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Review

# Novel approaches for vaccine development

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## SUMMARY

Vaccines are critical tools for maintaining global health. Traditional vaccine technologies have been used across a wide range of bacterial and viral pathogens, yet there are a number of examples where they have not been successful, such as for persistent infections, rapidly evolving pathogens with high sequence variability, complex viral antigens, and emerging pathogens. Novel technologies such as nucleic acid and viral vector vaccines offer the potential to revolutionize vaccine development as they are well-suited to address existing technology limitations. In this review, we discuss the current state of RNA vaccines, recombinant adenovirus vector-based vaccines, and advances from biomaterials and engineering that address these important public health challenges.

## INTRODUCTION

Vaccines play a critical role in global health by preventing infection and transmission of multiple diseases worldwide (Orenstein and Ahmed, 2017). The World Health Organization estimates that vaccines prevent the death of 2–3 million people every year (World Health Organization, 2019). Moreover, immunizations have enabled the eradication of smallpox and are now close to eradicating polio (Africa Regional Commission for the Certification of Poliomyelitis, 2020; Fenner et al., 1988). In addition, vaccines have a significant economic impact by reducing costs of illnesses and hospitalizations of over $500 billion (Orenstein and Ahmed, 2017; Ozawa et al., 2016).

Although traditional vaccines have been tremendously successful, there are many infectious diseases for which no efficacious vaccines have been developed. The development of vaccines for human pathogens such as human immunodeficiency virus (HIV), tuberculosis (TB), respiratory syncytial virus (RSV), cytomegalovirus (CMV), herpes simplex virus (HSV), and Epstein-Barr virus (EBV) has thus far been unsuccessful. HIV has caused 39 million deaths globally, and over 36 million people still live with virus today (Pandey and Galvani, 2019). Even with the availability of antiretroviral therapy (ART), approximately up to 2 million people become infected every year. Similarly, TB causes 1.6 million deaths annually (Singh et al., 2020b). RSV is a major cause of lower respiratory tract infections and hospital visits during infancy and childhood, with 59,600 in-hospital deaths occurring in 2015 globally (Shan et al., 2017). In the United States alone, each year, more than 40,000 infants are born with congenital CMV infection, with nearly a 20% of these children developing permanent hearing loss, brain damage, or neurodevelopmental delays (Johnson et al., 2012). In addition, emerging and re-emerging pathogens such as Ebola virus, Zika virus, and most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have become major global health threats. Combatting outbreaks requires the rapid development of vaccines that has not typically been possible with traditional vaccine platforms.

These challenges have sparked intense interest in the development of novel vaccine technologies. In this review, we will review three such platforms: mRNA vaccines, vector-based vaccines, and materials science approaches to vaccination. We will discuss the development of these platforms, including applications to the COVID-19 pandemic.

## BACKGROUND

The human immune system has several lines of defense that protect against infection. The first ones are physical and chemical barriers such as skin, mucous membranes, and gastric acid. If a pathogen bypasses these obstacles and causes infection, a series of sensors are present to detect foreign agents or antigens and to activate the innate immune system. These sensors have evolved to discriminate between self and non-self and to recognize conserved features of pathogens. For example, nucleic acid sequences and secondary structures of viruses are recognized by sensors, such as toll-like receptors (TLRs) (Bartok and Hartmann, 2020). Innate sensors trigger downstream signaling pathways, activating a non-specific, albeit rapid, innate immune response characterized by inflammation, cytokine production,

recruitment of immune cells, and activation of phagocytic cells that neutralize pathogens and infected cells. Further, inflammation draws antigen presenting cells (APCs) to the site of infection, where they take up antigens and traffic to secondary lymphoid organs, such as lymph nodes and spleen, to guide the activation of T and B cells, which constitute the adaptive immune response.

Although reliant on innate immune activation, the adaptive immune system is the classic pathogen-specific response. Within the secondary lymphoid tissues, APCs drive the expansion of different subsets of T cell populations. Notably, they trigger $CD8^+$ cytotoxic T cells, which have the ability to recognize and kill cells infected by specific pathogens. APCs also expand $CD4^+$ helper T cells, which aid in the differentiation of B cells that ultimately generate antigen or pathogen-specific antibodies. These antibodies are critical in clearing infections by either binding to microbes to prevent cellular entry or by tagging pathogens for destruction by complement or innate immune cells (Dimitrov and Lacroix-Desmazes, 2020; Lu et al., 2018). Eventually, these T and B cells adopt memory phenotypes, which leaves cells poised to expand and re-activate in response to a future pathogen encounter. Importantly, the next time the body is exposed to the same pathogen, this memory or recall can respond more rapidly and effectively.

Vaccines work by exposing a subject to a part or whole pathogen, thus activating the immune system. Traditional types of vaccines include live-attenuated, inactivated, and replication-defective pathogens as well as subunit and conjugate vaccines. Live-attenuated vaccines consist of weakened form of a pathogen. Attenuating pathogens usually involves passaging them through a series of cultures or animal embryos until they lose the ability to replicate efficiently in human cells. Some examples of clinically approved live-attenuated vaccines include those against smallpox, measles-mumps-rubella (MMR), and yellow fever. Although live-attenuated vaccines elicit strong immune responses, the administration of a live pathogen could pose a risk for people with weakened immune systems or other health complications. An alternative approach is to administer a whole inactivated pathogen to mitigate this safety risk. Inactivated vaccines include hepatitis A virus, poliovirus, and rabies virus vaccines. Of note, the development of both live-attenuated and inactivated vaccines requires large-scale growth of the pathogen posing a bio-safety risk. Finally, subunit vaccines are composed of a piece of a pathogen. Sub-unit vaccines have favorable safety profiles and eliminate the need to culture or grow live pathogen but often require booster immunizations as well as adjuvants. Advances in adjuvant technology as well as genomics have led to the introduction of a series of new vaccine approvals in the recent years, such as Heplisav (CpG adjuvanted hepatitis B vaccine) (Champion, 2020), Shingrix (recombinant zoster vaccine adjuvanted with AS01B) (Singh et al., 2020a), and Bexero (meningococcal B [MenB] vaccine incorporating recombinant protein and outer membrane vesicle adsorbed onto aluminum hydroxide adjuvant) (Rodrigues et al., 2020).

Limitations of traditional vaccine platforms have sparked the discovery and development of novel vaccine technologies. These approaches include viral vector vaccines (Barouch and Picker, 2014; Ertl, 2016) as well as nucleic acid vaccines (Bahl et al., 2017; John et al., 2018; Lindgren et al., 2017). These new technologies can address unmet medical needs, such as for vaccines that involve antigens that are difficult to manufacture or for novel pathogens for which rapid development is critical. In the ongoing COVID-19 pandemic, the four most advanced programs in Phase 3 trials in the United States are based on these technologies, including two mRNA vaccines and two adenovirus (Ad)-vectored vaccines (Folegatti et al., 2020; Jackson et al., 2020; Mulligan et al., 2020; Sadoff et al., 2020). This review will focus on the current status of mRNA vaccines and recombinant Ad vector-based vaccines as well as applications of engineering and materials science to vaccine delivery platforms.

## mRNA VACCINES

mRNA vaccines have gained considerable attention in the recent years, because they have the potential to expedite vaccine development, to have improved safety and efficacy, and to tackle diseases that have not been possible to prevent with other approaches. mRNA is non-infectious, non-integrating, and is degraded by normal cellular processes shortly after injection, decreasing the risk of toxicity and long-term side effects. Intracellular expression of the antigen by mRNA may lead to strong T cell responses typically seen with viral vector-based or replication-defective virus-based vaccines. However, mRNA vaccines have the advantage that they do not induce vector-specific immunity and do not contend with either pre-existing or newly raised vector immunity that could interfere with subsequent vaccinations. More than 10 mRNA vaccines were already at different stages of clinical testing before the beginning of the SARS-CoV-2 pandemic. These studies have demonstrated the safety and immunogenicity of mRNA vaccines in thousands of vaccinated subjects including children and elderly subjects.

mRNA vaccines enable precise antigen design and the generation of proteins with a ''native-like'' presentation (e.g., membrane bound with human glycosylation patterns), expression of proteins stabilized in a more immunogenic conformation or exposing key antigenic sites (e.g., prefusion stabilized) (Espeseth et al., 2020), and delivery of multiple mRNA to the same cell allowing the generation of multi-protein complexes (John et al., 2018) or protein antigens from different pathogens thus creating a single vaccine against several targets.

mRNA vaccines are manufactured using chemically defined, consistent processes, regardless of the antigen encoded by the mRNA, and this has the potential to simplify vaccine production, scale up, quality control, and the overall vaccine development timelines. These factors allow for multiple iterative cycles of antigen improvements and human evaluation thus shortening the overall development time to safe and efficacious vaccines (Figure 1). Finally, because this is such a rapidly developing field, there are many opportunities for innovation, improvements, and new developments.

A unique feature of mRNA vaccines is that they can be produced and scaled up in a predictable and consistent fashion and with well-established processes and reagents within weeks regardless of the antigen. This feature is advantageous during outbreaks caused by new viruses or pandemic situations where a rapid response is needed. The COVID-19 pandemic has

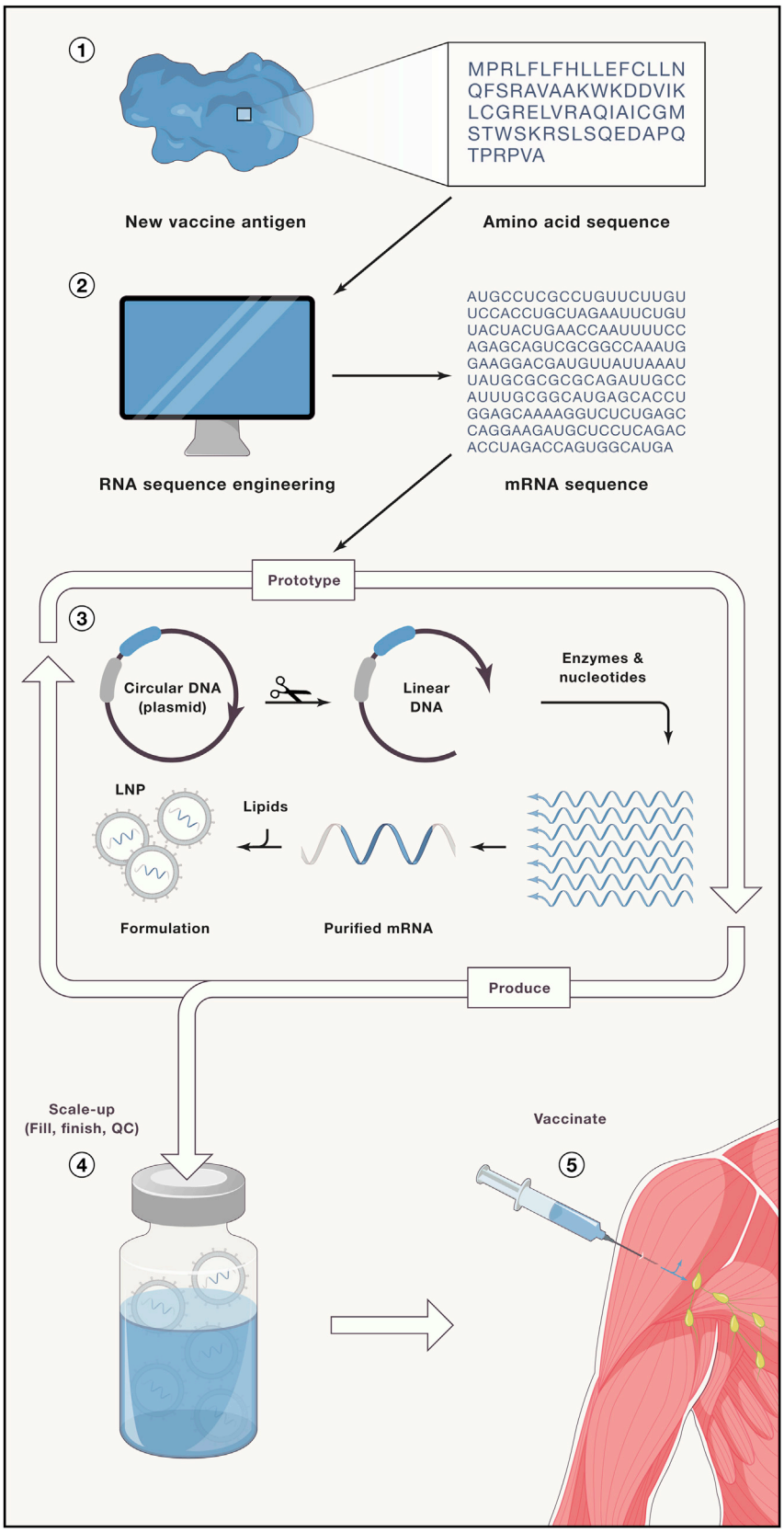


**Figure 1. Process for mRNA vaccine development from target identification to vaccination**

(1) It starts with the identification and design of a target antigen. (2) Digital sequence design based on propriety algorithm. (3) Manufacturing of plasmid, mRNA, and lipid nanoparticle (LNP). (4) Fill, finish, and quality control (QC). (5) intramuscular injection, cellular uptake, protein expression, and immune activation.

accelerated investment in manufacturing and scale-up of mRNA vaccines leading to commercial scale production. Two mRNA-based SARS-CoV-2 vaccines (Moderna mRNA-1273 and Pfizer/BioNTech BNT162b2) have been tested in large Phase 3 clinical trials and were demonstrated to be safe and highly efficacious in both adults and elderly subjects. Importantly, both manufacturers expect to produce hundreds of millions of doses of vaccine for deployment in 2021. These are the first licensed mRNA vaccines, and their wide acceptance and use would open the way to other novel mRNA vaccines against infectious disease targets.

