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REVIEW

COVID-19 Vaccine Frontrunners and Their Nanotechnology Design

Young Hun Chung, Veronique Beiss, Steven N. Fiering,* and Nicole F. Steinmetz*

Cite This: https://dx.doi.org/10.1021/acsnano.0c07197

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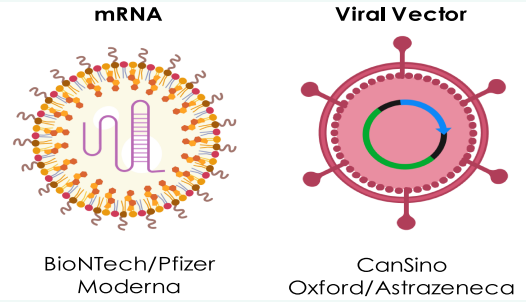
ABSTRACT: Humanity is experiencing a catastrophic pandemic. SARS-CoV-2 has spread globally to cause significant morbidity and mortality, and there still remain unknowns about the biology and pathology of the virus. Even with testing, tracing, and social distancing, many countries are struggling to contain SARS-CoV-2. COVID-19 will only be suppressible when herd immunity develops, either because of an effective vaccine or if the population has been infected and is resistant to reinfection. There is virtually no chance of a return to pre-COVID-19 societal behavior until there is an effective vaccine. Concerted efforts by physicians, academic laboratories, and companies around the world have improved detection and treatment and made promising early steps, developing many vaccine candidates at a pace that has been unmatched for prior diseases. As of August 11, 2020, 28 of these companies have advanced into clinical trials with Moderna, CanSino, the University of Oxford, BioNTech, Sinovac, Sinopharm, Anhui Zhifei Longcom, Inovio, Novavax, Vaxine, Zydus Cadila, Institute of Medical Biology, and the Gamaleya Research Institute having moved beyond their initial safety and immunogenicity studies. This review analyzes these frontrunners in the vaccine development space and delves into their posted results while highlighting the role of the nanotechnologies applied by all the vaccine developers.



KEYWORDS: *sars-cov-2, vaccine, moderna, biontech, pfizer, cansino, university of oxford, astrazeneca, mrna, viral vector*

BACKGROUND

In December 2019, the World Health Organization (WHO) Country Office in China was first alerted to an unknown outbreak of contagious and often severe lower respiratory illnesses originating from the city of Wuhan, the biggest city in and capital of China's Hubei province.[1] The cause of the respiratory illness is a virus of the betacoronavirus class now termed coronavirus infectious disease-19 (COVID-19). The virus was named SARS-CoV-2 due to its genetic and structural similarity with SARS-CoV.[1,2] On March 11, 2020, the WHO officially identified SARS-CoV-2 as a pandemic due to its quick global spread.[1] As of August 11, 2020, there are 19,936,210 confirmed cases worldwide and 732,499 deaths due to SARS-CoV-2.[3] The continued rise of both cases and deaths necessitates the rapid development of an effective SARS-CoV-2 vaccine. The second wave happening in some countries that have reopened their economies further accentuates this need.[4,5] While masking, social distancing, and contact tracing can slow the spread of this virus, it appears too infectious to be eliminated by these strategies, and a vaccine is essential to enable a return to normal human social interaction.

Fortunately, in the relatively few months since SARS CoV-2 was identified as the cause of COVID-19, over two hundred academic laboratories and companies have undertaken vaccine development, and many are making record time in advancing to clinical trials (Table S1).[6,7] Moderna reached clinical trials 63 days after their sequence selection.[8] It is striking that an unestablished nanotechnology formulation reached clinical testing almost a full month before established approaches (*i.e.*, inactivated and live-attenuated vaccines) entered clinical trials.[9,10] This highlights the opportunity for less developed technology platforms in vaccine development and, if proven successful, may enable a more rapid response to future emergent infectious diseases. It is also of note that in previous severe coronavirus outbreaks of SARS-CoV and MERS-CoV clinical trials were not reached until 25 and 22 months after the outbreaks began.[11] Older severe infectious disease outbreaks such as Dengue and Chikungunya did not reach clinical trials until 52 and 19 years after the outbreak.[11] The improved speed into clinical trials is hopeful, but despite the rapid progress, there are still reasons for concern.

