38[137] have highlighted their potential as vaccine targets that could be incorporated into Ad vaccines.

Hantaviridae: Sin Nombre virus (SNV) and Andes virus (ANDV)

The *Hantaviridae* family, order *Bunyavirales*, contains a number of viruses that cause diseases manifesting in vascular leakage: hantavirus fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). The virus is enveloped with a tripartite, negative-sense RNA genome (Figure 3C). Transmitted primarily by *Peromyscus maniculatus* (deer mice), infection occurs following exposure to infected rodents or by inhalation of aerosolized infectious material from rodent urine, droppings, or body fluids. Two strains, Sin Nombre virus (SNV) and Andes virus (ANDV), cause the most severe disease. Lethality ranges from 0.1%–15% for HFRS and up to 40%–50% for HPS (Table 1).[138,139] No licensed vaccine currently exists. The long incubation period, along with reports of human-to-human transmission during outbreaks of ANDV, are of increasing concern.[140] The precise correlates of protection from infection have not been conclusively identified. As for LASV and CCHFV, the surface GP precursor GPC, which is co-translationally cleaved into the Gn and Gc envelope proteins, is considered to be an important target for protective immunity. However, the multifunctional nucleoprotein (NP) can also elicit cellular and humoral immune responses and, as such, may represent an additional antigen target.

With this in mind, Maeda and Safronetz et al. engineered Ad5-based vaccines encoding SNV or ANDV N antigen.[134,141] Ad encoding SNV N elicited high levels of antigen-specific IFN-γ-producing T cells, which were superior to plasmid DNA or MVA vaccines encoding the same antigen.[141] In addition to N, Safronetz and colleagues also constructed Ads encoding -Gn or -Gc glycoproteins as separate Ad vaccines. Using mice to evaluate immunogenicity and a relevant Syrian hamster animal model to model HPS in humans,[134] the authors demonstrated that, when administered intraperitoneally (i.p.) as a single vaccine (1×10^8 PFUs), all vectors elicited detectable cellular and humoral immune responses that were capable of protecting hamsters from clinical disease. Interestingly, the authors determined that Ad5-N could completely protect animals from challenge despite a lack of NAbs. In fact, NAbs were not readily detected for any vaccine regimen, yet animals were completely protected from mortality. There was an association with increased control of ANDV replication following challenge in hamsters when immunized with Ad5-Gn > Ad5-Gc. When Ad5-Gn and Ad5-Gc were co-administered, ANDV RNA was undetectable in challenged animals.

Phenuiviridae: Rift Valley fever virus (RVFV)

First identified in the 1930s, RVFV is an arthropod-borne viral zoonosis that can be transmitted by multiple mosquito species. It has largely affected sub-Saharan Africa to date but is expanding geographically, with outbreaks spreading to the Arabian Peninsula and Madagascar. A member of *Phenuiviridae* family, order *Bunyavirales*, its structure is similar to CCHFV: it possesses an envelope and contains a tripartite, ambisense, negative-sense RNA viral genome (Figure 3C). RVFV infection predominantly affects ruminants with high rates of mortality, and it is responsible for mass spontaneous abortions and neonatal mortality, with substantial economic impact. The finding that RVFV can infect placental tissue[142] has raised concerns that infection may also be associated with risk of miscarriage in human pregnancy.[143] Infection of humans can be as a result of contact with infected animals or through mosquito bites during high density circulation in animals.[144] Clinical symptoms are wide ranging but can be severe, resulting in hemorrhagic fever with mortality rates of up to 35% in hospitalized patients (Table 1).[144] It is important to note that this virus also has biosecurity implications due to the fact that it can be lethal in aerosolized form, and thus, it represents a threat for bioterrorism.[144] There are no licensed vaccines for human use, and veterinary vaccines have been associated with some safety issues (i.e., fetal malformations and stillbirths) and are deemed unsafe for use in humans.[145] As such, a one-health approach for safe and effective vaccine development would be a worthy consideration.

NAbs are considered to be crucial for conferring sterilizing protection, in particular, NAbs directed toward the viral glycoproteins.[146,147] NAbs display a predominance in responses to Gn > Gc in recovered humans.[146] T cell responses to the viral glycoproteins have also been detected in RVFV-recovered patients. In 2009, Holman and colleagues evaluated an Ad5-based vaccine encoding Gc and Gc genes from RVFV in CD1 mice.[144] Animals were immunized i.p. with 1×10^8 PFUs, and some animals were administered with a homologous boost with the same dose at week 10 (W10). Serum Ab titers (ELISA) against Gn/Gc were detected 2 weeks post-immunization, and immunized mice were 100% protected from lethal challenge with 100 PFU ZH501 strain of RVFV 11 weeks post-immunization. Boosting of Gn- and Gc-specific Ab responses was detected upon homologous boosting with Ad5-GnGc, with Ab titers sustained out to week 26 post-immunization and complete protection from challenge at week 27. In the context of prior immunity to Ad5, the authors demonstrated that pre-existing immunity had a negative impact on Ab titers and survival when a low-dose Ad5-GnGc was used (10^6 PFUs), although this could be largely overcome by increasing the dose of vaccine used to immunize (10^8 PFUs).

Subsequent pre-clinical studies have evaluated ChAdOx1 as a vaccine against RVFV. A head-to-head comparison of immunogenicity and efficacy was described for i.m. immunization with 1×10^8 IUs of ChAdOx1 or Ad5 encoding Gn and Gc.[148] In addition, the effect of co-administration with commercial adjuvants AddaVax and Matrix-M was evaluated. As previously observed, humoral immune responses elicited by Ad5 were superior to ChAdOx1,[112,149] with higher NAb titers detected in Ad5-GnGc-immunized mice. However, adjuvants enhanced NAb responses elicited by ChAdOx1-GnGc, but not Ad5-GnGc. In contrast, AddaVax (but not Matrix-M) enhanced $CD8^+$ IFN-γ^+ and TNF responses in Ad5-GnGc-immunized mice but had no effect on the cytokine profile elicited by ChAdOx1-GnGc. These differences highlight that distinct, underlying mechanisms likely contribute to the induction of humoral or cellular immunity induced by these Ad platforms.[10] Despite differences, both platforms conferred 100% protection from challenge with 1×10^3 PFUs of RVFV strain 56/74 administered i.p.