## From DNA to mRNA vaccines

The earliest nucleic acid vaccine platform (late 1990s and early 2000s) used plasmid DNA generated after propagation in bacteria, extraction, and purification. The initial report by Ulmer et al. (1993) showed that naked DNA could generate potent immune responses against influenza virus after injection in mouse muscle with no need for additional formulation. In subsequent years, this technique did not translate to larger species (Jiao et al., 1992; Wolff and Budker, 2005). When tested in humans, immune responses were weak and not durable (Liu and Ulmer, 2005) and required doses >1 mg. Delivery was found to be a key limiting factor for plasmid DNA, because it needs to enter the cell then pass through the nuclear membrane—an inefficient process in nondividing cells. Device-mediated plasmid DNA delivery (such as electroporation and jet injectors) has recently shown some promise (Al-Dosari and Gao, 2009). These devices enhance delivery through physical deformation of the cell membrane facilitating nucleic acid cell entry. Nevertheless, the need for a device as well as high (e.g., milligram) dose levels are likely to limit broad use of plasmid DNA vaccines.

The use of mRNA as a vaccine platform has gained significant attention more recently. Like plasmid DNA, mRNA was first shown to be expressed in animals after naked injection in muscle (Wolff et al., 1990). However, until recently, there were many perceived limitations of mRNA such as vulnerability to nucleases, inherent instability, challenging manufacturing processes, and unwanted innate immune system stimulation that kept researchers and vaccine manufacturers away. Initial failure of DNA vaccines also raised concerns about the potency of nucleic acid vaccine approaches in humans. Progress over the past 10 years in mRNA science, delivery, and immunology has advanced the field to the point where multiple mRNA vaccines are now in advanced clinical trials.

## mRNA structure and design

Synthesis of mRNA is performed *in vitro* using a plasmid DNA as template, an RNA polymerase, nucleoside triphosphates, and buffers (*in vitro* transcription [IVT]) (Sahin et al., 2014). To be biologically active, mRNA requires addition of an inverted triphosphate cap (e.g., N7-methylated guanosine) to the 5′ end of the mRNA molecule. The cap can be added co-transcriptionally as with anti-reverse cap analog (ARCA) or enzymatically with an enzyme such as vaccinia capping enzyme (Shuman, 1990). Once produced, mRNA is then purified to remove impurities generated during IVT using chromatography techniques or affinity purification.

The final "active" mRNA consists of a cap at the 5′ end, a 5′ untranslated region (UTR), open reading frame (ORF), 3′ UTR, and poly-adenylation tail (Poly-A) resembling fully processed, mature, endogenous mRNA molecules present in the cytoplasm of eukaryotic cells (Figure 2). Each of these elements is critical for mRNA activity and provides targets for modifications to improve stability, translation efficiency, and ultimately, protein expression levels and duration. UTRs are important regulators of mRNA decay and translational efficiency via cellular RNA-binding proteins. The 3′ UTR sequence has been shown to modulate mRNA half-life (Guhaniyogi and Brewer, 2001; Orlandini von Niessen et al., 2019), whereas the 5′ UTR plays a role in stability and translation initiation efficiency (Leppek et al., 2018). Codon optimization of the ORF results in changes of secondary structure content and translation elongation rate both of which impact protein expression levels and potentially protein folding (Mauger et al., 2019). Finally, a long poly-A tail (i.e., >120 units) has been reported to increase protein expression (Holtkamp et al., 2006).

## mRNA mechanism and immunogenicity

Once administered intramuscularly, mRNA formulated in lipid nanoparticles (LNPs) are taken up by APCs, including dendritic cells, through endocytic pathways (Akinc et al., 2010; Liang et al., 2017). Next, the mRNA activates innate sensors present in the endosome and the cytosol and is also translated into protein antigen in the cytoplasm. The mRNA itself can be recognized by a variety of endosomal and cytosolic innate immune receptors (Chen et al., 2017), thus modulating the overall immune response to the vaccine. The newly synthesized antigen can engage both B cells and T cells, driven by the concomitant expression of antigen and innate activation of the immune system.

Innate immune stimulation is driven by mechanisms that immune cells have developed to enable responses against pathogens and are primarily driven by activation of cellular sensors against viral mRNA and DNA, bacterial lipids, and sugars. Different elements of mRNA vaccines can engage pattern-recognition receptors (PRRs) such as TLR3 and TLR7/8 in endosomes, cytosolic sensors like MDA5 and RIG-I, and NOD-like receptors (NLRs) (Chen et al., 2017). These sensors respond to pathogen-associated molecular pattern (PAMPs) like double-stranded RNA (dsRNA) and/or single-stranded RNA (ssRNA) present in the vaccine, resulting in robust type 1 interferon signaling (Zhang et al., 2016). Activation of the type 1 interferon pathway can be beneficial, because it can drive the activation and maturation of APCs, key to eliciting robust B cell and T cell immune responses, but it can also be detrimental as it can negatively impact protein translation and therefore immunogenicity as well as tolerability.

Some mRNA vaccine candidates use canonical bases without modifications (Rauch et al., 2020), an approach that has been shown to be immunogenic in *in vivo* studies but also demonstrated strong immune activation. Karikó et al. (2011) observed that base modifications lessened the degree of innate stimulation driven by mRNA. Recent reports have demonstrated that use of N1-methyl-pseudouridine modified mRNA results in robust protein translation (Corbett et al., 2020a, 2020b; Jackson et al., 2020; John et al., 2018). mRNA with this

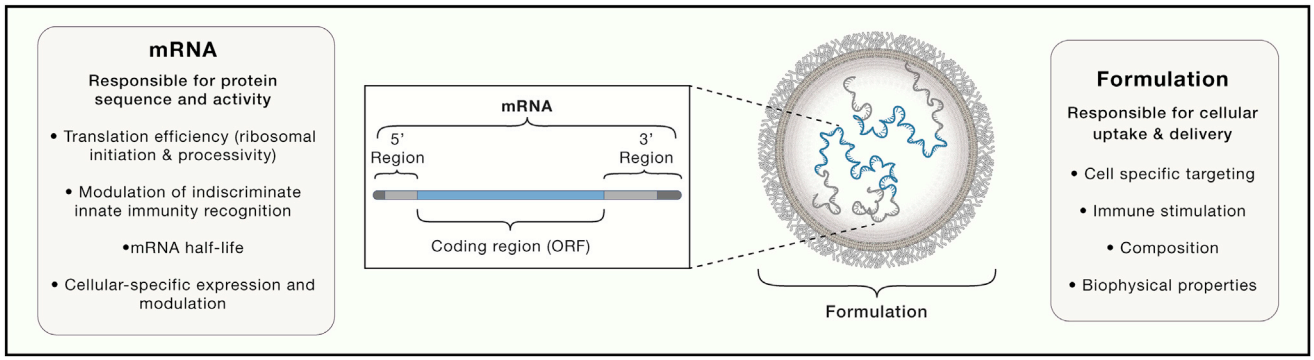


**Figure 2. mRNA vaccines are composed of proprietary lipid nanoparticle delivery systems and mRNA optimized for stability and translation**

nucleoside-modification was found to circumvent TLR7/8 activation, thus decreasing innate immune activation (Espeseth et al., 2020; Karikó et al., 2005), while still eliciting strong immune responses both preclinically and clinically (Corbett et al., 2020a, 2020b; Espeseth et al., 2020; Jackson et al., 2020; John et al., 2018). In summary, mRNA process changes and purification, codon optimization, and bases replacement can be used to modulate innate immune activation and therefore immunogenicity of mRNA vaccines (Linares-Fernández et al., 2020; Nelson et al., 2020).

The kinetics of antigen expression by mRNA vaccines also plays a role in the induction of immune response and durable immune memory. Studies have demonstrated that high antibody titers and germinal center (GC) B cell and T follicular helper ($T_{FH}$) cell responses are induced by sustained antigen availability resulting from mRNA vaccination (Liang et al., 2017; Lin et al., 2018; Lindgren et al., 2017). $T_{FH}$ cells are critical to the development of potent and durable neutralizing antibody responses and have been measured in response to vaccination by mRNA vaccines, most recently mRNA-1273 (Corbett et al., 2020b).

## Lipid nanoparticle mRNA delivery

In contrast to naked mRNA, formulated mRNA has been shown to result in higher protein expression *in vitro* and more potent immune responses *in vivo* (Pollard et al., 2013; Wolff et al., 1990). Formulations for mRNA vaccines perform many functions including stabilizing mRNA before injection and also facilitating mRNA entry into the cell to allow endosomal escape and delivery of the mRNA into the cytoplasm and subsequently degrade into metabolic intermediates. Although there are several approaches to introduce nucleic acids into cells, the field has converged recently on ionizable lipid-based systems or LNPs as the delivery system of choice.

Lipid nanoparticles are ~100 nm diameter delivery vehicles that approximate the size and composition of natural apolipoproteins such as VLDL. When injected into animals or humans, LNPs are rapidly opsonized by serum or interstitial proteins and trafficked similar to endogenous apolipoproteins to cells expressing lipid or scavenger receptors (e.g., LDLR, SR-B1). LNPs are typically composed of four components: an ionizable lipid, cholesterol, PEGylated lipid, and a helper lipid such as distearoylphosphatidylcholine (DSPC) (Adams et al., 2018; Jayaraman et al., 2012; Lutz et al., 2017) (Figure 2). The formulations are prepared through rapid precipitation and a self-assembly process at low pH (Hassett et al., 2019). LNPs can deliver RNAs very efficiently even when compared to a viral vector. When a viral replicon genome is delivered with an LNP and compared with a viral particle containing the same genome, both generated similar immune responses in rodents (Geall et al., 2012).

Among the components of the LNP, the amino lipid is key to their function. Early work relied on amino lipids used in other nucleic acid delivery systems, such as small interfering RNA (siRNA). These LNPs had some tolerability challenges related to biodegradability and composition. Subsequent efforts have led to the identification of biodegradable ionizable lipids that, when incorporated into the LNP, improved the potency of the vaccine (Hassett et al., 2019). In preclinical studies, the inclusion of a biodegradable lipid within an LNP also resulted in reduction in injection site inflammation resulting in vaccines with improved tolerability, because the lipid is cleared quickly from the site of injection, and other tissues have minimal exposure to the lipid due to metabolic breakdown and clearance. Importantly, for vaccines, the increased innate immune stimulation driven by LNPs does not equate to increased immunogenicity, illustrating that mRNA vaccine tolerability can be improved without affecting potency.

The first mRNA LNP vaccines to be evaluated in clinical studies were for H10 and H7 influenza hemagglutinin in 2015 and 2016, respectively. Phase I clinical data showed 100% seroconversion with 25- and 100-μg dose of modified uridine mRNA, respectively, with an adverse event profile similar to other approved vaccines (Feldman et al., 2019). From those first experiments, until the COVID-19 pandemic, 8 other mRNA vaccines have advanced into the clinic of which 5 rely on LNPs. The COVID-19 pandemic has led to 8 additional programs just in 2020.