Received: August 26, 2020
Accepted: October 5, 2020

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Vaccine development takes time as the vaccines must not only be protective but also safe. Unlike other drugs that are delivered into sick patients, vaccines are administered into healthy patients and require very high safety margins.[12] Therefore, the population should be carefully monitored if vaccine candidates are widely administered based on Emergency Use Authorization. This is especially vital as for past respiratory diseases such as SARS-CoV, MERS-CoV, respiratory syncytial virus, and measles it had been shown that antibodies can exacerbate disease severity through antibody-dependent enhancement.[13] Many of the vaccines that are frontrunners are preclinical nanotechnologies and have not been proven in clinical settings. For instance, mRNA vaccines have been in development and clinical testing for the past 30 years, but the technology has not been previously approved.[14] The platform technology offers speed and adaptability, so these vaccine candidates can be rapidly developed by repurposing previously developed nanostructures as shown by Moderna.[15] Likewise, Novavax's vaccine is also modeled off of their previous vaccine against influenza.[16] Even so, the vaccines must be rigorously tested for safety before widespread vaccination can occur, which Moderna and Novavax have accomplished through their Phase I studies.[17,18] Beyond Moderna and Novavax, several other companies have moved beyond their safety and immunogenicity Phase I and II clinical studies and have released pertinent data corresponding to these trials.[17,19−23] This review analyzes these posted results and highlights the nanotechnological aspects of the vaccines from these leading companies as well as summarizes the potential of other rapidly developed vaccines in clinical trials.

VACCINATION IMMUNOLOGY

To gain a better understanding of the clinical data, it is important to understand concepts in vaccine immunology. There is no "one size fits all" protective antiviral immune response. Every virus is different with different routes of infection, different range of infectable cell types, and different associated pathology. Accordingly, the immune response best suited for protection against each virus will also be variable.[24] Other factors such as sex, age, pregnancy, and route of infection can also influence the immune response.[24,25] It is widely reported that some people become heavily infected with SARS-CoV-2, but remain asymptomatic, and that some become critically ill and succumb to the disease. This extreme variability in response to infection underscores the variability of individual immune responses to this virus, suggesting that there may not be a single perfect strategy that will achieve uniform long-lasting immunity in everyone. The specific immune responses that elicit the most rapid and dependable viral clearance need to be understood and replicated by the vaccines. Major unanswered questions are whether humoral and/or cellular cytotoxic responses are required, what types of helper T cells are most effective (*e.g.*, Th1 vs Th2 vs Th17) as well as what isotype of antibody response (*e.g.* IgG vs IgA) most effectively protects against this virus.[26−28] Most of these questions are being answered through laboratory studies as well as through analysis of serum and circulating cells from recovered patients.

Given the variability of host immune responses, there is unfortunately no guarantee that a vaccine, even if it has progressed into advanced clinical trials, will protect against SARS-CoV-2. While a single vaccination can confer lifelong protection against small pox[29] or poliovirus,[30] HIV continues to evade protection by vaccination despite a major worldwide effort to develop an effective HIV vaccine.[31] Additionally, there are indications that respiratory viruses are especially difficult to protect against with vaccines. The respiratory syncytial virus is a prime example in which there are no approved vaccines, despite considerable efforts to develop one.[32]

One reason for vaccine failure against respiratory viruses is that the respiratory tract, including the lungs, is an external mucosal surface that is protected by the generation of secreted IgA antibodies; yet, the antibodies measured to determine whether an experimental subject has "responded" to a vaccine often focus on IgG, IgM, or total immunoglobulin in the blood.[33,34] Most vaccines are delivered intramuscularly, and mucosal immunity and IgA secretion is thereby minimal.[35] Furthermore, eliciting IgA production from conventional vaccines is difficult, and vaccines may lack the immunogenicity required to elicit necessary IgA protection.[36] Regardless, there are efforts and reports on development of SARS-CoV-2 vaccine candidates that can elicit IgA responses (see Table S1). For instance, Altimmune, an adenovirus (Ad)-based nonreplicating viral vector vaccine administered intranasally, showed 29-fold IgA induction in mice.[37] Other companies such as Stabilitech Biopharma Limited and Quadram Institute Biosciences are also developing mucosal vaccines.[38,39] The value of IgA or other immunoglobulin isotypes in protection against SARS-CoV-2 has not been fully elucidated, but it is believed that IgA can prevent SARS-CoV-2 binding to the airway epithelium thereby helping to block both initial infection and subsequent transmission.[34,37] It is important to note that it is not known what role, if any, IgA plays in protection against SARS-CoV-2 and that many of the current vaccines are not specifically looking to activate IgA responses. Of course, it is possible that IgA production will not be important for an effective vaccine, or may even be harmful, as IgA production was negatively correlated to increased severity in COVID-19 patients.[40]