Considering that a one-health approach is an appealing strategy for a vaccine against RVFV, studies have shown that the ChAdOx1-GnGc vaccine can elicit protective immunity in sheep, goats, and cattle[150] and, importantly, in pregnant sheep and goats.[145] A single-shot i.m. immunization with 1×10^9 IUs elicited NAb responses in all three species and conferred 100% protection from challenge with no detectable viremia. In pregnant ruminants immunized in the first trimester, the vaccine was shown to elicit robust NAb titers, provide protection against viremia, and prevent fetal loss, although the latter was incomplete in goats, with 2 out of 23 fetuses found to be autolyzed (1/5 and 1/3 in two does with multi-fetal pregnancies). Interestingly, NAb titers were higher in goats with fetal loss as compared with sheep where no fetal loss was observed, suggesting that species-specific differences in mechanisms of *in utero* infection or the phenotype of protective immunity may play a role in vaccine efficacy.

The TPP for vaccines against RVFV include three options: (1) a human vaccine for reactive, emergency use to be deployed during outbreaks and in regions in close proximity to outbreaks that is safe for use during pregnancy; (2) a vaccine that could confer longer term protection for individuals with high risk of infection due to their occupation (i.e., slaughterhouse workers, veterinarians, and farmers); and (3) a vaccine suitable for use in ruminants that could prevent transmission between animals and humans. The latter TPP should be affordable, suitable for use in pregnant animals, independent of cold-chain storage requirements, and compatible with differentiating infected from vaccinated animals (DIVA) principles. In terms of a vaccine for humans, the optimal criteria include at least 90% efficacy in preventing disease, rapid onset of immunity (2 weeks), the ability to confer protection against all RVFV lineages for at least 1 year following a single-dose regimen, and suitability for co-administration with other relevant vaccines. Considering the demonstrated immunogenicity and efficacy of Ad vaccines in diverse animal species, in addition to their now extensively documented use in humans, Ad-based platforms would be well suited to future vaccine development for RVFV and other "one-health" vaccine applications.

Flaviviridae: Zika virus (ZIKV)

ZIKV was discovered in Africa in 1947 and subsequently in Asia in 1966.[151] Major outbreaks occurred in the Pacific between 2007 and 2015, with a substantial outbreak in the Americas in 2016, resulting in spread to over 70 countries and its declaration as a public health emergency of international concern by the WHO. Transmitted by infected mosquitoes of the *Aedes* species, the majority of cases are asymptomatic, but the virus can cause a spectrum of fetal and birth defects collectively known as congenital Zika syndrome, and infection has been associated with Guillain-Barré syndrome (GBS). ZIKV is an enveloped, positive-sense RNA virus in the family *Flaviviridae* and order *Amarillovirales* (Figure 3D). The family includes other viruses that can cause hemorrhagic fever or encephalitis, such as Dengue virus (DENV), West Nile virus (WNV), or Japanese encephalitis virus (JEV). In 2016, a dramatic increase in cases of microcephaly and other congenital or neurological disorders was associated with infection with ZIKV during pregnancy in Brazil. As the arthropod vector, *Aedes* mosquitoes, has a broad geographic distribution, there is concern that ZIKV could spread to the northern hemisphere (Table 1). As such, the development of an effective vaccine that is safe for use in individuals of child-bearing age or in pregnant people is a public-health priority.

Antigens that have been evaluated as vaccine targets for ZIKV include the pre-membrane (prM) or envelope (E) proteins that are exposed on the surface of the virion (Figure 2D). In late 2016, Abbink and colleagues described the construction of a species G simian Ad vaccine platform, RhAd52,[152] encoding ZIKV prM-Env.[153] A single-shot regimen was tested in rhesus monkeys following i.m. immunization with 1×10^{11} vps. The Ad vaccine rapidly induced ZIKV Env-specific NAbs 2 weeks post-immunization with broad epitope recognition, along with Env-specific T cell responses. Importantly, 100% protection from complete protection against subcutaneous (s.c.) challenge with 10^3 PFUs of ZIKV-BR was observed. Subsequently, an Ad26-based vaccine, a species D Ad vector encoding membrane (M) and Env was evaluated in mice and NHP. In both species, Env binding and NAbs were detected and a single-shot low dose of Ad26 (4×10^7 vps) was capable of providing complete protection from challenge in mice. In NHP, a comparable human dose of 1×10^{11} vps elicited robust protective immune responses and conferred complete protection from viremia. The latter vaccine, Ad26.ZIKV.001, has recently been evaluated in phase I clinical trials (NCT03356561), where it was tested in a single-shot or homologous prime:boost regimen (Table 3). Eight weeks following the prime immunization, homologous boosting of both NAbs and IFN-γ^+ ELISpot responses were detected.[58]

Several other approaches have included vaccines based on Ad5 and ChAdOx1, in which the encoded antigen formulation was modified to identify the optimal transgene cassette. A study by Kim and colleagues encoded the extracellular portion of E, in which the transmembrane domain (TM) was removed and replaced with a heterologous trimerization domain from T4 bacteriophage, fibritin foldon.[105] This modified antigen was encoded within Ad5 and used to immunize mice s.c. with 1×10^{11} vps, with a homologous vector boost

administered via i.n. or intradermal (i.d.) route on D14 post-prime. As described for other Ad platforms,[104,112] rapid induction of ZIKV-specific Abs was detected 2 weeks post-immunization, and NAbs were high 1 month following the boost immunization. Interestingly, this study evaluated protection from disease in ZIKV-challenged pups born to immunized mice. Complete survival was observed in pups from immunized mice versus 12.5% survival pups from PBS-immunized mothers. Furthermore, in the Ad-immunized groups, pups displayed only mild to no symptoms of neurological disease (i.e., hindlimb paralysis), whereas all pups of PBS-immunized dams had neurological disease symptoms.