## Self-amplifying mRNA (saRNA) versus mRNA

In addition to conventional mRNA constructs, saRNAs based on linear, nonsegmented, single-stranded, positive-sense viral genomes are being evaluated as vaccines. In this approach, the viral genome encoding the nonstructural replication-specific proteins are retained and the structural ORFs are replaced with ORFs coding for the antigen of interest. The goal is to mimic a

viral infection, but without the ability to create an infectious particle due to the lack of structural viral proteins. However, compared to an mRNA encoding for the same vaccine antigen, the size of saRNA is greatly increased due to the addition of the viral non-structural replication machinery.

A number of reports have compared conventional, unmodified mRNA, and saRNA for vaccination (Bernstein et al., 2009; Maruggi et al., 2017; Probst et al., 2007). Dose sparing was observed with the saRNA, however, none of these reports include the extensive mRNA sequence modification approaches that can be used to optimize protein expression, minimize nuclease degradation, or modulate stimulation of innate immunity. The only clinical data published to date are for the modified mRNA vaccines that are currently in Phase 3 studies. A recent Phase I trial for COVID-19 vaccines sponsored by Pfizer/BioNTech could provide the first clinical comparison of unmodified mRNA, saRNA, and modified mRNA (https://ClinicalTrials.gov/show/NCT04380701). It is important to note that saRNA is more difficult to scale up due to construct length, purification challenges, and enzymatic reaction inefficiencies and is intrinsically far less stable than mRNA, because 1 strand breakage every 10,000 bases inactivates saRNA versus 1 per every ~2,500 bases for mRNA.

### mRNA vaccines for SARS-CoV-2

mRNA vaccines are well-suited to respond to new emerging pathogens and infectious disease outbreaks with pandemic potential. Indeed, the use of methods and processes that are antigen-independent make this approach intrinsically faster and more reliable than other technologies. A striking example of the flexibility, speed, and scalability of the mRNA/LNP technology is exemplified by the development of the Pfizer/BioNTech BNT162b2 and Moderna mRNA-1273 vaccine candidates, both encoding the SARS-CoV-2 pre-fusion stabilized S2P protein, which showed very high efficacy in Phase 3 trials (Baden et al., 2021; Jackson et al., 2020; Mulligan et al., 2020; Polack et al., 2020). The mRNA-1273 antigen design was facilitated by previous pre-clinical work on an mRNA vaccine against MERS CoV (Corbett et al., 2020a). mRNA-1273 was produced, vialed, and ready for clinical testing within 42 days from the time of virus RNA sequence was made available. Sixty-four days from sequence availability, an NIH-sponsored Phase 1 study initiated dosing and was followed by a Phase 2 study and then a 30,000 subject placebo-controlled Phase 3 efficacy study 2 months later. The Phase 1 study demonstrated that the vaccine is safe and well-tolerated and elicited comparable immune responses across different age groups (18–55, 55–71, and 71+ years old) (Anderson et al., 2020). A large-scale effort was made to scale up mRNA/LNP production, purification, analytical methods, development, and testing of the vaccines to stand to the task of producing hundreds of millions of doses per year. In addition to these 2 late stage candidates, there are other mRNA/LNP vaccines in preclinical or early clinical evaluation from Curevac (CVnCoV, unmodified mRNA, currently in Phase 2), Arcturus (ARCT-021, SAM in LNP, currently in Phase 1), Translate Bio/Sanofi (MRT5500, unmodified mRNA, currently being evaluated preclinically). It is therefore conceivable that in the future, a pandemic response with mRNA vaccines may be even faster as a suitable infrastructure, safety, and regulatory framework have been established.

## ADENOVIRUS VECTOR-BASED VACCINES

One strategy to drive *in vivo* vaccine antigen expression is to harness natural carriers of genetic instructions, such as viruses. During infection, viruses multiply by hijacking the host cellular machinery to self-replicate. Thus, viruses are intrinsically equipped to enter target cells, deliver genetic instructions to key intracellular compartments, and drive efficient protein expression. An idea that has been pursued in many fields—spanning gene therapy, immunotherapy, vaccine design, and more—is to harness these intrinsic features of viruses to direct *in vivo* gene expression. Briefly, cassettes encoding these genes of interest are inserted into the viral genome, often by replacing key viral genes. This replacement serves dual purpose: (1) rendering the virus replication-incompetent or less virulent for safety purposes, and (2) freeing space to insert genes without significantly changing the inherent genome size (Lee et al., 2017). Over the past several decades, different viruses have been developed into vectors including Ads (Barouch and Picker, 2014; Ertl, 2016), cytomegaloviruses (Barouch and Picker, 2014), adeno-associated viruses (AAVs) (El Andari and Grimm, 2020), poxviruses (Conrad and Liu, 2019), herpesviruses (Artusi et al., 2018), and retroviruses (Chen et al., 2018b; Luis, 2020; Stephenson et al., 2016). For the purposes of this review, we will focus on the current state of Ad vector development.

### Ad vectors

Ads often cause mild or asymptomatic respiratory infections, although infections can be more severe, or even life-threatening, in immunocompromised individuals. Ads constitute a double-stranded DNA genome encased in a protein capsid (Usman and Suarez, 2020). The capsid structure includes fibrous projections that extend from the core and bind to receptors expressed on host cells mediating cellular entry (Baker et al., 2019).

Ads initially emerged as a promising platform for gene therapy to deliver and induce persistent expression of absent or mutated genes in patients suffering from genetic diseases. The unique ability of Ads to infect a wide range of cell types, including hepatocytes, myoblasts, as well as epithelial and endothelial cells, was especially beneficial for broadly inducing gene expression. An Ad vector was used for the first time in 1992 to treat two genetic disorders; alpha-1 antitrypsin deficiency (Lemarchand et al., 1992) and cystic fibrosis (Rosenfeld et al., 1992). In these studies, Ad vectors were demonstrated to deliver therapeutic genetic material to host cells efficiently. However, Ad vectors triggered inflammatory host innate immune pathways that recognize foreign patterns of viral structures (Shirley et al., 2020). This innate immune trigger limited Ad vector transduction and transgene expression. Consequently, it also induced an adaptive immune response against the vector that limited the efficiency of subsequent vector administrations needed for persistent therapeutic gene expression. Thus, the use of Ad vectors in gene therapy was limited by their potent immunogenicity (Zaiss et al., 2009).

## Immunogenicity of Ad vector vaccines

Although the intrinsic immunogenicity of Ad vectors presented limitations for applications in gene therapy, these properties encouraged the development of Ad vectors as a platform for vaccines. In the context of vaccination, simultaneous innate immune activation and antigen expression are key in promoting downstream expansion of antigen-specific T cells and B cells required for vaccine efficacy. Underscoring this feature of vector-based vaccines is the fact that other platforms, such as subunit or protein-based vaccines, typically require additional adjuvants or components to elicit potent, effective immune responses. In contrast, Ads are self-adjuvanting, simplifying the vaccine composition and formulation process. Ads intrinsically induce host innate immune response by activating cellular sensors that detect viral components (Coughlan, 2020; Rathinam and Fitzgerald, 2011). The primary innate immune trigger of Ad vectors is the viral genome, which contains foreign patterns and sequences that are not common in host genetic material. Ad capsids lacking DNA have been shown to induce reduced innate responses (Iacobelli-Martinez and Nemerow, 2007). Thus, foreign DNA sensors such as TLR9 and cyclic guanosine monophosphate-AMP synthase (cGAS) play a critical role in Ad vector innate immunity. This innate sensor activation leads to inflammation, production of cytokines such as interferons, and tissue infiltration by immune cells at the site of vaccination (Coughlan, 2020). Concurrent expression of antigen in this inflammatory environment can upregulate co-stimulatory markers on APCs. Consequently, APCs drive the expansion of adaptive T and B cells that are critical for vaccine immunity and immune memory.

In addition to their self-adjuvanting capability, another property that makes Ads suitable vaccine vectors is their tropism. They have an intrinsic cell transduction capability and can efficiently express transgene antigens both in dividing and non-dividing cells (Vorburger and Hunt, 2002). This flexibility increases the probability of successful, high level transgene expression across multiple cell types, leading to efficient production of vaccine antigen *in situ* required for robust antigen-specific vaccine immunity.

## Ad pre-existing immunity

Although Ad vectors efficiently mediate broad and efficient transduction of vaccine transgenes in cells, this capability hinges on the absence of substantial preexisting Ad immunity in the host. At the population level, successful Ad vector immunization could be thwarted by preexisting immunity if people have already been infected with the type of Ad used as a vaccine vector. Most human Ads are ubiquitous around the world and have infected the majority of the population by adulthood (Barouch et al., 2011; Mennechet et al., 2019). This induces neutralizing antibodies (NAbs) against Ad vaccine vectors and interferes with cellular entry. Various strategies have been developed to address this concern. These include the use of rare human or non-human Ads as well as vector capsid engineering to evade neutralization.

Human Ads can be classified into seven groups A–G and contain at least 67 serotypes that are phylogenetically distinct (Crenshaw et al., 2019). Although most of these serotypes are common, rare human Ads such as Ad26 and Ad35 have lower seroprevalence in humans than Ad5 (Barouch et al., 2011; Chen et al., 2010). Both serotypes have been demonstrated to evade the prevalent Ad5 immunity and induce robust and protective antigen-specific cellular and humoral immune responses (Geisbert et al., 2011). In the developing world, baseline Ad26 immunity is common, but baseline Ad26 neutralizing antibody titers remain low and do not appear to suppress Ad26-based vaccines.

The initial cloning and vectorization of Ad26 and several other Ad serotypes allowed a comparison of seroprevalence and immunogenicity of various candidate Ad vector platforms (Abbink et al., 2007). In addition to low titers of baseline vector-specific antibodies and high immunogenicity, Ad26 vectors can grow to high titers allowing for large scale production (Abbink et al., 2007). Ad26 vaccines have successfully advanced from preclinical studies to clinical trials for HIV and other pathogens (Baden et al., 2020; Barouch et al., 2018; Stephenson et al., 2020), which set the stage for the utilization of Ad26 in the development for vaccines for SARS-CoV-2 as well as other pathogens (Mercado et al., 2020; Sadoff et al., 2021).

Similarly, non-human Ads have also been utilized as vaccine vectors to address anti-vector immunity. Non-human serotypes are genetically and structurally similar to human Ads, making them easily adaptable as vaccine vectors. Commonly used non-human Ads include chimpanzee (ChAd), gorilla (GAd), and rhesus macaque (RhAd) serotypes. Safety and efficacy of ChAd vaccine vectors has been demonstrated in preclinical and clinical studies against SARS-COV-2 (Folegatti et al., 2020), HIV (Hayton et al., 2014), and rabies (Zhou et al., 2006). Nevertheless, baseline seroprevalence to certain ChAds has been shown to be up to 20% in developing countries (Xiang et al., 2006). This has sparked interest in the discovery of alternative carriers such as RhAds, which have greater phylogenetic distance from human Ads (Abbink et al., 2018; Pantó et al., 2015). RhAds have been evaluated in preclinical studies for HIV (Iampietro et al., 2018) and Zika (Larocca et al., 2019).

Alternatively, strategies to re-engineer vectors to circumvent pre-existing immunity have also emerged. These strategies seek to identify and replace specific regions, sequences, or epitopes targeted by pre-existing immunity. Anti-vector antibodies and T cells primarily target surface antigens of Ad vectors to dampen vaccine efficacy. Of the surface antigens, the hexon protein is a primary target of NAbs. Thus, pre-existing immunity can be evaded by replacing the hexon sequence of the exposed epitopes. This method has been applied to Ad5 and has allowed for enhanced transgene expression (Roberts et al., 2006; Sumida et al., 2005).