SARS-CoV-2 is unusual for a respiratory virus in that it binds to a receptor, angiotensin converting enzyme 2 (ACE2), expressed in virtually all organs,[40] but especially in the lungs,[41] brain,[42] and gut.[43] Therefore, unlike most respiratory viruses, SARS-CoV-2 has broader biodistribution and can cause considerable damage outside the respiratory system. It adversely affects the digestive, urogenital, central nervous, and circulatory systems, and the pervasiveness of the ACE2 receptor is why symptoms are highly variable and can range among dyspnea, diarrhea, headache, high blood pressure, venous thromboembolism, and more.[40] Therefore, since much of the pathology is outside the airway due to systemic viral infection, a vaccine that elicits IgG antibodies could protect patients from systemic circulation of the virus. IgG antibodies opsonize the targeted antigens presenting the opsonized products to phagocytes while also activating the complement system.[44]

Another hallmark of vaccine development is T-cell involvement, and differences in T-cell responses can influence generation of high affinity and neutralizing antibodies (NAbs) as well as elimination of infected cells.[45] Immune memory and generation of high affinity class-switched antibodies are highly dependent on T-cells and normally do not develop without proper T-cell involvement.[46,47] Immune memory is the main driver of long-term immunoprotection, and studies have shown that immune titers from patients infected with the first SARS-CoV can have significant antibody levels for up to 3 years postinfection.[48] Such antibody maintenance would be

https://dx.doi.org/10.1021/acsnano.0c07197
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extremely beneficial in the fight against SARS-CoV-2, and this prolonged immune memory could potentially confer long-term protection by a vaccine. However, it is likely that periodic booster vaccination will be necessary in areas of rebounding cases as is done for other infectious diseases.

It is currently unclear whether any of the tested vaccines will confer protection against SARS-CoV-2. Fortunately, as noted below, there are encouraging early results from multiple vaccines that are safe and immunogenic in limited patients. This early success warrants the progression into Phase III clinical trials, and expectations are that 20,000−40,000 subjects would be involved. There is a daunting task left in the effort to develop effective vaccine(s) against SARS-CoV-2, and no guarantee of success, but we are encouraged by the early testing success and rapid development of so many candidate vaccines.

NANOTECHNOLOGY OFFERS OPPORTUNITIES IN VACCINE DESIGN

Nanoparticles and viruses operate at the same size scale; therefore, nanoparticles have an ability to enter cells to enable expression of antigens from delivered nucleic acids (mRNA and DNA vaccines) and/or directly target immune cells for delivery of antigens (subunit vaccines). Many vaccine technologies employ these direct benefits by encapsulating genomic material or protein/peptide antigens in nanoparticles such as lipid nanoparticles (LNPs) or other viruses such as Ads. BioNTech/Pfizer and Moderna encapsulate their mRNA vaccines within LNPs while the University of Oxford/Astrazeneca (from here on out referred to as Oxford/Astrazeneca) and CanSino incorporate antigen-encoding sequences within the DNA carried by Ads.[17,19,22,23] Novavax decorates recombinant S proteins of SARS-CoV-2 onto their proprietary virus like particle (VLP) nanoparticles.[49] The nanoparticles are described in further detail in the discussion below.