Although prior studies with DNA-based vaccines identified the optimal prM-Env cassette for use in RhAd52-prM-Env (which retains the TM domain of Env), a separate study determined that prM-EnvΔTM was the optimal antigen configuration when used in ChAdOx1.[154] ChAdOx1-based vaccines, with various iterations of the prM-Env cassette, were tested i.m. at a dose of 1×10^8 IUs in mice. ChAdOx1-prM-EnvΔTM elicited NAbs that were maintained for 16 weeks following a single shot, and immunization conferred 100% protection from challenge. In a more recent study, the authors evaluated the same ChAdOx1 vaccine platform, encoding the envelope protein domain III (EDIII) as a sole antigen, on the basis that this domain has previously been reported to be an effective immunogen for other flaviviruses.[155] However, despite inducing anti-ENV Abs, NAbs were not elicited and the vaccine candidate failed to control viremia or completely protect against challenge in two mouse models,[156] suggesting that EDIII is not an optimal vaccine target for protection against ZIKV.

The WHO TPP for a priority vaccine against ZIKV is one that could be used predominantly in an outbreak response, with the main objective being the prevention of pre-natal ZIKV infection and in prevention of clinical illness, namely congenital malformations or complications in pregnancy. The ideal vaccine would be expected to prevent virologically confirmed disease in >80% of the population in a single-dose formulation using a non-replicating platform and should be capable of neutralizing both the Asian and African ZIKV lineages. Additional considerations include suitability for co-administration with other appropriate licensed vaccines (i.e., the WHO EPI program), manufacturing processes in place for rapid scale up, affordability, and shelf-life stability, allowing cold-chain free distribution. Although NAbs are considered to be an important correlate of protection, there is growing appreciation that $CD8^+$ T cells might also contribute to protection.[157,158] Ad-based vaccines are known to elicit potent $CD8^+$ responses[10,159,160] in addition to Ab and, as previously stated, can exhibit breadth of reactivity. Furthermore, the platform fulfills requirements for co-administration with EPI vaccines[79] and the capacity for rapid scale up to meet demand during outbreak scenarios.

EVIDENCE FOR THE SAFETY OF ADENOVIRAL VACCINES

The urgent need for rapid-response vaccines to curtail the global spread of SARS-CoV-2 put unprecedented pressure on the pharmaceutical industry to develop and test vaccine candidates that would provide protection against disease severity and death while simultaneously demonstrating safety in vaccine recipients. Building upon existing blueprints from Ad vaccines to combat HIV,[76,161] EBOV,[87,91,162,163] influenza virus,[75,94,122] respiratory syncytial virus (RSV),[164,165] and Middle Eastern respiratory syndrome coronavirus (MERS-CoV),[166] which had already been evaluated clinically, vaccines based on Ad5, Ad26, and ChAdOx1 were constructed, manufactured, and rapidly advanced to safety and efficacy studies in early 2020. Several reviews describing the immunogenicity and efficacy of Ad-based vaccines against SARS-CoV-2 have been published[23,86] and will not be covered in detail in this review. However, as global-scale evaluation of Ad vaccines has provided a wealth of information regarding the clinical safety profile of distinct Ad-based vectors, we will summarize the latter findings, as they will inform the design of next-generation Ad vaccine platforms for emerging infectious diseases.

Safety data for chimpanzee Ad vector ChAdOx1

The rationale for use of ChAdOx1 as a vaccine against SARS-CoV-2 was based on its low seroprevalence in humans,[167] its prior evaluation in phase I clinical trials as a vaccine for other viral pathogens,[55,59,93] and promising findings in animal models for a ChAdOx1-based vaccine against a related coronavirus, MERS.[168–170] Initial findings from interim analyses of phase I/II and later phase III clinical trials for Ad-vectored SARS-CoV-2 vaccines reported good tolerability and a lack of serious adverse events (SAEs) related to vaccine administration. Most importantly, these vaccines also provided near-complete protection from death, with significant reductions in the severity of disease and the need for hospitalization. The FDA recommends a toxicity grading scale for measuring adverse events in healthy adults (https://www.fda.gov/media/73679/download), ranging from mild (grade 1), to moderate (grade 2), to severe (grade 3), or potentially life-threatening (grade 4). A preliminary report of the data collected from a single-blind, randomized, controlled phase I/II clinical trial of the AZD1222 SARS-CoV-2 vaccine, based on ChAdOx1 (manufactured by AstraZeneca) showed that both single- and two-dose (28-day interval) vaccine regimens administered i.m. at a dose of 5×10^{10} vps were well tolerated,[77] with a profile of adverse reactions similar to prior reports for Ad vaccines (ClinicalTrials.Gov ID: NCT04324606). In this relatively small study, 534 participants were administered with the ChAdOx1 nCoV-19 vaccine, and 533 participants were administered with the meningococcal conjugate vaccine, MenACWY vaccine.[77] Solicited local and systemic adverse reactions were recorded at day 3, 7, 14, 28, and 56 post-vaccination, with a follow-up evaluation for safety and efficacy on days 184 and 364. Among solicited local adverse responses recorded during the first 7 days post-vaccination, the most common were mild tenderness, which was reported by 83% of participants, and pain at the injection site (reported by 67% of participants). Mild-to-moderate fatigue (70% of participants), headache (68%), malaise (61%), and muscle ache (60%), followed by chills and feeling feverish, were among the most common systemic adverse reactions reported within 7 days of ChAdOx1 vaccine administration.[77] In a two-dose regimen, where prime

vaccine administration was followed by a boost 28 days later, mild-to-moderate pain and tenderness remained the most common local adverse reaction, while headache, feeling feverish, chills, malaise, and muscle pain were reported as the most common systemic adverse reactions, similar to participants who received only a single dose of the vaccine. In a two-dose cohort, it was noticed that the reactogenicity profile (or the severity of adverse reactions) after administration of a booster dose of the vaccine was less severe, as compared with the severity of adverse reactions after the prime dose administration.