## Ad vector design, production, and application

Ads contain a 34–43 kilobase (kb) pair of double-stranded DNA, a relatively small viral genome size that enables facile manipulation (Figure 3). The construction of adenoviral vectors typically involves the deletion of a key viral gene, E1, to render the virus replication-deficient (McGrory et al., 1988). As noted above, this modification enhances the safety profile and allows the insertion of a vaccine antigen transgene of interest. Further design refinement has identified the E3 region as an additional

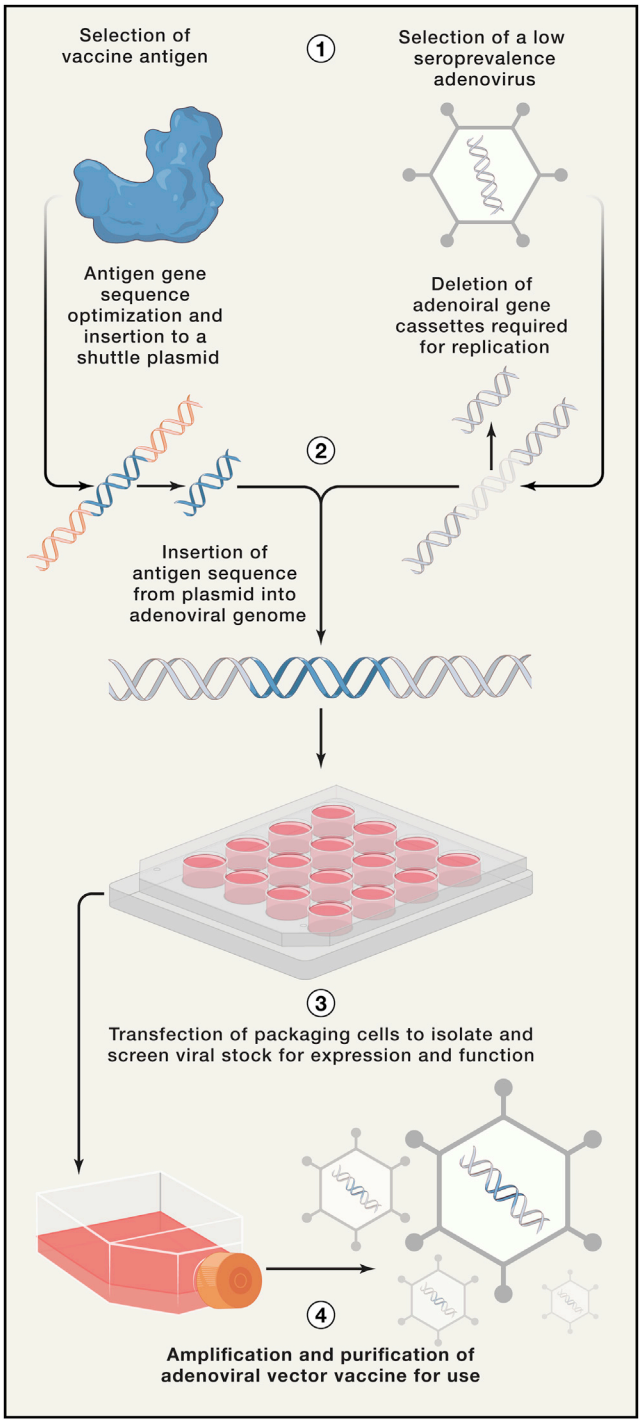


**Figure 3. Process of adenovirus vector vaccine development**
(1) First a vaccine antigen as well as an adenovirus vector with low seroprevalence are identified. (2) The gene sequence of the viral antigen is optimized and cloned into a shuttle plasmid. This plasmid is used to insert the antigen sequence into an adenoviral backbone in place of viral gene cassettes. (3) A primary viral stock is made in virus packaging cells and screened for antigen expression and function. (4) Last, the viral stock is amplified and purified for immunizations.

target for deletion, freeing up space to accommodate large transgenes. Because the E3 protein has been demonstrated to dampen ideal cellular innate immune responses, deletion of the gene also offers optimal vector innate immunogenicity (Windheim et al., 2004). Once an Ad vaccine genome is constructed, it is used to transfect packaging cells, such as HEK293, to prepare a primary viral stock that is screened for antigen expression and function. Packaging cells are transfected or supplemented with the E1 gene to allow the virus vector to replicate. Lastly, the primary stock goes through large-scale production and purification for clinical use.

Critically, this approach to incorporate genetic instructions into the Ad genome enables flexible and rapid vaccine development. For example, Ad vectors offer the potential to incorporate and screen different sequence variants of the same antigen to identify optimal vaccine candidates quickly. In addition to ease and speed of vaccine development, Ad vector production is also scalable and does not require high level biosafety containment and infrastructure, particularly compared to vaccine approaches that would require large-scale production of highly contagious and virulent pathogens prior to inactivation (Kamen and Henry, 2004). For example, Janssen's AdVac and PER.C6 high density cell production technology has allowed for the accelerated and large-scale production of millions of doses of an Ad26-Ebola vaccine that has been used extensively in Democratic Republic of the Congo (DRC) and Rwanda (Goldstein et al., 2020; Kitonsa et al., 2020) and has recently received regulatory approval in Europe.

These advantages of Ad vectors have made them promising vaccine platforms for a wide range of pathogens (Figure 4), including persistent infections such as HIV (Baden et al., 2020; Barouch et al., 2018; Stephenson et al., 2020). For HIV, Ad vaccines have allowed for the use of multivalent or mosaic sequences of HIV antigens that could be expressed together to elicit markedly enhanced immune responses (Barouch et al., 2010). Ad vector vaccines have also been developed for other persistent infections such as hepatitis C (Hartnell et al., 2019) and influenza (Matsuda et al., 2019). Most Ad vaccines have been developed for viral diseases, because the vectors mimic a viral infection by triggering similar innate immune sensors and inducing potent antibodies as well as CD8 cytotoxic responses, which are critical in clearing virus and lysing virally infected cells (Fields et al., 2000). However, Ad vaccines have also been developed for bacterial and parasitic diseases such as tuberculosis (Walsh et al., 2016) and malaria (Tiono et al., 2018). The rapid and scalable development potential of Ad vector-based vaccines have also made them well-suited for outbreak scenarios when rapid vaccine development and widespread distribution are critical. Consequently, adenoviral vaccines have been developed for outbreak viruses such as Ebola (Tapia et al., 2020) and Zika (Abbink et al., 2016; Bullard et al., 2020; Larocca et al., 2019).

## Ad vector-based vaccines for SARS-CoV-2

After the coronavirus genome sequence was made available in January 2020, multiple Ad-based COVID-19 vaccine candidates were developed and entered clinical trials in a matter of months. AstraZeneca and the University of Oxford developed a

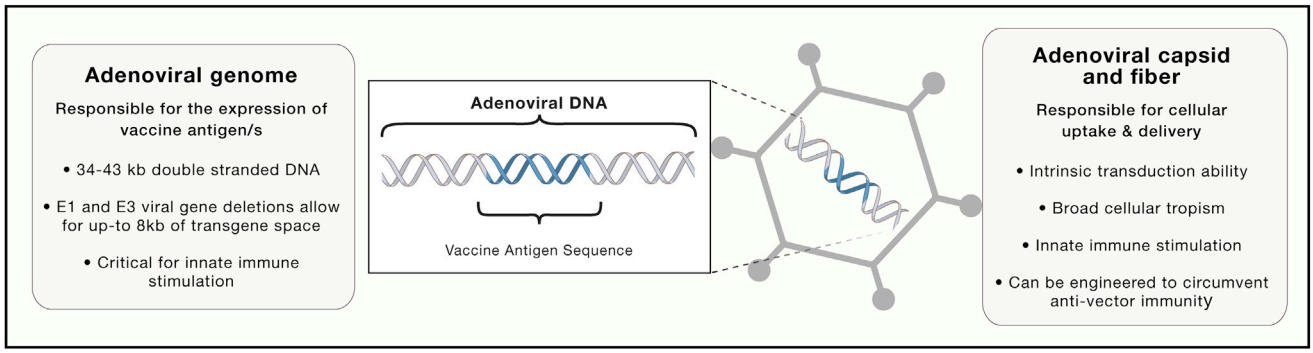


**Figure 4. Characteristics of adenoviral vaccine vectors**
Adenoviral vaccine vectors include a viral genome containing genes of antigen(s) of interest for expression as well as a viral capsid and fiber for cellular delivery.

chimpanzee Ad-based COVID-19 vaccine (ChAdOx1). The Phase 1/2 trial involved a prime-boost immunization and demonstrated that the vaccine was safe and immunogenic with both T cell and neutralizing antibody responses (Folegatti et al., 2020; van Doremalen et al., 2020). A global Phase 3 trial for the vaccine enrolled 23,848 participants and demonstrated robust efficacy (Voysey et al., 2021).

CanSino Biologics developed an Ad5-based COVID-19 vaccine, which has been shown to be safe, tolerable, and immunogenic (Zhu et al., 2020b). By July 2020, a Phase 2 clinical trial for this vaccine demonstrated immunogenicity in the majority of 508 eligible participants (Zhu et al., 2020a). In August 2020, CanSino began Phase 3 trials in 40,000 participants in multiple countries.

Johnson & Johnson developed an Ad26-based COVID-19 vaccine in collaboration with Beth Israel Deaconess Medical Center. A single immunization of this vaccine induced neutralizing antibody responses that correlated with protection against SARS-CoV-2 challenge in rhesus macaques (Mercado et al., 2020). This vaccine entered Phase 1/2 trials in July of 2020 and demonstrated safety and immunogenicity (Sadoff et al., 2021). The Phase 3 clinical trial in 44,000 participants, which began in September of 2020, demonstrated 85% efficacy in preventing severe COVID-19 disease 28 days after a single immunization, including in Latin America and in South Africa against the B.1.351 variant. Additional features of this vaccine are that it is protective with a single shot and does not require subzero freezing, which should facilitate vaccine campaigns.

The Gamaleya Research Institute developed a COVID-19 vaccine that combined two Ads, Ad5 and Ad26. The vaccine was shown to be safe and to generate both humoral and cell-mediated responses in a Phase 1 clinical trial (Logunov et al., 2020). The Phase 2/3 trial was launched mid-October 2020 and has reported high efficacy.

## BIOMATERIALS-BASED VACCINES

Engineering strategies have been employed to optimize carrier efficacy for vaccines (e.g., by tuning lipid composition and physiochemical properties to design LNPs for mRNA vaccines) (Hassett et al., 2019). More generally, approaches that combine immunogen design with materials science and engineering techniques have emerged as a promising strategy for multiple vaccine platforms (Chung et al., 2020; Fries et al., 2020). Bioengineering-based platforms, therefore, offer potential to tune the magnitude and nature of responses elicited, ideally skewing toward cell phenotypes, functions, and frequencies known to protect from infection or disease. Several reviews have described the application of various materials platforms in applications for not only infectious diseases (Fries et al., 2020; Yenkoidiok-Douti and Jewell, 2020) but also for cancer immunotherapy (Karlsson et al., 2018; Wang and Mooney, 2018) or to combat autoimmune diseases (Gammon and Jewell, 2019; Northrup et al., 2016; Pearson et al., 2019).

During infection, the physical structure and composition of pathogens engage both the innate and adaptive arms of the immune system. Yet conventional vaccine platforms, such as heat or chemical inactivation or delivery of subunits, can alter how antigens interact with immune cells and tissues. For example, protein-based vaccines have excellent safety profiles but are often plagued by poor immunogenicity if delivered without adjuvant (Fan and Moon, 2017; Kuai et al., 2018). One strategy to augment immune responses is to incorporate molecular adjuvants to trigger key innate inflammatory pathways (e.g., TLRs) that are typically activated by pathogens, but not by proteins alone (Iwasaki and Medzhitov, 2004). These strategies often rely on co-delivery of signals to the same tissues or even to the same cell. The cargos of interest—protein antigen, nucleic acid-based adjuvants, small molecule immunomodulatory drugs, and recombinant cytokines—have vastly different physiochemical properties and, therefore, exhibit disparate biodistribution and half-life following injection (Kuai et al., 2018; Lynn et al., 2015). This variance can present a significant challenge, because encounter of protein without adjuvant may limit expansion of antigen-specific pro-inflammatory or effector responses; in parallel, adjuvant in the absence of antigen may drive high levels of non-specific, systemic inflammation (Ilyinskii et al., 2014).