Beyond antigen delivery, nanoparticles can codeliver adjuvants to help prime the desired immune responses. Adjuvants are immunostimulatory molecules administered together with the vaccine to help boost immune responses mainly by activating additional molecular receptors that predominantly recognize pathogens or danger signals. These pathways function primarily within the innate immune system, and each adjuvant generally has a different range of stimulation of these pathogen or danger receptors. While the vaccine goal is to stimulate recognition and response by lymphocytes, not innate cells, the activation of the innate immune cells is required to activate the lymphocytes to obtain both B and T-cell responses.[50,51] Encapsulation and/or conjugation of both the adjuvant and antigen within the same nanoparticle enables targeted, synchronous delivery to the same antigen presenting cell (APC). Many adjuvants have previously failed in the clinic due to toxicity issues, and this codelivery can help to direct antigen and adjuvant activity only in APCs that have taken up the antigen thereby reducing off-target side effects.[52] Targeted delivery of appropriate adjuvants can also reduce the necessary antigen dose for immune protection thereby producing a dose-sparing effect.[52] This effect would be abundantly helpful practically and financially in the current pandemic due to the enormous number of doses needed for global vaccination. Furthermore, when adjuvants and antigens are not codelivered they may dissociate quickly within the body, which may lead to off-target effects and/or rapid degradation of the adjuvant reducing the potency of the vaccine.[53] Both Moderna and BioNTech encapsulate their mRNA vaccines within LNPs to protect the mRNA from nuclease degradation.[17,19] Loss of temporal synchronization, *i.e.*, uptake of the antigen and adjuvant by APCs at separate times, can also lead to autoimmunity against host proteins, as the adjuvant can activate APCs that are not primed against the antigen but rather primed against self-antigens.[52] Therefore, nanotechnology offers an opportunity in vaccine design, and there are several strategies that enable codelivery of SARS-CoV-2 antigens and adjuvants. The three main methods are (i) codelivery through encapsulation within or conjugation onto a nanoparticle, (ii) direct antigen-adjuvant conjugation, and (iii) utilizing the delivery vehicle as an adjuvant.[53,54] Another benefit that nanoparticles can confer is multivalent antigen presentation and orientation of subunit antigens in their native form.[55] For example, BioNTech/Pfizer, one of the frontrunner companies producing a SARS-CoV-2 vaccine, formulates their receptor binding domain (RBD) antigens onto a T4 fibritin-derived "foldon" trimerization base to better resemble the trimeric form of the spike (S) protein of SARS-CoV-2.[19,56] Furthermore, display of different RBD epitopes of influenza A on multivalent ferritin nanoparticles can increase production of cross-reactive B-cells against influenza A, and produce a more diverse and effective antibody response than ferritin nanoparticles with homotypic RBD display.[57] The study also found that the multivalent, heterotypic nanoparticles induced a broadly NAb response, which makes the generation of an all-encompassing, universal influenza A vaccine possible.

Lastly, due to the "nano" scale of nanomaterials as well as their composition, they can traffic *in vivo* differently from other materials. The lymphatic system is critical in initiating immune responses as APCs, and other lymphocytes travel from peripheral organs to nearby lymph nodes using the lymphatic system.[58] Accessing the lymphatic system can be challenging, but nanomaterials can traverse the interstitial spaces and access nearby lymph nodes. For instance, inhaled radiolabeled solid lipid nanoparticles were shown to traffic from the alveoli into nearby lymph nodes *via* the lymphatic system, while the free radiotracers trafficked *via* the systemic circulation.[59] Lymphatic drainage especially into lymph nodes near the lungs could be extremely beneficial in the fight against respiratory diseases such as SARS-CoV-2. Companies such as etheRNA and Intravacc are developing intranasally delivered vaccines delivered into the respiratory system that may target such nearby lymph nodes.[60,61]

For further reading on the opportunities of nanotechnology in SARS-CoV-2 vaccine design, we would like to refer the reader to the following review.[62] This review also discusses challenges and opportunities of the manufacturing processes and delivery platforms that are necessary for global vaccination.

THE LANDSCAPE OF COVID-19 VACCINE CANDIDATES

According to the WHO and the Milken Institute, as of August 11, 2020, there are 202 companies and universities worldwide working on a coronavirus vaccine (Table S1).[7,63] The vaccine types vary from well-established vaccines (*e.g.*, inactivated and live-attenuated) to vaccines that have recently gained clinical approval (*e.g.*, subunit) to those that have not yet made the transition into the clinic (*e.g.*, mRNA, DNA, nonreplicating viral vector, replicating viral vector) (Figure 1). Inactivated vaccines are similar to the native pathogen but are replication

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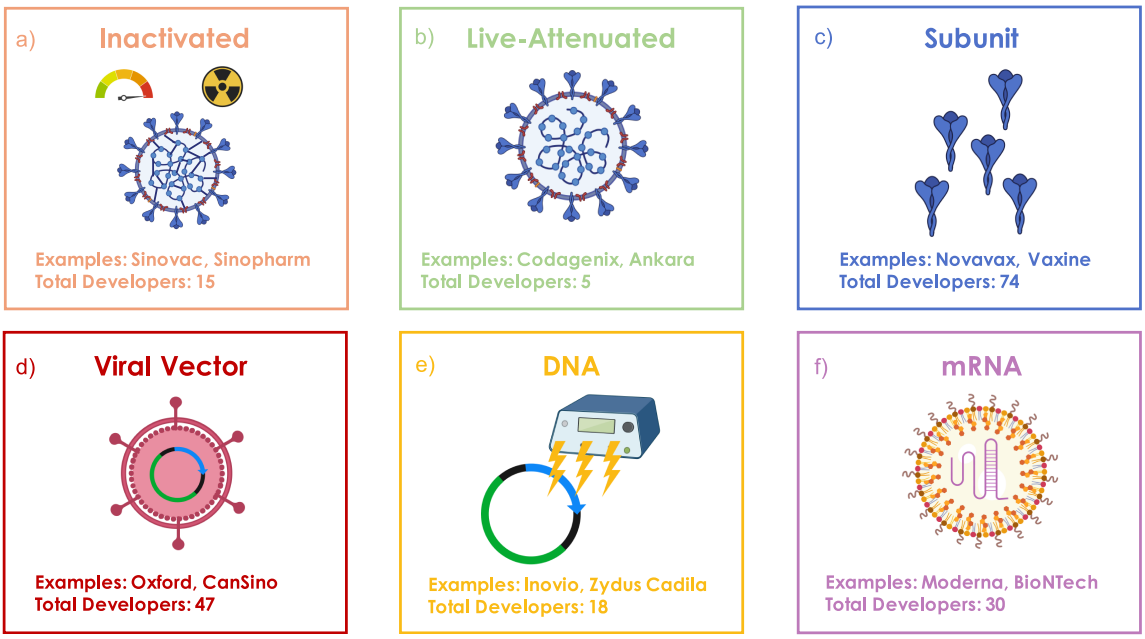