In a recent report of data collected from the ongoing pivotal double-blind, placebo-controlled phase III study, the safety and efficacy of AZD1222 was evaluated in 21,587 participants who received AZD1222 in a prime:boost regimen and in 10,792 participants who received placebo (NCT04516746).[171] Unsolicited adverse events (AEs) were recorded for a duration of 28 days after each dose of vaccine or placebo, while solicited local and systemic AEs were monitored for 7 days post-administration of vaccine or placebo. This study evaluated the safety and efficacy of a vaccine dose of 5×10^{10} vps following i.m. administration, with a 4-week interval between prime and boost immunizations. Participants were also stratified by age into those who were 18–65 years old and those who were over 65 years of age. The majority of participants in this study had comorbidities that are known to increase coronavirus disease 2019 (COVID-19) disease severity, including a history of obesity, type 1 and type 2 diabetes, high blood pressure, and history of smoking, among others. Similar to findings from earlier trials, the majority of solicited local AEs were mild to moderate in intensity, with tenderness (68.7%) and pain at the injection site (58.3%). Upon analysis of systemic AEs, in addition to mild and moderate fatigue, muscle pain, and headache observed in earlier clinical trials with AZD1222, in this larger trial, severe fatigue, muscle pain, headache, and malaise were observed in a subset of participants aged 18–65 years after the first vaccine dose.[171] However, the majority of local and systemic AEs were self-limiting and resolved within 1 to 2 days after the onset.

The analysis of vaccine reactogenicity in this larger cohort of participants revealed a spectrum of rare unsolicited AEs, which were observed within 28 days after vaccine administration. Out of 21,587 participants who received at least one dose of the vaccine, 225 participants reported AEs of grade 3 or higher, 1,288 participants (6%) experienced medically attended AEs, and AEs of special interest observed in 58 participants were judged to be related to trial intervention. However, it is important to note that grade 3 AEs, medically attended AEs, and AEs of special interest were also observed in participants receiving the placebo at similar frequencies. Furthermore, the absolute majority of various types of medically attended AEs were experienced only by a single patient and were observed in both the vaccine and placebo arms, making formal association of each particular type of AE with vaccine reactogenicity impossible. Vaccine reactogenicity was stronger after the first administration, compared with a subsequent boost dose, and was less severe in participants over 65 years of age, compared with vaccines from the 18–65 years old group. Overall, this and other clinical trials that analyzed the safety and efficacy of AZD1222 vaccine concluded that the vaccine was safe and effective at preventing symptomatic and severe COVID-19.[172,173]

Safety data for species D Ad26 vectors

Similar to ChAdOx1, the rationale for use of Ad26 as a vaccine platform for SARS-CoV-2 was based on its low seroprevalence in humans,[152,174] its established use in clinical trials,[76,157,161,164,165] and its prior approval by the EMA as a component in a vaccine against EBOV.[82,87,90,91] The safety and efficacy of a single-dose COVID-19 vaccine Ad26.COV2.S, based on rare human adenovirus Ad26 (developed and distributed by Janssen and Johnson & Johnson), was evaluated in a series of randomized, placebo-controlled clinical trials. Initial studies analyzed two different doses of the vaccine as well as one- or two-dose regimens, where vaccines were administered 56 days apart (NCT04436276).[175] In the report of the interim results of a phase I/IIa trial of Ad26.COV2.S vaccine, the reactogenicity of vaccine doses of 5×10^{10} vps (low dose, 323 participants) and 1×10^{11} vps (high dose, 319 participants) was compared with placebo (163 participants). In this trial, participants were also stratified by age into those 18–55 years old and those who were 65 years and older. Similar to findings reported upon analysis of reactogenicity for the AZD1222 vaccine, administration of the Ad26.COV2.S vaccine triggered transient, self-limiting AEs, with the majority recorded as grade 1 and 2 in severity.[175] Pain at the injection site was the most frequently reported local AE after Ad26.COV2.S administration, whereas fatigue, headache, myalgia, and nausea were the most frequent systemic AEs reported by the participants in both the low- and the high-dose groups. In this study, participants of 18–55 years of age reported fever as a frequent solicited AE. In this age group, 15% of participants in the low-vaccine-dose group and 39% of participants in the high-dose group reported grade 1 and 2 fevers. Grade 3 fever was reported by 5% and 9% of participants after receiving low and high vaccine doses, respectively. In a group of 65 years and older, grade 3 fever was not observed in participants who received low dose of the vaccine and was observed in 1% of participants who received high vaccine dose. After the second dose of the vaccine, no grade 3 fever was observed in any of the groups and there was no participant discontinuation due to an AE.[175]

In a subsequent randomized, double-blind, placebo-controlled phase III clinical trial of the Ad26.COV2.S COVID-19 vaccine administered at a single dose of 5×10^{10} vps, the safety and efficacy was evaluated in 19,630 participants who received the vaccine and in 19,691 participants who received placebo (NCT04505722).[57] In this study, participants were also stratified by age into two groups: 18–59 years old and 60 years and older. Similar to earlier studies, in this trial, solicited local and systemic AEs were recorded for 7 days and unsolicited AEs were observed and recorded for 28 days after vaccine administration. While unsolicited AEs were monitored in all participants, solicited AEs were monitored in subpopulations that included 3,356 participants who received the vaccine and 3,380 participants who received placebo. In the vaccine group, pain at the injection site was reported by 48.6% of participants and was the most commonly observed local solicited AE. Consistently with earlier observations, headache

(reported by 38.9% of participants), fatigue (38.2%), myalgia (33.2%), and nausea (14.2%) were the most common systemic solicited AEs in participants who received the vaccine. Twenty out of 21,895 participants who received the vaccine and 11 out of 21,888 participants who received placebo reported unsolicited AEs of grade 3 or higher, which were considered to be related to the intervention.[57] Although an imbalance in the number of unsolicited AEs between vaccine and placebo groups was noted, the majority of these events were only observed in a single patient, making definitive conclusion regarding association of the event with vaccine administration impossible. Similar to findings for the AZD1222 vaccine, the reactogenicity of a single dose of the Ad26.COV2.S vaccine was less pronounced in participants aged 60 years and older, as compared with a cohort of participants 18–59 years old. In summary, a single dose of the Ad26.COV2.S vaccine was found to be well tolerated and safe in humans.