Biomaterials can aid to overcome this hurdle, through co-encapsulation or entrapment of cargos in polymer particles or lipid carriers (Ilyinskii et al., 2014; Thompson et al., 2018), by tethering signals to the surface of spherical carriers (e.g., gold nanoparticles, polystyrene beads) (Niikura et al., 2013; Tao and Gill, 2015), or via self-assembly of cues into nano- or micro-complexes, virus-like particles, or polyelectrolyte multilayers (Lynn et al., 2015; Tostanoski and Jewell, 2017; Yenkoidiok-Douti

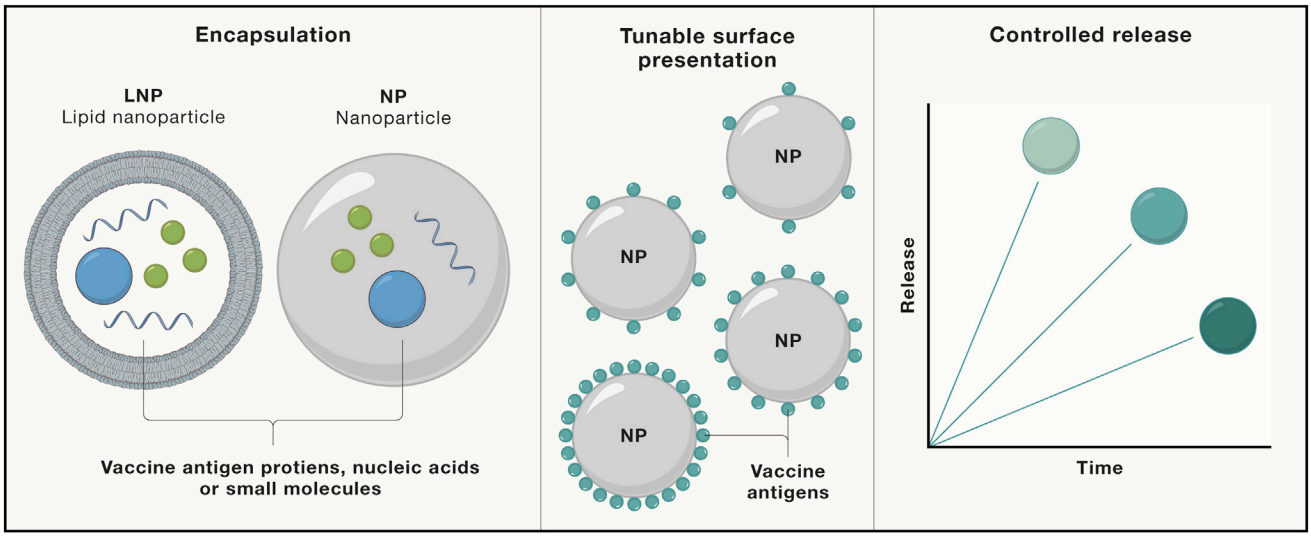


**Figure 5. Advantages of biomaterials in vaccine design**

Encapsulation: allows for enhanced cellular uptake of vaccine antigen proteins, nucleic acids or small molecules as well as improved lymphoid organ drainage. Surface presentation: allows improved and regulated presentation of multiple copies of antigens for enhanced immune stimulation. Controlled release: allows for the regulation of the kinetics and availability of immunogens at different times post injection. LNP, lipid nanoparticle; NP, nanoparticle.

and Jewell, 2020) (Figure 5). These approaches have been shown to enhance co-delivery of signals to the same cell, increase uptake by APCs, promote drainage of signals to lymph nodes, and minimize off-target effects. Materials-based approaches have also been engaged to administer several TLRs with antigen (Kasturi et al., 2011; Kuai et al., 2018; Thompson et al., 2018). A synergistic effect on expansion of IgG was observed when lipoprotein nanodiscs co-assembled antigen with both TLR4 and TLR9 agonists, compared with ad-mixed formulations or particles carrying one TLR agonist alone (Kuai et al., 2018); this effect also appears to translate across materials platforms, because similar synergistic effects of inclusion of multiple TLR agonists have also been observed in polymer nanoparticle-based approaches (Kasturi et al., 2011). This potential to incorporate multiple classes of cargo may be of particular interest for vaccine design for persistent infectious diseases, because many experimental regimens incorporate several signals—viral vector, protein, and adjuvant—in different sequences and combinations (Barouch et al., 2018).

Materials science, namely polymer chemistry, has also been employed to tune the kinetics of vaccine and immune cue delivery *in vivo*. Traditional vaccine approaches deliver a bolus injection, in which a high dose of immunogen is administered, and the concentration wanes as the signal is taken up by cells and cleared. In contrast, in next generation approaches that drive *in situ* expression of vaccine antigen, the bioavailability of the cue or cues correspond to the expression profiles in cells. Importantly, antigen kinetics have been shown to be tightly linked to responses elicited, motivating the development of tools to regulate the timing and duration of antigen delivery in a programmable manner (Cirelli et al., 2019; Irvine et al., 2020). Toward this goal, several approaches explore the use of degradable polymers. For example, polyesters like poly(lactic-co-glycolic acid) (PLGA) have been extensively employed in the drug delivery field and, more recently, translated to encapsulate and deliver vaccine cargo (Ben-Akiva et al., 2018; Irvine et al., 2020). The degradation rate of these carriers can be controlled through manipulation of polymer composition, including molecular weight, monomer ratio, and chemical end group. The link between antigen and adjuvant release kinetics and the magnitude and speed of immune responses has also recently been demonstrated using a degradable dextran-based microparticle platform (Chen et al., 2018a). Together, these examples demonstrate the potential for application of controlled release to magnify or tune immune responses or to develop tools to study the link between signal kinetics and the resultant responses to inform vaccine design.

In addition to exploring biomaterials platforms to encapsulate signals, several groups have demonstrated applications to decorate the surface of a particulate carrier or to engineer a multivalent scaffold to present multiple copies of an antigen (Irvine and Read, 2020; Karch and Burkhard, 2016). This approach may more closely mimic the typical presentation of surface antigens on viruses or bacteria, recapturing a feature of pathogens typically lost in vaccine production. Further, tunable approaches have demonstrated the potential to control the valency and spacing these signals. For example, Kato et al. (2020) recently demonstrated that high valency presentation of HIV-1 immunogens on nanoparticles dramatically enhanced both the magnitude and breadth of antibody responses compared with low valency presentation of the same immunogen. The King Lab has also developed a library of self-assembling protein nanoparticles of different architectures (Brouwer et al., 2019; Marcandalli et al., 2019) demonstrated to drive a substantial increase in neutralizing antibody titer compared with soluble immunogen for RSV. More generally, these approaches demonstrate how nano-assemblies of different structures can be used to capture features of pathogens—for example, the relatively sparse density of

envelope spikes on the HIV virion, compared with the tightly packed expression of hemagglutinin in the surface of influenza. This variable can be tuned to study and identify the physical arrangement of cues that is most immunogenic, independent of the typical pathogen architecture.

This concept of harnessing materials to probe fundamental questions about the immune system also extends beyond exploring the role of antigen valency and spacing. As noted above, screening different combinations of signals—antigen, TLR ligand or ligands, STING agonists, etc.—can facilitate the study of how engaging different inflammatory pathways skews resultant T and B cell function. Similarly, carrier size, shape, and stability can impact the persistence and localization of these cues following injection (Chen et al., 2018a; Niikura et al., 2013). Furthermore, several recent papers demonstrate intrinsic immunogenic properties of many materials employed in nano- and micro-scale assemblies, including PLGA, polystyrene, chitosan, alginate, and more (Andorko and Jewell, 2017; Demento et al., 2009; Park and Babensee, 2012; Sharp et al., 2009). These findings underscore the opportunity to study and select a carrier that not only enhances control over *in vivo* delivery, but also contributes to skew immune responses down a particular path of cell differentiation and expansion. Finally, many of these questions and ideas could be difficult to access without the inclusion of a biomaterial component, suggesting the power and exciting potential of combining engineering and immunology to develop new delivery platforms, inform novel vaccine design, and expand our understanding of the immune system.

## CONCLUSIONS

Incredible progress has been made in the field of mRNA vaccines in the last decade. Optimization in mRNA design, LNP composition, as well as in manufacturing processes have led to mRNA vaccines that are well-tolerated and immunogenic in humans, stable, and can be scaled up to hundreds of millions of doses. The use of standardized processes and reagents, the ability to combine multiple mRNA antigens in the same LNP therefore targeting multi-pathogens simultaneously, the lack of vector immunity, and the robust immune responses confirmed in several clinical studies make mRNA vaccines a disruptive technology that may change vaccine development in the incoming years. In addition, due to the relative recent application of mRNA for large-scale vaccine applications, there is much room for improvements and new developments.

Ads vector vaccines have also evolved to become promising vaccine platforms. Optimal Ad vaccine vector design involves the selection of non-prevalent vector serotypes, and the structural components of Ad vectors can be harnessed and modified for enhanced tropism, efficient transduction, and optimal antigen expression. Ad vectors can be developed rapidly and manufactured at commercial scale, and vector potency and stability characteristics support single-shot vaccines that do not require a frozen cold chain. The development of Ad vectors against multiple pathogens illustrates their flexibility and their promise for current and future vaccine applications.

Finally, the application of biomaterials and engineering to enhance control of vaccine delivery has shown promise to enhance vaccine efficacy and to tune the nature of responses elicited. Taken together, these innovations in vaccine science have the potential to address many shortcomings of conventional vaccine technologies and will likely play a major role in the development of future vaccines for both existing and novel pathogens.

## DECLARATION OF INTERESTS

L.A.B., D.K.E., and A.C. are employees of Moderna Inc. and hold equities from the company. D.H.B. is a co-inventor on SARS-CoV-2 vaccine patents that have been licensed.

## REFERENCES

Abbink, P., Lemckert, A.A., Ewald, B.A., Lynch, D.M., Denholtz, M., Smits, S., Holterman, L., Damen, I., Vogels, R., Thorner, A.R., et al. (2007). Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. J. Virol. *81*, 4654–4663.

Abbink, P., Larocca, R.A., De La Barrera, R.A., Bricault, C.A., Moseley, E.T., Boyd, M., Kirilova, M., Li, Z., Ng'ang'a, D., Nanayakkara, O., et al. (2016). Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. Science *353*, 1129–1132.

Abbink, P., Kirilova, M., Boyd, M., Mercado, N., Li, Z., Nityanandam, R., Nanayakkara, O., Peterson, R., Larocca, R.A., Aid, M., et al. (2018). Rapid Cloning of Novel Rhesus Adenoviral Vaccine Vectors. J. Virol. *92*, e01924-17.

Adams, D., Gonzalez-Duarte, A., O'Riordan, W.D., Yang, C.C., Ueda, M., Kristen, A.V., Tournev, I., Schmidt, H.H., Coelho, T., Berk, J.L., et al. (2018). Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N. Engl. J. Med. *379*, 11–21.

Africa Regional Commission for the Certification of Poliomyelitis Eradication (2020). Certifying the interruption of wild poliovirus transmission in the WHO African region on the turbulent journey to a polio-free world. Lancet Glob. Health *8*, e1345–e1351.

Akinc, A., Querbes, W., De, S., Qin, J., Frank-Kamenetsky, M., Jayaprakash, K.N., Jayaraman, M., Rajeev, K.G., Cantley, W.L., Dorkin, J.R., et al. (2010). Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. Mol. Ther. *18*, 1357–1364.

Al-Dosari, M.S., and Gao, X. (2009). Nonviral gene delivery: principle, limitations, and recent progress. AAPS J. *11*, 671–681.

Anderson, E.J., Rouphael, N.G., Widge, A.T., Jackson, L.A., Roberts, P.C., Makhene, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruijssers, A.J., et al. (2020). Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. N. Engl. J. Med. *383*, 2427–2438.

Andorko, J.I., and Jewell, C.M. (2017). Designing biomaterials with immunomodulatory properties for tissue engineering and regenerative medicine. Bioeng. Transl. Med. *2*, 139–155.

Artusi, S., Miyagawa, Y., Goins, W.F., Cohen, J.B., and Glorioso, J.C. (2018). Herpes Simplex Virus Vectors for Gene Transfer to the Central Nervous System. Diseases *6*, 74.

Baden, L.R., El Sahly, H.M., Essink, B., Kotloff, K., Frey, S., Novak, R., Diemert, D., Spector, S.A., Rouphael, N., Creech, C.B., et al. (2021). Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N. Engl. J. Med. *384*, 403–416.

Baden, L.R., Stieh, D.J., Sarnecki, M., Walsh, S.R., Tomaras, G.D., Kublin, J.G., McElrath, M.J., Alter, G., Ferrari, G., Montefiori, D., et al.; Traverse/HVTN 117/HPX2004 Study Team (2020). Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVERSE): a randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. Lancet HIV *7*, e688–e698.