Figure 1. Vaccine types currently under development for SARS-CoV-2. (a) Inactivated vaccine that uses the native virus rendered replication deficient from heat or chemical treatment, (b) live-attenuated vaccine that can replicate, but in a limited manner that cannot cause the disease, (c) subunit vaccine that incorporates subsections of the native virus such as the S protein, (d) viral vector vaccine that encapsulates the genome of a different weakly pathogenic virus with additional DNA that encodes the target viral antigen, (e) DNA vaccine using a DNA plasmid that encodes the target antigen, often administered by electroporation, (f) RNA vaccine of RNA encapsulated within a LNP to decrease RNA degradation and increase translation efficiency. Graphics created with Biorender.com.

deficient due to chemical or heat treatment.[64] Live-attenuated vaccines are weakened forms of the virus that can replicate in a limited manner unable to cause the actual disease,[65] and subunit vaccines confer immunoprotection using portions of the virus.[66] Subunit vaccines are usually less immunogenic and require an adjuvant to stimulate the immune recognition of the antigens in the vaccine. Nucleic acid based vaccines can be mRNA or DNA based, and rather than directly injecting the antigen, express it within host cells using the genomic material.[67] Lastly, viral vector vaccines contain engineered genomes to encode the antigen of the target pathogen. When administered *in vivo*, the viral vectors enter target cells and the genomic material is transcribed and translated for *in vivo* antigen production.[68] They can, but do not always, possess the ability to replicate within the host. Replication deficient vaccines may impart better safety, but immune memory is not as long-lasting. Currently, there are no viral vector vaccines used in the clinic for humans, but there are some that have been utilized for veterinary applications.[69] Of the 202 companies, only a select few have advanced into clinical trials; as of August 11, 2020, the WHO indicates there are 29 vaccine candidates in clinical trials (Figure 2a,b).[7] Of these select few vaccines, even fewer have released data of their initial safety and immunogenicity from completed Phase I and II studies (Table 1). It is noteworthy that every company that has released data has reported positive results from their early Phase clinical trials, allowing advancement into wider and more broadly encompassing efficacy studies.

In this review, we discuss the vaccine nanotechnologies employed by the companies that have released their results in publication form. Two of the companies that have released early results are Moderna and the BioNTech/Pfizer partnership. While Moderna has already published their data in the *New England Journal of Medicine*, BioNTech/Pfizer's data is currently prepublished in *medRxiv* and is awaiting peer review.[17,19] Both employ similar techniques for their vaccine, utilizing mRNA that encodes for subunits of the SARS-CoV-2 S protein (Table 2). On the other hand, Oxford/Astrazeneca and CanSino are both developing vaccines based on non-replicating viral vectors.[7,22,23]