Safety of species C Ad5 vectors

Vaccines to combat COVID-19 based on the common human adenovirus serotype, Ad5, were developed (CanSino Biologics) and evaluated for safety and efficacy in China (NCT04313127).[176] In a phase I dose-escalation, open-label, non-randomized clinical trial, reactogenicity was evaluated in three cohorts of participants who received 5×10^{10} vps (low-dose cohort, 36 participants), 1×10^{11} vps (middle-dose cohort, 36 participants), and 1.5×10^{11} vps (high-dose cohort, 36 participants).[176] Although the frequency of all reported AEs was similar in all cohorts, grade 3 AEs were more common in participants who received the highest vaccine dose. While pain at the injection site was the most frequent local AE, observed in 47% of participants in the low-dose, 56% of participants in the middle-dose, and 58% of participants in the high-dose cohorts, fever, followed by headache, fatigue, and muscle pain, was the most frequently recorded systemic AEs. Specifically, fever was observed in 42% of participants who received the low, 42% who received the middle, and 56% of participants who received the high vaccine dose. In this trial, grade 3 fever was observed in 14% of participants who received the high dose of the vaccine, while 6% of participants who received low and middle vaccine doses reported grade 3 fever. Overall, the reactogenicity of the Ad5-based vaccine was judged to be dose dependent, and subsequent phase II and III clinical trials were initiated with only low and middle doses of the vaccine.

In the subsequent randomized, double-blind, placebo-controlled phase II clinical trial, the Ad5-based COVID-19 vaccine was administered at a dose of 5×10^{10} vps (129 participants) or 1×10^{11} vps (253 participants), and vaccine reactogenicity was compared with placebo (126 participants). In this larger trial, the dose-dependent increase in the severity of AEs was documented and determined to be highly statistically significant (NCT04341389).[176] Specifically, while the majority of reported adverse reactions were mild to moderate in severity, grade 3 adverse reactions were noted in 9% of participants who received vaccine dose of 1×10^{11} vps, which was significantly higher than in participants who received 5×10^{10} vps of the vaccine (1% of participants; $p = 0.0011$) or placebo (0% participants; $p = 0.0004$). Similar to findings in the phase I trial described above (NCT04313127),[177] fever was the most frequently reported grade 3 adverse reaction, while fatigue was reported as the most frequent systemic adverse reaction: observed in 42% of participants who received 1×10^{11} vps vaccine dose and in 34% of participants who received 5×10^{10} vps dose of the vaccine. In this study, it was noted that high levels of pre-existing anti-Ad5 immunity, older age, and male sex were associated with a significantly lower frequency of fever after vaccination. All grade 3 reactions resolved within 72–96 h without medical intervention. No differences in the occurrence of unsolicited AEs between vaccine and placebo groups were noted during the 14-day observation period post-immunization with either vaccine or placebo. No SAEs were observed within 28 days of vaccination in this trial.[176] Data from an ongoing placebo-controlled phase III clinical trial of this Ad5-based COVID-19 vaccine in 20,000 participants who will receive a single dose of vaccine or placebo are due for reporting in early 2022 (NCT04526990).

Safety of heterologous prime:boost with Ad26 and Ad5 vectors

A heterologous Ad vaccine regimen for COVID-19, Gam-COVID-Vac (Sputnik V), was developed, tested, and approved for use in Russia.[178] Gam-COVID-Vac consists of a replication-deficient Ad26 vector, which is used for the prime, and an Ad5-based vector used for the boost. Gam-COVID-Vac is administered at a dose of 1×10^{11} vps for each vector component, and the prime and boost vaccine doses are administered 21 days apart. The largest datasets on the safety and efficacy of this vaccine were reported upon interim analysis of findings from a randomized, double-blind, placebo-controlled phase III clinical trial. In this study, 16,427 participants received one dose of the vaccine and were monitored for AEs and SAEs, with the nature and frequency of AEs and SAEs compared with 5,435 participants who received placebo (NCT04530396).[178] From 16,427 participants who received the prime dose of the vaccine, 14,964 participants subsequently received a second, boost vaccine dose. AEs were recorded during observational visits on days 28, 42, and 180 post-immunization. The most frequent systemic AEs reported by participants were flu-like illness, headache, and asthenia. Most of the reported AEs were grade 1 and 2 in severity. Grade 3 AEs were observed in 0.38% of the participants. Severe AEs were observed in 0.274% of participants who received the vaccine and in 0.423% of participants who received placebo. None of the severe AEs were considered to be associated with vaccination in this trial.[178]