Bahl, K., Senn, J.J., Yuzhakov, O., Bulychev, A., Brito, L.A., Hassett, K.J., Laska, M.E., Smith, M., Almarsson, Ö., Thompson, J., et al. (2017). Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. Mol. Ther. *25*, 1316–1327.

Baker, A.T., Greenshields-Watson, A., Coughlan, L., Davies, J.A., Uusi-Kerttula, H., Cole, D.K., Rizkallah, P.J., and Parker, A.L. (2019). Diversity within the adenovirus fiber knob hypervariable loops influences primary receptor interactions. Nat. Commun. *10*, 741.

Barouch, D.H., and Picker, L.J. (2014). Novel vaccine vectors for HIV-1. Nat. Rev. Microbiol. *12*, 765–771.

Barouch, D.H., O'Brien, K.L., Simmons, N.L., King, S.L., Abbink, P., Maxfield, L.F., Sun, Y.H., La Porte, A., Riggs, A.M., Lynch, D.M., et al. (2010). Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. Nat. Med. *16*, 319–323.

Barouch, D.H., Kik, S.V., Weverling, G.J., Dilan, R., King, S.L., Maxfield, L.F., Clark, S., Ng'ang'a, D., Brandariz, K.L., Abbink, P., et al. (2011). International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations. Vaccine *29*, 5203–5209.

Barouch, D.H., Tomaka, F.L., Wegmann, F., Stieh, D.J., Alter, G., Robb, M.L., Michael, N.L., Peter, L., Nkolola, J.P., Borducchi, E.N., et al. (2018). Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). Lancet *392*, 232–243.

Bartok, E., and Hartmann, G. (2020). Immune Sensing Mechanisms that Discriminate Self from Altered Self and Foreign Nucleic Acids. Immunity *53*, 54–77.

Ben-Akiva, E., Est Witte, S., Meyer, R.A., Rhodes, K.R., and Green, J.J. (2018). Polymeric micro- and nanoparticles for immune modulation. Biomater. Sci. *7*, 14–30.

Bernstein, D.I., Reap, E.A., Katen, K., Watson, A., Smith, K., Norberg, P., Olmsted, R.A., Hoeper, A., Morris, J., Negri, S., et al. (2009). Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. Vaccine *28*, 484–493.

Brouwer, P.J.M., Antanasijevic, A., Berndsen, Z., Yasmeen, A., Fiala, B., Bijl, T.P.L., Bontjer, I., Bale, J.B., Sheffler, W., Allen, J.D., et al. (2019). Enhancing and shaping the immunogenicity of native-like HIV-1 envelope trimers with a two-component protein nanoparticle. Nat. Commun. *10*, 4272.

Bullard, B.L., Corder, B.N., Gordon, D.N., Pierson, T.C., and Weaver, E.A. (2020). Characterization of a Species E Adenovirus Vector as a Zika virus vaccine. Sci. Rep. *10*, 3613.

Champion, C.R. (2020). Heplisav-B: A Hepatitis B Vaccine With a Novel Adjuvant. Ann. Pharmacother. Published online September 28, 2020. https://doi.org/10.1177/1060028020962050.

Chen, H., Xiang, Z.Q., Li, Y., Kurupati, R.K., Jia, B., Bian, A., Zhou, D.M., Hutnick, N., Yuan, S., Gray, C., et al. (2010). Adenovirus-based vaccines: comparison of vectors from three species of adenoviridae. J. Virol. *84*, 10522–10532.

Chen, N., Xia, P., Li, S., Zhang, T., Wang, T.T., and Zhu, J. (2017). RNA sensors of the innate immune system and their detection of pathogens. IUBMB Life *69*, 297–304.

Chen, N., Johnson, M.M., Collier, M.A., Gallovic, M.D., Bachelder, E.M., and Ainslie, K.M. (2018a). Tunable degradation of acetalated dextran microparticles enables controlled vaccine adjuvant and antigen delivery to modulate adaptive immune responses. J. Control. Release *273*, 147–159.

Chen, Y.H., Keiser, M.S., and Davidson, B.L. (2018b). Viral Vectors for Gene Transfer. Curr. Protoc. Mouse Biol. *8*, e58.

Chung, Y.H., Beiss, V., Fiering, S.N., and Steinmetz, N.F. (2020). COVID-19 Vaccine Frontrunners and Their Nanotechnology Design. ACS Nano *14*, 12522–12537.

Cirelli, K.M., Carnathan, D.G., Nogal, B., Martin, J.T., Rodriguez, O.L., Upadhyay, A.A., Enemuo, C.A., Gebru, E.H., Choe, Y., Viviano, F., et al. (2019). Slow Delivery Immunization Enhances HIV Neutralizing Antibody and Germinal Center Responses via Modulation of Immunodominance. Cell *177*, 1153–1171.e28.

Conrad, S.J., and Liu, J. (2019). Poxviruses as Gene Therapy Vectors: Generating Poxviral Vectors Expressing Therapeutic Transgenes. Methods Mol. Biol. *1937*, 189–209.

Corbett, K.S., Edwards, D.K., Leist, S.R., Abiona, O.M., Boyoglu-Barnum, S., Gillespie, R.A., Himansu, S., Schäfer, A., Ziwawo, C.T., DiPiazza, A.T., et al. (2020a). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature *586*, 567–571.

Corbett, K.S., Flynn, B., Foulds, K.E., Francica, J.R., Boyoglu-Barnum, S., Werner, A.P., Flach, B., O'Connell, S., Bock, K.W., Minai, M., et al. (2020b). Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N. Engl. J. Med. *383*, 1544–1555.

Coughlan, L. (2020). Factors Which Contribute to the Immunogenicity of Non-replicating Adenoviral Vectored Vaccines. Front. Immunol. *11*, 909.

Crenshaw, B.J., Jones, L.B., Bell, C.R., Kumar, S., and Matthews, Q.L. (2019). Perspective on Adenoviruses: Epidemiology, Pathogenicity, and Gene Therapy. Biomedicines *7*, 61.

Demento, S.L., Eisenbarth, S.C., Foellmer, H.G., Platt, C., Caplan, M.J., Mark Saltzman, W., Mellman, I., Ledizet, M., Fikrig, E., Flavell, R.A., and Fahmy, T.M. (2009). Inflammasome-activating nanoparticles as modular systems for optimizing vaccine efficacy. Vaccine *27*, 3013–3021.

Dimitrov, J.D., and Lacroix-Desmazes, S. (2020). Noncanonical Functions of Antibodies. Trends Immunol. *41*, 379–393.

El Andari, J., and Grimm, D. (2020). Production, Processing, and Characterization of synthetic AAV Gene Therapy Vectors. Biotechnol. J. *16*, e2000025.

Ertl, H.C. (2016). Viral vectors as vaccine carriers. Curr. Opin. Virol. *21*, 1–8.

Espeseth, A.S., Cejas, P.J., Citron, M.P., Wang, D., DiStefano, D.J., Callahan, C., Donnell, G.O., Galli, J.D., Swoyer, R., Touch, S., et al. (2020). Modified mRNA/lipid nanoparticle-based vaccines expressing respiratory syncytial virus F protein variants are immunogenic and protective in rodent models of RSV infection. NPJ Vaccines *5*, 16.

Fan, Y., and Moon, J.J. (2017). Particulate delivery systems for vaccination against bioterrorism agents and emerging infectious pathogens. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. *9*, 10.1002/wnan.1403.

Feldman, R.A., Fuhr, R., Smolenov, I., Mick Ribeiro, A., Panther, L., Watson, M., Senn, J.J., Smith, M., Almarsson, Ö., Pujar, H.S., et al. (2019). mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials. Vaccine *37*, 3326–3334.

Fenner, F., Henderson, D.A., Arita, I., Jezek, Z., and Ladnyi, I.D. (1988). Smallpox and Its Eradication, *Volume 6* (World Health Organization).

Fields, P.A., Kowalczyk, D.W., Arruda, V.R., Armstrong, E., McCleland, M.L., Hagstrom, J.N., Pasi, K.J., Ertl, H.C., Herzog, R.W., and High, K.A. (2000). Role of vector in activation of T cell subsets in immune responses against the secreted transgene product factor IX. Mol. Ther. *1*, 225–235.

Folegatti, P.M., Ewer, K.J., Aley, P.K., Angus, B., Becker, S., Belij-Rammerstorfer, S., Bellamy, D., Bibi, S., Bittaye, M., Clutterbuck, E.A., et al.; Oxford COVID Vaccine Trial Group (2020). Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet *396*, 467–478.

Fries, C.N., Curvino, E.J., Chen, J.-L., Permar, S.R., Fouda, G.G., and Collier, J.H. (2020). Advances in nanomaterial vaccine strategies to address infectious diseases impacting global health. Nat. Nanotechnol. Published online August 17, 2020. https://doi.org/10.1038/s41565-020-0739-9.

Gammon, J.M., and Jewell, C.M. (2019). Engineering Immune Tolerance with Biomaterials. Adv. Healthc. Mater. *8*, e1801419.

Geall, A.J., Verma, A., Otten, G.R., Shaw, C.A., Hekele, A., Banerjee, K., Cu, Y., Beard, C.W., Brito, L.A., Krucker, T., et al. (2012). Nonviral delivery of self-amplifying RNA vaccines. Proc. Natl. Acad. Sci. USA *109*, 14604–14609.

Geisbert, T.W., Bailey, M., Hensley, L., Asiedu, C., Geisbert, J., Stanley, D., Honko, A., Johnson, J., Mulangu, S., Pau, M.G., et al. (2011). Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge. J. Virol. *85*, 4222–4233.

Goldstein, N., Bockstal, V., Bart, S., Luhn, K., Robinson, C., Gaddah, A., Callendret, B., and Douoguih, M. (2020). Safety and Immunogenicity of Heterologous and Homologous Two Dose Regimens of Ad26- and MVA-Vectored

Ebola Vaccines: A Randomized, Controlled Phase 1 Study. J. Infect. Dis. Published online September 16, 2020. https://doi.org/10.1093/infdis/jiaa586.

Guhaniyogi, J., and Brewer, G. (2001). Regulation of mRNA stability in mammalian cells. Gene *265*, 11–23.

Hartnell, F., Brown, A., Capone, S., Kopycinski, J., Bliss, C., Makvandi-Nejad, S., Swadling, L., Ghaffari, E., Cicconi, P., Del Sorbo, M., et al. (2019). A Novel Vaccine Strategy Employing Serologically Different Chimpanzee Adenoviral Vectors for the Prevention of HIV-1 and HCV Coinfection. Front. Immunol. *9*, 3175.

Hassett, K.J., Benenato, K.E., Jacquinet, E., Lee, A., Woods, A., Yuzhakov, O., Himansu, S., Deterling, J., Geilich, B.M., Ketova, T., et al. (2019). Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. Mol. Ther. Nucleic Acids *15*, 1–11.

Hayton, E.J., Rose, A., Ibrahimsa, U., Del Sorbo, M., Capone, S., Crook, A., Black, A.P., Dorrell, L., and Hanke, T. (2014). Safety and tolerability of conserved region vaccines vectored by plasmid DNA, simian adenovirus and modified vaccinia virus ankara administered to human immunodeficiency virus type 1-uninfected adults in a randomized, single-blind phase I trial. PLoS ONE *9*, e101591.

Holtkamp, S., Kreiter, S., Selmi, A., Simon, P., Koslowski, M., Huber, C., Türeci, O., and Sahin, U. (2006). Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. Blood *108*, 4009–4017.

Iacobelli-Martinez, M., and Nemerow, G.R. (2007). Preferential activation of Toll-like receptor nine by CD46-utilizing adenoviruses. J. Virol. *81*, 1305–1312.

Iampietro, M.J., Larocca, R.A., Provine, N.M., Abbink, P., Kang, Z.H., Bricault, C.A., and Barouch, D.H. (2018). Immunogenicity and Cross-Reactivity of Rhesus Adenoviral Vectors. J. Virol. *92*, e00159-18.

Ilyinskii, P.O., Roy, C.J., O'Neil, C.P., Browning, E.A., Pittet, L.A., Altreuter, D.H., Alexis, F., Tonti, E., Shi, J., Basto, P.A., et al. (2014). Adjuvant-carrying synthetic vaccine particles augment the immune response to encapsulated antigen and exhibit strong local immune activation without inducing systemic cytokine release. Vaccine *32*, 2882–2895.