In the clinic, both viral vector vaccines and mRNA vaccines have enjoyed variable successes with neither vaccine type currently approved for a specific use. mRNA vaccines and viral vector vaccines both depend on nucleic acids that encode the target antigen(s) but differ in their approach to vaccination. Instead of mRNA, viral vectors use DNA to encode the antigen of interest. Viral vector vaccines can impart high gene transduction capabilities due to their ability to enter into cells using the virus' own receptor for infection, and efficient intracellular trafficking enables high production of target gene expression.[70] However, immunogenicity of the viral vectors and other adverse effects bear hurdles to safe use. The immunogenicity of the viral vector may decrease vaccine efficiency caused by NAbs against the viral vector in patients that either are developed during the course of vaccination or are pre-existing due to previous exposure to the Ad vectors they use.[70] Viral vectors that humans are not commonly exposed to such as the chimpanzee Ad utilized by Oxford/Pfizer can reduce neutralization.[23] Another safety concern for viral vectors is possible host genome integration, which may cause cancer if integrated into oncogenes and other regulatory sequences.[70]

mRNA vaccines are generally encapsulated within nanoparticles. Both BioNTech/Pfizer and Moderna encapsulate their RNA vaccines within LNPs, which may enable cytoplasmic delivery *via* fusogenic mechanisms.[17,19] However, neither Moderna nor BioNTech/Pfizer specifically mention the use of fusogenic LNPs although BioNTech/Pfizer does

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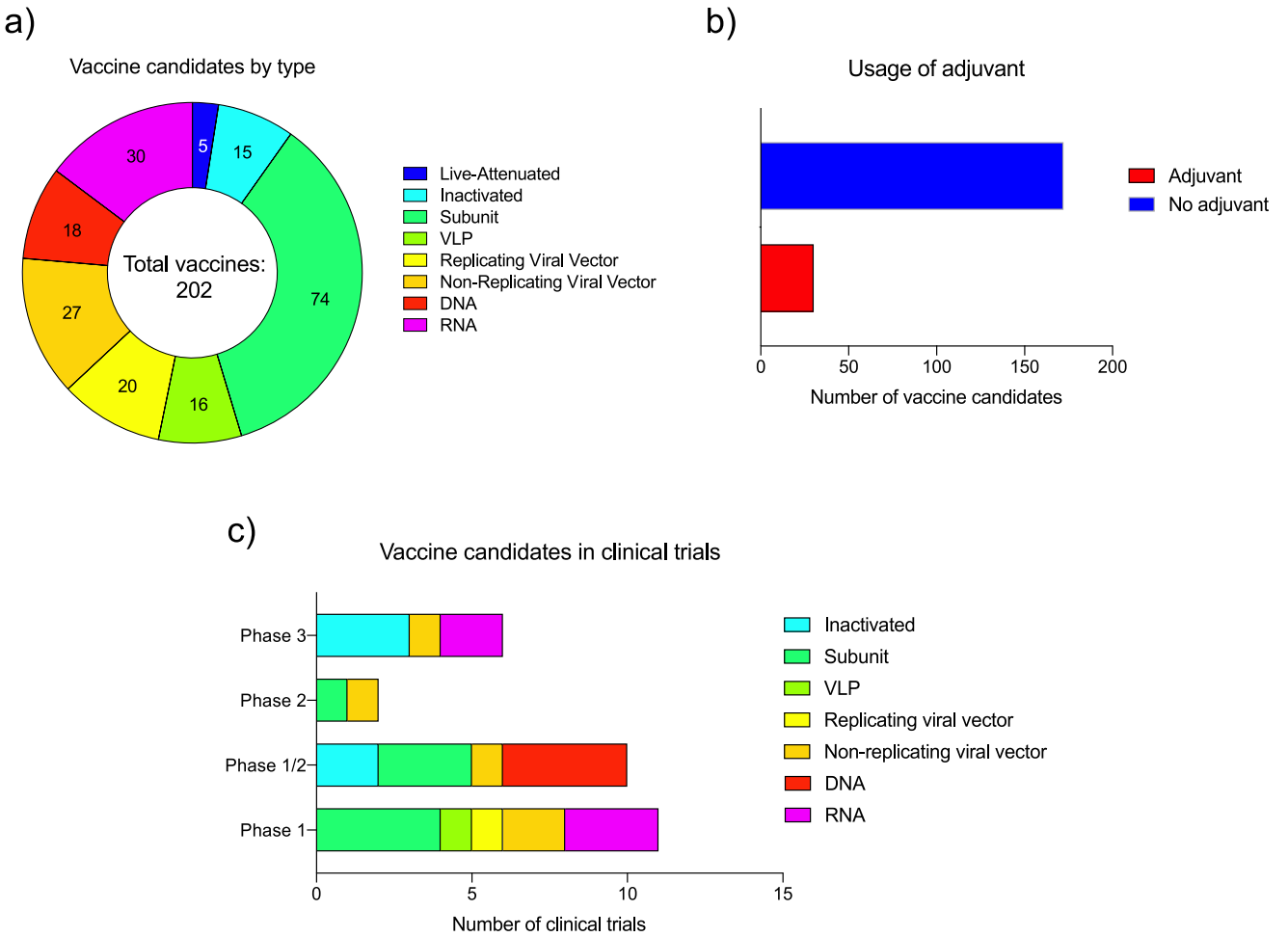