Detailed information on the reactogenicity of Gam-COVID-Vac was recently reported by Babamahmoodi and colleagues.[179] The authors analyzed side effects and immunogenicity following administration of the Gam-COVID-Vac vaccine in 13,435 healthcare workers in Iran.[179] This observational study reported solicited AE data collected from vaccinated participants during the first 8 days post-administration of each of the vaccine doses, in which 3,236 out of 13,435 participants reported AEs post-vaccination. Pain at the injection site was the most frequently reported local AE, reported by 58.2% of respondents after receiving the first dose of the vaccine and by 54.1% of respondents after receiving the second vaccine dose. For vaccinees who reported systemic AEs, the most frequently reported were fatigue (reported by 54.2% after the first and by 44% after the second vaccine doses), body pain (48.6% after the first and 38% after the second vaccine

dose), weakness (46.6% after the first and 38.1% after the second vaccine doses), headache (38.1% after the first and 30.8% after the second vaccine dose), and fever (36.5% after the first and 23.8% after the second vaccine dose). The majority of these self-reported AEs subsided within 3 days after the onset, less than 10% of the events lasted 3–7 days, and about 3% of events lasted more than 7 days following either the first or the second vaccine doses.[179] Overall, these data are in line with findings from an observational study by Pagotto et al.,[180] who reported AEs observed within 72 h of administration of Gam-COVID-Vac vaccine to 707 healthcare workers in Argentina. Out of 683 participants who responded to an AE questionnaire, 57% reported pain at the injection site. Among systemic AEs observed after the first dose of the vaccine, 40% reported fever, 33% reported headache, and 20% reported muscle pain, all of which were mild in severity. However, in this group of vaccinees, muscle pain was reported by 27% of responders as moderate, 10% as severe, and 1% as grade 4. However, because this observational study was small and did not include a placebo group, a definitive conclusion on the association of these severe AEs with vaccine administration could not be drawn.

Safety concerns and considerations for future use of Ad-based vaccines: Vaccine-induced immune thrombotic thrombocytopenia

Although Ad-based vaccines displayed good tolerability and safety in clinical trials that included tens of thousands of participants, administering Ad26.COV2.S and ChAdOx1 (but not Ad5) vaccines to millions of people revealed some extremely rare serious AEs that were suspected to be causally associated with vaccination. On April 9, 2021, two reports published in the *New England Journal of Medicine* documented cases of unusual thrombosis and thrombocytopenia in recipients of the ChAdOx1 nCov-19 vaccine in Norway[181] and in Germany and Austria.[182] The report from Norway reported cerebral venous thrombosis (CVT) with thrombocytopenia, observed in 5 out of 132,686 recipients of the ChAdOx1 nCov-19 vaccine. For patients who developed this complication, the time from vaccine administration to hospital admission ranged from 7 to 10 days. All patients had high levels of anti-platelet factor 4 (PF4) Abs in the blood.[181] Similarly, high levels of anti-PF4 Abs were found in the blood of 11 patients who developed thrombosis and thrombocytopenia after vaccination with the ChAdOx1 nCov-19 vaccine in Germany and Austria.[182] The symptom onset for the latter group of patients ranged from 5 to 13 days after receiving the first dose of the vaccine. Based on the unusual clinical presentation and suspected association with vaccine administration, the syndrome was named vaccine-induced immune thrombotic thrombocytopenia (VITT)[181] or thrombosis with thrombocytopenia syndrome (TTS) (Centers for Disease Control [CDC], USA).

Disseminated intravascular coagulation (DIC) was earlier observed after administration of an Ad5 vector during a gene therapy clinical trial that led to the death of a patient in 1999.[183] Therefore, it was immediately debated whether the same mechanisms that triggered DIC in that gene therapy trial were responsible for triggering VITT following administration of Ad-based vaccines. Despite both DIC and VITT being thrombotic events, similarities in the mechanisms that trigger these two SAEs are highly unlikely. First, DIC in the gene therapy clinical trial was observed within 36 h after intravenous (i.v.) administration of Ad vector directly into the bloodstream, whereas VITT occurs 5–13 days after i.m. administration of Ad-based vaccines. Second, the dose of vector that triggered DIC was over 500-fold higher, as compared with doses of Ad vectors used for vaccination. Importantly, i.v. administration of Ad vectors in cancer patients at doses 60-fold higher than used in vaccination has not triggered DIC.[184] Third, i.v. administration of extremely high doses of Ad vectors, in addition to DIC, triggers cytokine-storm syndrome, involving the systemic activation of innate immune defense mechanisms. In contrast, the onset of clinical presentation with VITT 5–13 days after vaccination suggests activation of adaptive immunity,[185] where vaccine administration could trigger a recall response (symptom onset 5–7 days post-vaccination) or a primary PF4-targeted Ab response (symptom onset 7–13 days post-vaccination) upon activation of PF4-specific autoreactive B cells.

While the exact mechanism triggering VITT is currently unknown, should an autoimmune nature be definitively implicated, one might speculate that the incidence of VITT could vary greatly among vaccine recipients in different parts of the world, where genetic and environmental factors that underlie the development of many autoimmune diseases differ dramatically from those present in Western Europe and the United States. The incidence of VITT after ChAdOx1 AZD1222 vaccination is estimated to be ~10 per million for individuals aged >50, ~20 per million for individuals <50 years,[186] or ~24.9 per million, as reported in Norway and Denmark.[187] These rates are lower following immunization with Ad26.COV2.S, which is estimated at ~1.7 per million.[188,189] However, it is critical to note that infection with SARS-COV-2 that results in symptomatic COVID-19 triggers CVT at an estimated rate of ~42.8 per million.[190] Once again, this provides a rationale for continuing vaccination to limit COVID-19-associated morbidity and mortality.[190,191] In-depth observational studies and intervention strategies are currently being developed to promptly diagnose and effectively mitigate the severity of VITT following immunization, as well as to optimize vaccination options for populations most at risk for developing VITT.[192]

Safety concerns and considerations for future use of Ad-based vaccines: Guillain-Barré syndrome