Irvine, D.J., and Read, B.J. (2020). Shaping humoral immunity to vaccines through antigen-displaying nanoparticles. Curr. Opin. Immunol. *65*, 1–6.

Irvine, D.J., Aung, A., and Silva, M. (2020). Controlling timing and location in vaccines. Adv. Drug Deliv. Rev. *158*, 91–115.

Iwasaki, A., and Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. Nat. Immunol. *5*, 987–995.

Jackson, L.A., Anderson, E.J., Rouphael, N.G., Roberts, P.C., Makhene, M., Coler, R.N., McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., et al. (2020). An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N. Engl. J. Med. *383*, 1920–1931.

Jayaraman, M., Ansell, S.M., Mui, B.L., Tam, Y.K., Chen, J., Du, X., Butler, D., Eltepu, L., Matsuda, S., Narayanannair, J.K., et al. (2012). Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. Angew. Chem. Int. Ed. Engl. *51*, 8529–8533.

Jiao, S., Williams, P., Berg, R.K., Hodgeman, B.A., Liu, L., Repetto, G., and Wolff, J.A. (1992). Direct gene transfer into nonhuman primate myofibers in vivo. Hum. Gene Ther. *3*, 21–33.

John, S., Yuzhakov, O., Woods, A., Deterling, J., Hassett, K., Shaw, C.A., and Ciaramella, G. (2018). Multi-antigenic human cytomegalovirus mRNA vaccines that elicit potent humoral and cell-mediated immunity. Vaccine *36*, 1689–1699.

Johnson, J., Anderson, B., and Pass, R.F. (2012). Prevention of maternal and congenital cytomegalovirus infection. Clin. Obstet. Gynecol. *55*, 521–530.

Kamen, A., and Henry, O. (2004). Development and optimization of an adenovirus production process. J. Gene Med. *6* (*Suppl 1*), S184–S192.

Karch, C.P., and Burkhard, P. (2016). Vaccine technologies: From whole organisms to rationally designed protein assemblies. Biochem. Pharmacol. *120*, 1–14.

Karikó, K., Buckstein, M., Ni, H., and Weissman, D. (2005). Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity *23*, 165–175.

Karikó, K., Muramatsu, H., Ludwig, J., and Weissman, D. (2011). Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res. *39*, e142.

Karlsson, J., Vaughan, H.J., and Green, J.J. (2018). Biodegradable Polymeric Nanoparticles for Therapeutic Cancer Treatments. Annu. Rev. Chem. Biomol. Eng. *9*, 105–127.

Kasturi, S.P., Skountzou, I., Albrecht, R.A., Koutsonanos, D., Hua, T., Nakaya, H.I., Ravindran, R., Stewart, S., Alam, M., Kwissa, M., et al. (2011). Programming the magnitude and persistence of antibody responses with innate immunity. Nature *470*, 543–547.

Kato, Y., Abbott, R.K., Freeman, B.L., Haupt, S., Groschel, B., Silva, M., Menis, S., Irvine, D.J., Schief, W.R., and Crotty, S. (2020). Multifaceted Effects of Antigen Valency on B Cell Response Composition and Differentiation In Vivo. Immunity *53*, 548–563.e8.

Kitonsa, J., Ggayi, A.B., Anywaine, Z., Kisaakye, E., Nsangi, L., Basajja, V., Nyantaro, M., Watson-Jones, D., Shukarev, G., Ilsbroux, I., et al. (2020). Implementation of accelerated research: strategies for implementation as applied in a phase 1 Ad26.ZEBOV, MVA-BN-Filo two-dose Ebola vaccine clinical trial in Uganda. Glob. Health Action *13*, 1829829.

Kuai, R., Sun, X., Yuan, W., Ochyl, L.J., Xu, Y., Hassani Najafabadi, A., Scheetz, L., Yu, M.Z., Balwani, I., Schwendeman, A., and Moon, J.J. (2018). Dual TLR agonist nanodiscs as a strong adjuvant system for vaccines and immunotherapy. J. Control. Release *282*, 131–139.

Larocca, R.A., Mendes, E.A., Abbink, P., Peterson, R.L., Martinot, A.J., Iampietro, M.J., Kang, Z.H., Aid, M., Kirilova, M., Jacob-Dolan, C., et al. (2019). Adenovirus Vector-Based Vaccines Confer Maternal-Fetal Protection against Zika Virus Challenge in Pregnant IFN-$\alpha\beta R^{-/-}$ Mice. Cell Host Microbe *26*, 591–600.e4.

Lee, C.S., Bishop, E.S., Zhang, R., Yu, X., Farina, E.M., Yan, S., Zhao, C., Zheng, Z., Shu, Y., Wu, X., et al. (2017). Adenovirus-Mediated Gene Delivery: Potential Applications for Gene and Cell-Based Therapies in the New Era of Personalized Medicine. Genes Dis. *4*, 43–63.

Lemarchand, P., Jaffe, H.A., Danel, C., Cid, M.C., Kleinman, H.K., Stratford-Perricaudet, L.D., Perricaudet, M., Pavirani, A., Lecocq, J.P., and Crystal, R.G. (1992). Adenovirus-mediated transfer of a recombinant human alpha 1-antitrypsin cDNA to human endothelial cells. Proc. Natl. Acad. Sci. USA *89*, 6482–6486.

Leppek, K., Das, R., and Barna, M. (2018). Functional 5′ UTR mRNA structures in eukaryotic translation regulation and how to find them. Nat. Rev. Mol. Cell Biol. *19*, 158–174.

Liang, F., Lindgren, G., Lin, A., Thompson, E.A., Ols, S., Röhss, J., John, S., Hassett, K., Yuzhakov, O., Bahl, K., et al. (2017). Efficient Targeting and Activation of Antigen-Presenting Cells In Vivo after Modified mRNA Vaccine Administration in Rhesus Macaques. Mol. Ther. *25*, 2635–2647.

Lin, A., Liang, F., Thompson, E.A., Vono, M., Ols, S., Lindgren, G., Hassett, K., Salter, H., Ciaramella, G., and Loré, K. (2018). Rhesus Macaque Myeloid-Derived Suppressor Cells Demonstrate T Cell Inhibitory Functions and Are Transiently Increased after Vaccination. J. Immunol. *200*, 286–294.

Linares-Fernández, S., Lacroix, C., Exposito, J.Y., and Verrier, B. (2020). Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends Mol. Med. *26*, 311–323.

Lindgren, G., Ols, S., Liang, F., Thompson, E.A., Lin, A., Hellgren, F., Bahl, K., John, S., Yuzhakov, O., Hassett, K.J., et al. (2017). Induction of Robust B Cell Responses after Influenza mRNA Vaccination Is Accompanied by Circulating Hemagglutinin-Specific ICOS+ PD-1+ CXCR3+ T Follicular Helper Cells. Front. Immunol. *8*, 1539.

Liu, M.A., and Ulmer, J.B. (2005). Human clinical trials of plasmid DNA vaccines. Adv. Genet. *55*, 25–40.

Logunov, D.Y., Dolzhikova, I.V., Zubkova, O.V., Tukhvatulin, A.I., Shcheblyakov, D.V., Dzharullaeva, A.S., Grousova, D.M., Erokhova, A.S., Kovyrshina, A.V., Botikov, A.G., et al. (2020). Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two

formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet *396*, 887–897.

Lu, L.L., Suscovich, T.J., Fortune, S.M., and Alter, G. (2018). Beyond binding: antibody effector functions in infectious diseases. Nat. Rev. Immunol. *18*, 46–61.

Luis, A. (2020). The Old and the New: Prospects for Non-Integrating Lentiviral Vector Technology. Viruses *12*, 1103.

Lutz, J., Lazzaro, S., Habbeddine, M., Schmidt, K.E., Baumhof, P., Mui, B.L., Tam, Y.K., Madden, T.D., Hope, M.J., Heidenreich, R., and Fotin-Mleczek, M. (2017). Unmodified mRNA in LNPs constitutes a competitive technology for prophylactic vaccines. NPJ Vaccines *2*, 29.

Lynn, G.M., Laga, R., Darrah, P.A., Ishizuka, A.S., Balaci, A.J., Dulcey, A.E., Pechar, M., Pola, R., Gerner, M.Y., Yamamoto, A., et al. (2015). In vivo characterization of the physicochemical properties of polymer-linked TLR agonists that enhance vaccine immunogenicity. Nat. Biotechnol. *33*, 1201–1210.

Marcandalli, J., Fiala, B., Ols, S., Perotti, M., de van der Schueren, W., Snijder, J., Hodge, E., Benhaim, M., Ravichandran, R., Carter, L., et al. (2019). Induction of Potent Neutralizing Antibody Responses by a Designed Protein Nanoparticle Vaccine for Respiratory Syncytial Virus. Cell *176*, 1420–1431.e17.

Maruggi, G., Chiarot, E., Giovani, C., Buccato, S., Bonacci, S., Frigimelica, E., Margarit, I., Geall, A., Bensi, G., and Maione, D. (2017). Immunogenicity and protective efficacy induced by self-amplifying mRNA vaccines encoding bacterial antigens. Vaccine *35*, 361–368.

Matsuda, K., Huang, J., Zhou, T., Sheng, Z., Kang, B.H., Ishida, E., Griesman, T., Stuccio, S., Bolkhovitinov, L., Wohlbold, T.J., et al. (2019). Prolonged evolution of the memory B cell response induced by a replicating adenovirus-influenza H5 vaccine. Sci. Immunol. *4*, eaau2710.

Mauger, D.M., Cabral, B.J., Presnyak, V., Su, S.V., Reid, D.W., Goodman, B., Link, K., Khatwani, N., Reynders, J., Moore, M.J., and McFadyen, I.J. (2019). mRNA structure regulates protein expression through changes in functional half-life. Proc. Natl. Acad. Sci. USA *116*, 24075–24083.

McGrory, W.J., Bautista, D.S., and Graham, F.L. (1988). A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology *163*, 614–617.

Mennechet, F.J.D., Paris, O., Ouoba, A.R., Salazar Arenas, S., Sirima, S.B., Takoudjou Dzomo, G.R., Diarra, A., Traore, I.T., Kania, D., Eichholz, K., et al. (2019). A review of 65 years of human adenovirus seroprevalence. Expert Rev. Vaccines *18*, 597–613.

Mercado, N.B., Zahn, R., Wegmann, F., Loos, C., Chandrashekar, A., Yu, J., Liu, J., Peter, L., McMahan, K., Tostanoski, L.H., et al. (2020). Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature *586*, 583–588.

Mulligan, M.J., Lyke, K.E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Raabe, V., Bailey, R., Swanson, K.A., et al. (2020). Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature *586*, 589–593.

Nelson, J., Sorensen, E.W., Mintri, S., Rabideau, A.E., Zheng, W., Besin, G., Khatwani, N., Su, S.V., Miracco, E.J., Issa, W.J., et al. (2020). Impact of mRNA chemistry and manufacturing process on innate immune activation. Sci. Adv. *6*, eaaz6893.

Niikura, K., Matsunaga, T., Suzuki, T., Kobayashi, S., Yamaguchi, H., Orba, Y., Kawaguchi, A., Hasegawa, H., Kajino, K., Ninomiya, T., et al. (2013). Gold nanoparticles as a vaccine platform: influence of size and shape on immunological responses in vitro and in vivo. ACS Nano *7*, 3926–3938.

Northrup, L., Christopher, M.A., Sullivan, B.P., and Berkland, C. (2016). Combining antigen and immunomodulators: Emerging trends in antigen-specific immunotherapy for autoimmunity. Adv. Drug Deliv. Rev. *98*, 86–98.

Orenstein, W.A., and Ahmed, R. (2017). Simply put: Vaccination saves lives. Proc. Natl. Acad. Sci. USA *114*, 4031–4033.

Orlandini von Niessen, A.G., Poleganov, M.A., Rechner, C., Plaschke, A., Kranz, L.M., Fesser, S., Diken, M., Löwer, M., Vallazza, B., Beissert, T., et al. (2019). Improving mRNA-Based Therapeutic Gene Delivery by Expression-Augmenting 3′ UTRs Identified by Cellular Library Screening. Mol. Ther. *27*, 824–836.