Figure 2. Graphs detailing the vaccines currently in development for SARS-CoV-2 according to the WHO and the Milken Institute as of August 11, 2020. (a) Pie chart of the vaccines by type, (b) bar graph showing the number of vaccines using adjuvants, (c) bar graph of the vaccine candidates in clinical trials.

mention using cationic lipids.[71] Cytoplasmic delivery may improve translation efficiency, but it may also decrease RNA immunostimulation. RNA stimulates the immune system, and therefore acts as an adjuvant, by activating specific toll like receptors (TLRs), mainly TLRs 3, 7, and 8, which are all located within the cell's endosomes.[72] TLRs 7 and 8 are especially important for mRNA vaccines as they recognize single stranded RNA and engage in virus recognition.[73] Encapsulation within nanoparticles improves RNA phagocytosis by APCs with subsequent localization within the endosomes. Failure of the RNA to be endocytosed can lead to nuclease degradation and weak immune stimulation. While advances in nanoparticle design have enabled cytoplasmic delivery of mRNA, synthetic nanoparticles do not match the efficiency of the machinery evolved by the viral vectors that enables trafficking inside the cell. Once inside the cell, the mRNA is translated directly within the cytoplasm; in contrast, DNA plasmids from the viral vectors need to be translocated into the nucleus, transcribed, and exported back to the cytoplasm.[74] This means that mRNA vaccines may produce greater amounts of antigen from smaller doses, but a caveat is that DNA tends to be more stable than mRNA meaning mRNA expression is generally shorter lived. The interplay between stability and translation efficiency can be a big determinant in effective antigen production.

RESULTS—MRNA VACCINES: MODERNA AND BIONTECH/PFIZER

As mentioned above, Moderna and BioNTech/Pfizer utilize mRNA vaccines that encode for the S protein of SARS-CoV-2. The S protein is the viral protein that binds to ACE2 on cells to mediate infection and is a frequent vaccine target since it is expected that antibodies binding to the correct epitope on the S protein could be neutralizing and therefore block intercellular viral spread.[2] The S protein has two subsections: S1 and S2. The S1 subunit contains the RBD and is responsible for initial attachment to the host cell through the ACE2 receptor, while the S2 subunit promotes viral fusion with cells to initiate infection.[2] Moderna's vaccine, mRNA-1273, was codeveloped with the National Institute of Allergy and Infectious Diseases and specifically encodes the prefusion form of the S antigen (named S-2P) that includes a transmembrane anchor and an intact S1−S2 cleavage site.[17] Two proline substitutions in the vaccine mRNA at amino acids 986 and 987, which are within the central helix of the S2 subunit, keep the protein stable in its prefusion conformation.[56] The mRNA is encapsulated within an LNP composed of four lipids (Table 2). The exact formulation is not provided; however, inferences can be made based on previous LNP vaccines by Moderna, which utilize formulations of ionizable lipid, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, cholesterol,

https://dx.doi.org/10.1021/acsnano.0c07197
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Table 1. Summaries of Clinical Trials That Have Been Completed by Companies in the Vaccination Effort Against SARS-CoV-2[a]