Among other selected AEs that were reported after Ad-based COVID-19 vaccine administration through the vaccine adverse event reporting system (VAERS), the US CDC lists GBS. GBS is a rare, immune-mediated disorder that can present following non-viral or viral infection[193] (including SARS-CoV-2), in which the immune system attacks neurons, leading to acute inflammatory demyelinating polyneuropathy, muscle weakness, and, in rare cases, paralysis. The majority of GBS patients fully recover. GBS events reported to VAERS occurred within 2 weeks of immunization. As of October 27, 2021, the US CDC reported that 244 suspected GBS events were observed after 15.5 million administrations of Ad26.COV2.S vaccine, corresponding to incidence rate of 15.7 cases per million. However, this incidence rate falls within the 8.1–19.1 per million person years range

of GBS incidence in the general population, which was determined based on population-based studies from North America and Europe.[193] In an observational study and systematic review of GBS reports after vaccination, Shao et al.[194] identified 39 cases of GBS that occurred within 2 weeks of vaccination. Out of these 39 confirmed cases of GBS, 25 were reported after vaccination with the ChAdOx1 AZD1222 vaccine, 12 were reported after vaccination with BNT162b2 (Pfizer-BioNTech vaccine), 1 was reported after vaccination with Ad26.COV2.S vaccine, and 1 was reported after administration of the CoronaVac vaccine (inactivated vaccine).[194] Due to the very low incidence and the observational nature of studies that reported GBS events after vaccination, definitive conclusions regarding the association of GBS events with vaccine administration cannot be drawn. Because the majority of GBS cases respond well to available pharmaceutical interventions and recover, the benefits of vaccination far outweigh the risk of GBS.

Safety concerns and considerations for future use of Ad-based vaccines: HIV acquisition risk

With the global use of Ad-vectored COVID-19 vaccines, concerns were raised regarding a risk of potential increase in HIV acquisition rates in vaccinated populations, in regions where HIV infections are prevalent.[195] This concern is based on the results of two historical, randomized, double-blind, placebo-controlled clinical trials in the Americas, Caribbean, and Australia (STEP trial)[196] and in South Africa (Phambili trial).[197] These clinical studies were designed to test the efficacy of a preventive HIV-1 vaccine based on the human Ad5 vector in cohorts at high risk for HIV exposure. Early results from these trials, as well as extended follow-up, demonstrated an enhanced risk of HIV acquisition in vaccine recipients, with uncircumcised men with pre-existing Ad5-specific immunity found to be at highest risk.[198–200] Extended follow-up analyses of participants in the STEP trial have shown that the risk of enhanced HIV acquisition wanes 18 months post-vaccination,[199] and in the Phambili trial, the risk of enhanced HIV acquisition was observed in men (hazard ratio [HR] = 2.75; 95% confidence interval [CI] 1.49, 5.06; $p = 0.001$), but not in women (HR = 1.12; 95% CI 0.73, 1.72; $p = 0.62$).[201] Although several potential mechanisms have been proposed,[202–204] the exact factors responsible for the trend toward increased HIV acquisition after vaccination with Ad5-based vectored vaccines remain unknown. It is also unknown whether the same mechanisms that led to enhanced HIV acquisition after vaccination with vectors based on Ad5 are also activated after administration of vaccine vectors based on alternate types, specifically Ad26 or ChAdOx1 (Y25). As of the time of submission of this review for publication, no evidence was found that administration of Ad26.COV2.S or ChAdOx1 COVID-19 vaccines leads to enhanced HIV acquisition. In further support of this, follow-on studies in participants from the STEP trial, in addition to other clinical trials, determined that seropositivity to Ad5 and a range of other Ad types did not present an increased risk for HIV acquisition.[205,206] Nevertheless, particular attention to changes in HIV infection in at-risk populations receiving Ad-vectored vaccines or in geographical regions with high HIV prevalence is certainly warranted.[96,195]

VACCINE EFFICACY AND REAL-WORLD EVIDENCE FOR PROTECTION FROM SEVERE COVID-19 INFECTION AND DEATH

A hierarchy in immunogenicity has been observed when comparing two-dose mRNA vaccines (mRNA-1273 and BNT162b2) versus single-dose Ad26.COV2.S, with mRNA-1273 > BNT162b2 > Ad26.COV2.S.[207] In agreement with this, Ad-based vaccination regimens have been shown to elicit lower NAb titers (when measured 4 weeks after full vaccination) than mRNA-based vaccines, with reduced NAb titers against variants of concern (VOCs) also observed for Ad platforms versus mRNA.[208] Overall efficacy in preventing symptomatic infection for Ad-based vaccines has also been reported to be lower than for mRNA vaccines (60%–70% versus >90%).[171,208–211] However, importantly, protection from severe-critical disease, hospitalization, or death is high for all vaccine platforms. In a phase III efficacy study with a single dose of Ad26.COV2.S, protection against severe-critical COVID-19 disease >28 days post-immunization was 85.4%[57] and efficacy against severe disease (i.e., emergency department visits) was reported to be ∼94% for ChAdOx1 AZD1222[171] and 91.6% for Sputnik V.[178] Furthermore, real-world protection data are now emerging. A non-randomized study of US insurance claim data reported vaccine effectiveness (VE) of 81% against COVID-19 hospitalization following a single shot of Ad26.CoV2.S.[212] Another study comparatively evaluated the VE of Moderna, Pfizer-BioNTech (both two-dose regimens), and Janssen Ad26.COV2.S (one-dose regimen) vaccines against COVID-19 hospitalizations at 21 US hospitals from March to August 2021 (immunocompromised patients were excluded).[213] VE was highest for Moderna (93%) followed by Pfizer-BioNTech (88%), with the single-shot Janssen vaccine having 71% protection against hospitalization.[213]

More recently, the Janssen Ad26.COV2.S vaccine has been tested in a homologous boost regimen. Press releases for the ENSEMBLE 2 study, in which a boost of Ad26.COV2.S was administered at a 2-month interval, have suggested VE of 100% against COVID-19 hospitalization. Very recently, results from the Sisonke 2 phase 3b study were reported, indicating that a booster shot of Ad26.COV2.S administered 6–9 months following initial immunization of South African healthcare workers has a VE >84% against hospital admission when the Omicron variant was dominant.[214] Although overall, the VE of Ad26.CoV2.S has been reported to be lower than mRNA vaccines,[207] there is evidence of waning protection from infection or hospitalization for mRNA vaccines, whereas VE for Ad26.COV2.S appears to be durable.[215] Data are still emerging regarding efficacy and VE against new VOCs. Nonetheless, the robust protection from severe disease for each vaccine, despite differences in immunological potency, suggests a need to better understand the qualitative differences between the immune response elicited by Ad and mRNA platforms in the future and how those parameters translate into correlates of protection.