Ozawa, S., Clark, S., Portnoy, A., Grewal, S., Brenzel, L., and Walker, D.G. (2016). Return On Investment From Childhood Immunization In Low- And Middle-Income Countries, 2011-20. Health Aff. (Millwood) *35*, 199–207.

Pandey, A., and Galvani, A.P. (2019). The global burden of HIV and prospects for control. Lancet HIV *6*, e809–e811.

Pantó, L., Podgorski, I.I., Jánoska, M., Márkó, O., and Harrach, B. (2015). Taxonomy proposal for Old World monkey adenoviruses: characterisation of several non-human, non-ape primate adenovirus lineages. Arch. Virol. *160*, 3165–3177.

Park, J., and Babensee, J.E. (2012). Differential functional effects of biomaterials on dendritic cell maturation. Acta Biomater. *8*, 3606–3617.

Pearson, R.M., Podojil, J.R., Shea, L.D., King, N.J.C., Miller, S.D., and Getts, D.R. (2019). Overcoming challenges in treating autoimmuntity: Development of tolerogenic immune-modifying nanoparticles. Nanomedicine (Lond.) *18*, 282–291.

Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Pérez Marc, G., Moreira, E.D., Zerbini, C., et al.; C4591001 Clinical Trial Group (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N. Engl. J. Med. *383*, 2603–2615.

Pollard, C., Rejman, J., De Haes, W., Verrier, B., Van Gulck, E., Naessens, T., De Smedt, S., Bogaert, P., Grooten, J., Vanham, G., and De Koker, S. (2013). Type I IFN counteracts the induction of antigen-specific immune responses by lipid-based delivery of mRNA vaccines. Mol. Ther. *21*, 251–259.

Probst, J., Weide, B., Scheel, B., Pichler, B.J., Hoerr, I., Rammensee, H.G., and Pascolo, S. (2007). Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent. Gene Ther. *14*, 1175–1180.

Rathinam, V.A., and Fitzgerald, K.A. (2011). Innate immune sensing of DNA viruses. Virology *411*, 153–162.

Rauch, S., Roth, N., Schwendt, K., Fotin-Mleczek, M., Mueller, S.O., and Petsch, B. (2020). mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents. bioRxiv. https://doi.org/10.1101/2020.10.23.351775.

Roberts, D.M., Nanda, A., Havenga, M.J., Abbink, P., Lynch, D.M., Ewald, B.A., Liu, J., Thorner, A.R., Swanson, P.E., Gorgone, D.A., et al. (2006). Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. Nature *441*, 239–243.

Rodrigues, F.M.P., Marlow, R., Simões, M.J., Danon, L., Ladhani, S., and Finn, A. (2020). Association of Use of a Meningococcus Group B Vaccine With Group B Invasive Meningococcal Disease Among Children in Portugal. JAMA *324*, 2187–2194.

Rosenfeld, M.A., Yoshimura, K., Trapnell, B.C., Yoneyama, K., Rosenthal, E.R., Dalemans, W., Fukayama, M., Bargon, J., Stier, L.E., Stratford-Perricaudet, L., et al. (1992). In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. Cell *68*, 143–155.

Sadoff, Le Gars, J.M., Shukarev, G., Heerwegh, D., Truyers, C., de Groot, A.M., Stoop, J., Tete, S., Van Damme, W., Leroux-Roels, I., et al. (2020). Safety and immunogenicity of the Ad26.COV2.S COVID-19 vaccine candidate: interim results of a phase 1/2a, double-blind, randomized, placebo-controlled trial. medRxiv. https://doi.org/10.1101/2020.09.23.20199604.

Sadoff, J., Le Gars, M., Shukarev, G., Heerwegh, D., Truyers, C., de Groot, A.M., Stoop, J., Tete, S., Van Damme, W., Leroux-Roels, I., et al. (2021). Interim Results of a Phase 1-2a Trial of Ad26.COV2.S Covid-19 Vaccine. N. Engl. J. Med. Published online January 13, 2021. https://doi.org/10.1056/NEJMoa2034201.

Sahin, U., Karikó, K., and Türeci, Ö. (2014). mRNA-based therapeutics–developing a new class of drugs. Nat. Rev. Drug Discov. *13*, 759–780.

Shan, C., Muruato, A.E., Nunes, B.T.D., Luo, H., Xie, X., Medeiros, D.B.A., Wakamiya, M., Tesh, R.B., Barrett, A.D., Wang, T., et al. (2017). A live-attenuated Zika virus vaccine candidate induces sterilizing immunity in mouse models. Nat. Med. *23*, 763–767.

Sharp, F.A., Ruane, D., Claass, B., Creagh, E., Harris, J., Malyala, P., Singh, M., O'Hagan, D.T., Pétrilli, V., Tschopp, J., et al. (2009). Uptake of particulate

vaccine adjuvants by dendritic cells activates the NALP3 inflammasome. Proc. Natl. Acad. Sci. USA *106*, 870–875.

Shirley, J.L., de Jong, Y.P., Terhorst, C., and Herzog, R.W. (2020). Immune Responses to Viral Gene Therapy Vectors. Mol. Ther. *28*, 709–722.

Shuman, S. (1990). Catalytic activity of vaccinia mRNA capping enzyme subunits coexpressed in Escherichia coli. J. Biol. Chem. *265*, 11960–11966.

Singh, G., Song, S., Choi, E., Lee, P.B., and Nahm, F.S. (2020a). Recombinant zoster vaccine (Shingrix®): a new option for the prevention of herpes zoster and postherpetic neuralgia. Korean J. Pain *33*, 201–207.

Singh, R., Dwivedi, S.P., Gaharwar, U.S., Meena, R., Rajamani, P., and Prasad, T. (2020b). Recent updates on drug resistance in Mycobacterium tuberculosis. J. Appl. Microbiol. *128*, 1547–1567.

Stephenson, K.E., D'Couto, H.T., and Barouch, D.H. (2016). New concepts in HIV-1 vaccine development. Curr. Opin. Immunol. *41*, 39–46.

Stephenson, K.E., Wegmann, F., Tomaka, F., Walsh, S.R., Tan, C.S., Lavreys, L., Ansel, J.L., Kanjilal, D.G., Jaegle, K., Nkolola, J.P., et al. (2020). Comparison of shortened mosaic HIV-1 vaccine schedules: a randomised, double-blind, placebo-controlled phase 1 trial (IPCAVD010/HPX1002) and a preclinical study in rhesus monkeys (NHP 17-22). Lancet HIV *7*, e410–e421.

Sumida, S.M., Truitt, D.M., Lemckert, A.A., Vogels, R., Custers, J.H., Addo, M.M., Lockman, S., Peter, T., Peyerl, F.W., Kishko, M.G., et al. (2005). Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. J. Immunol. *174*, 7179–7185.

Tao, W., and Gill, H.S. (2015). M2e-immobilized gold nanoparticles as influenza A vaccine: Role of soluble M2e and longevity of protection. Vaccine *33*, 2307–2315.

Tapia, M.D., Sow, S.O., Ndiaye, B.P., Mbaye, K.D., Thiongane, A., Ndour, C.T., Mboup, S., Ake, J.A., Keshinro, B., Akintunde, G.A., et al.; Zaire EBola Research Alliance group (2020). Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in adults in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect. Dis. *20*, 707–718.

Thompson, E.A., Ols, S., Miura, K., Rausch, K., Narum, D.L., Spångberg, M., Juraska, M., Wille-Reece, U., Weiner, A., Howard, R.F., et al. (2018). TLR-adjuvanted nanoparticle vaccines differentially influence the quality and longevity of responses to malaria antigen Pfs25. JCI Insight *3*, e120692.

Tiono, A.B., Nébié, I., Anagnostou, N., Coulibaly, A.S., Bowyer, G., Lam, E., Bougouma, E.C., Ouedraogo, A., Yaro, J.B.B., Barry, A., et al. (2018). First field efficacy trial of the ChAd63 MVA ME-TRAP vectored malaria vaccine candidate in 5-17 months old infants and children. PLoS ONE *13*, e0208328.

Tostanoski, L.H., and Jewell, C.M. (2017). Engineering self-assembled materials to study and direct immune function. Adv. Drug Deliv. Rev. *114*, 60–78.

Ulmer, J.B., Donnelly, J.J., Parker, S.E., Rhodes, G.H., Felgner, P.L., Dwarki, V.J., Gromkowski, S.H., Deck, R.R., DeWitt, C.M., Friedman, A., et al. (1993). Heterologous protection against influenza by injection of DNA encoding a viral protein. Science *259*, 1745–1749.

Usman, N., and Suarez, M. (2020). Adenoviruses (StatPearls).

van Doremalen, N., Lambe, T., Spencer, A., Belij-Rammerstorfer, S., Purushotham, J.N., Port, J.R., Avanzato, V.A., Bushmaker, T., Flaxman, A., Ulaszewska, M., et al. (2020). ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. Nature *586*, 578–582.

Vorburger, S.A., and Hunt, K.K. (2002). Adenoviral gene therapy. Oncologist *7*, 46–59.

Voysey, M., Clemens, S.A.C., Madhi, S.A., Weckx, L.Y., Folegatti, P.M., Aley, P.K., Angus, B., Baillie, V.L., Barnabas, S.L., Bhorat, Q.E., et al.; Oxford COVID Vaccine Trial Group (2021). Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet *397*, 99–111.

Walsh, D.S., Owira, V., Polhemus, M., Otieno, L., Andagalu, B., Ogutu, B., Waitumbi, J., Hawkridge, A., Shepherd, B., Pau, M.G., et al.; Aeras C-012-402 Study Team (2016). Adenovirus type 35-vectored tuberculosis vaccine has an acceptable safety and tolerability profile in healthy, BCG-vaccinated, QuantiFERON(®)-TB Gold (+) Kenyan adults without evidence of tuberculosis. Vaccine *34*, 2430–2436.

Wang, H., and Mooney, D.J. (2018). Biomaterial-assisted targeted modulation of immune cells in cancer treatment. Nat. Mater. *17*, 761–772.

World Health Organization (2019). Immunization. https://www.who.int/health-topics/vaccines-and-immunization#tab=tab_1.

Windheim, M., Hilgendorf, A., and Burgert, H.G. (2004). Immune evasion by adenovirus E3 proteins: exploitation of intracellular trafficking pathways. Curr. Top. Microbiol. Immunol. *273*, 29–85.

Wolff, J.A., and Budker, V. (2005). The mechanism of naked DNA uptake and expression. Adv. Genet. *54*, 3–20.

Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Acsadi, G., Jani, A., and Felgner, P.L. (1990). Direct gene transfer into mouse muscle in vivo. Science *247*, 1465–1468.

Xiang, Z., Li, Y., Cun, A., Yang, W., Ellenberg, S., Switzer, W.M., Kalish, M.L., and Ertl, H.C. (2006). Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. Emerg. Infect. Dis. *12*, 1596–1599.

Yenkoidiok-Douti, L., and Jewell, C.M. (2020). Integrating Biomaterials and Immunology to Improve Vaccines Against Infectious Diseases. ACS Biomater. Sci. Eng. *6*, 759–778.

Zaiss, A.K., Machado, H.B., and Herschman, H.R. (2009). The influence of innate and pre-existing immunity on adenovirus therapy. J. Cell. Biochem. *108*, 778–790.

Zhang, Z., Ohto, U., Shibata, T., Krayukhina, E., Taoka, M., Yamauchi, Y., Tanji, H., Isobe, T., Uchiyama, S., Miyake, K., and Shimizu, T. (2016). Structural Analysis Reveals that Toll-like Receptor 7 Is a Dual Receptor for Guanosine and Single-Stranded RNA. Immunity *45*, 737–748.

Zhou, D., Cun, A., Li, Y., Xiang, Z., and Ertl, H.C. (2006). A chimpanzee-origin adenovirus vector expressing the rabies virus glycoprotein as an oral vaccine against inhalation infection with rabies virus. Mol. Ther. *14*, 662–672.

Zhu, F.C., Guan, X.H., Li, Y.H., Huang, J.Y., Jiang, T., Hou, L.H., Li, J.X., Yang, B.F., Wang, L., Wang, W.J., et al. (2020a). Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet *396*, 479–488.

Zhu, F.C., Li, Y.H., Guan, X.H., Hou, L.H., Wang, W.J., Li, J.X., Wu, S.P., Wang, B.S., Wang, Z., Wang, L., et al. (2020b). Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet *395*, 1845–1854.