LESSONS LEARNED FROM THE SARS-CoV-2 PANDEMIC

Through the course of the pandemic, several priority areas for pandemic preparedness have become apparent. First, it is clear

that we need to (1) optimize and develop vaccines capable of conferring broad, protective immunity that could address the emergence of variants. In the context of Ad-based vaccines, this could be achieved by applying strategies used in "universal" vaccine development, such as focusing on highly conserved viral antigens or epitopes and domains, incorporating molecular or genetic adjuvants, or engineering multi-valent vectors that encode more than one vaccine antigen to increase breadth of protection. Related to this is (2) the crucial importance of antigen selection and the potential for use of stabilized immunogens—optimized through structure-guided approaches—to elicit humoral immune responses against antigenically authentic viral proteins. This was highlighted by reports of a 2P stabilization modification in the spike (S) of SARS-CoV-2, which locked it into a pre-fusion structure and enhanced expression.[216,217] This approach was used by both mRNA platforms, in addition to Johnson & Johnson's Ad26.COV2.S vaccine[217] but was not used in the ChAdOx1 AZD1222 platform. Although it is difficult to evaluate how differences in antigen design could contribute to differences in efficacy when comparing between two distinct Ad platforms, these questions can be considered for the design of next-generation vaccines against viruses that represent a future emerging pandemic threat.

In addition to these points, questions also arose that were related to (3) increasing our understanding of the precise, step-by-step mechanism of action of vaccines, i.e., can we design optimized vaccines that maximize prevention of infection as well as vaccines that prevent disease? Further to this is the need to better understand how homologous or heterologous Ad prime:boost regimens work (and indeed heterologous Ad + mRNA or + protein)[218–222] in terms of the phenotype of immune response they elicit, how varying intervals between prime:boost affects the downstream immunogenicity or durability of immunity, and how pre-existing immunity (i.e., to Ad vectors) affects subsequent homologous boosting. In addition, there is growing interest in (4) advancing research to develop mucosal vaccines in the future. Ads have an established pre-clinical[112,223,224] and clinical track record for mucosal administration[74,94,122,225–228] and may therefore represent useful vaccines for i.n. immunization and protection against a broad range of respiratory pathogens.[229] With their low cost, scalability, and suitability for thermostabilization or storage independent of specialized cold-chain, Ad-based vaccines represent an ideal platform for equitable vaccine distribution. Efforts to build capacity in local vaccine manufacturing within LMICs will undoubtedly help to overcome supply issues and will be vital for future pandemic preparedness.

FUTURE DIRECTIONS

A broad range of approaches can be taken to enhance the safety, immunogenicity, and ultimately the efficacy of Ad-based vaccines. A recent study reported direct binding of PF4 to the hexon of the ChAdOx1, Ad26, and Ad5 capsids,[230] which may have implications for the induction of Abs directed toward PF4 and the development of VITT in a small number of individuals following immunization. Reassuringly, in the past, it has been possible to successfully modify the hexon of Ad vectors to eliminate the binding of various molecules.[231] As such, targeted genetic engineering of the hexon could be used to construct Ads that do not interact with PF4 (or other molecules), thereby potentially enhancing their clinical safety profile. Alternatively, the evaluation of a wider range of Ad vectors could identify platforms that inherently lack off-target interactions with PF4 and other factors and possess biological characteristics optimal for vaccine applications (i.e., robust immunogenicity, high titer growth, genetic stability, etc.).[10] Another means of indirectly improving the safety profile of Ad-based vaccines includes strategies to maximize the immunogenicity of the encoded transgene antigen, facilitating the use of reduced vector doses. Such approaches could include using built-in molecular and genetic adjuvants,[112] re-targeting the tropism of Ad vectors to specific APCs (including defined DC subsets), or incorporating peptide antigens into the capsid,[69,70] in addition to encoding them as a transgene. Furthermore, there is now interest in better understanding how mix-and-match heterologous immunization regimens using Ads and mRNA- or nanoparticle-based vaccine platforms could help maximize VE.[218–222,232] Data emerging on heterologous immunization regimens using Ad-based platforms as a prime and mRNA or nanoparticle boost have been promising, showing increases in the magnitude and breadth of humoral and cellular immune responses.[220,222]

SUMMARY

In summary, safety evaluations of COVID-19 vaccines based on different Ad types in tens of thousands of clinical trial participants, and in billions of vaccinees globally, now provide clear evidence that these vector-based vaccines are well tolerated. Ad-based vaccines have been a crucial public health intervention during this pandemic[233] and have emerged as one of the front-running vaccine platforms due to their cost relative to mRNA platforms ($3–$10 per dose as compared with $19.50–$37, respectively).[234] This is reflected in their widespread global distribution: with use of AZD1222 in 168–182 countries[235] and use of Ad26.COV2.S in 71 countries worldwide (source: Covid19trackvaccines website/NYT COVID Tracker). In fact, vaccine coverage in Africa, Asia, Russia, and South America has been dominated by Ad-based vaccines (i.e., AstraZeneca, Johnson & Johnson, CanSino, Gamaleya-Sputnik V, and Covishield). Based on the collective safety profile and demonstrated high efficacy at preventing severe disease and death, the risk-benefit analysis strongly points to the exceptional public health benefit of global introduction of Ad-based COVID-19 vaccines and supports their future investment as vaccines for new emerging viral diseases with pandemic potential.

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DECLARATION OF INTERESTS

L.C. declares no competing interests. E.J.K. is a member of the EMA vaccine advisory panel and was president of the user supervisory panel of TransVac II. D.M.S. is a paid consultant of Merck and a founder, officer, and shareholder of AdCure Bio, which develops adenovirus technologies for therapeutic use.

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