Company	Phase	# of Participants	Common Symptoms	Neutralizing Antibody Response?	T-cell Response?	Advancement into Next Phase?	Clinical Trial Registry	Reference
Moderna	I	45	• Pain • Headache • Chills	Yes	Yes	Yes	NCT04283461	17,75
BioNTech, Pfizer (United States)	I/II	45	• Pain • Fatigue • Headache	Yes	Yes	Yes	NCT04368728	19,76
BioNTech, Pfizer (Germany)	I/II	60	• Pain • Fatigue • Headache	Yes	Yes	Yes	NCT04380701	19,77
University of Oxford, Astrazeneca	I/II	1077	• Pain • Fatigue • Headache	Yes	Yes	Yes	NCT04324606	23,78
CanSino Biologics	I	108	• Pain • Fever • Fatigue	Yes	Yes	Yes	NCT04313127	21,79
CanSino Biologics	II	508	• Pain • Fatigue • Headache	Yes	Yes	Yes	NCT04341389	22,80
Inovio Pharmaceuticals	I	40	N/A	Yes	Yes	Yes	NCT04336410	81,82
Sinovac Biotech Ltd.	I/II	744	N/A	Yes	N/A	Yes	NCT04352608	83,84
Sinovac Biotech Ltd.	I/II	422	N/A	N/A	N/A	Yes	NCT04383574	83,84
Sinopharm, Wuhan Institute of Biological Products,	I/II	320	N/A	Yes	N/A	Yes	ChiCTR2000031809	9,85
Sinopharm, Beijing Institute of Biological Products	I/II	1,120	N/A	Yes	N/A	Yes	ChiCTR2000032459	7,86,87
Gamaleya Research Institute	I/II	38	N/A	N/A	N/A	Yes	NCT04436471	88
Gamaleya Research Institute	I/II	38	N/A	N/A	N/A	Yes	NCT04437875	89
Anhui Zhifei Biological Products, Institute of Microbiology of the Chinese Academy of Sciences	I	50	N/A	N/A	N/A	Yes	NCT04445194	90,91
Novavax	I/II	131	• Pain • Headache • Fatigue	Yes	Yes	Yes	NCT04368988	18,92
Vaxine	I	40	N/A	N/A	N/A	Yes	NCT04453852	93,94
Zydus Cadila	I/II	48	N/A	N/A	N/A	Yes	CTRI/2020/07/026352	95,96
Institute of Medical Biology, Chinese Academy of Medical Sciences	I	471	N/A	N/A	N/A	Yes	NCT04470609	97,98

[a]Legend: blue = publicly released data from journals, green = unpublished publicly announced data, N/A = no answer, company did not report.

and polyethylene glycol-lipid.[100] The exact lipids used are not stated. The mRNA-containing LNPs are solubilized and injected directly into the deltoid muscle. Moderna does not explicitly state the use of an adjuvant, but the LNP carrier may be an adjuvant since other lipids have been reported to have adjuvant properties (see the Discussion).[101]

The mRNA utilized by BioNTech/Pfizer encodes for the RBD.[19] Named BNT162b1, the mRNA is modified with single nucleoside incorporations of 1-methylpseudouridine (Table 2), which not only reduces the immunogenicity of the mRNA *in vivo* but also increases its translation.[102] The exact mechanism for increased translation has not been entirely elucidated, but one hypothesis is that the nucleoside modification improves

https://dx.doi.org/10.1021/acsnano.0c07197
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Table 2. Descriptions of Vaccines That Have Moved Beyond Their Initial Safety and Immunogenicity Phase I Studies[a]

Company	Vaccine Type	Vaccine Name	Vaccine Description	Contemporary /Unestablished Vaccine	Reference
Moderna	mRNA	mRNA-1273	mRNA vaccine encoding for the prefusion form of the S antigen that includes a transmembrane anchor and an intact S1-S2 cleavage site. Two proline substitutions keep protein stable in its prefusion form. Encapsulated within an LNP.	Unestablished	17
BioNTech, Pfizer	mRNA	BNT162b1	mRNA vaccine encoding for the RBD of the S1 protein. Single nucleoside incorporations of 1-methyl-pseudouridine. RBD antigen contains a T4 fibritin-derived "foldon" trimerization domain. Encapsulated within an LNP.	Unestablished	19
University of Oxford, Astrazeneca	Non-replicating viral vector	AZD1222	Ad derived from chimpanzee with E1 and E3 deletions encoding for the full-length S protein with a tissue plasminogen activator signal peptide	Unestablished	23
CanSino Biologics	Non-replicating viral vector	Ad5-nCoV	Ad5 with E1 and E3 deletions encoding for the full-length S protein. Gene was derived from the Wuhan-Hu-1 sequence for SARS-CoV2 and contains a tissue plasminogen activator signal peptide	Unestablished	22
Inovio Pharmaceuticals	DNA	INO-4800	Optimized DNA plasmid-based vaccine administered intradermally using a CELLECTRA® 2000 device encoding for the full-length S protein of SARS-CoV-2	Unestablished	107
Sinovac Biotech Ltd.	Inactivated	Coronavac	Formalin-inactivated whole virus particles	Contemporary	108
Sinopharm	Inactivated	N/A	Inactivated vaccine of SARS-CoV-2	Contemporary	7
Gamaleya Research Institute	Non-replicating viral vector	Gam-Covid-Vac Lyo or Sputnik-V	Adenovirus based vaccine combining two adenoviruses: Ad5 and Ad26	Unestablished	109,110
Anhui Zhifei Biological Products, Institute of Microbiology of the Chinese Academy of Sciences	Subunit	N/A	RBD-dimer recombinant subunit vaccine administered with adjuvant	Unestablished	7
Novavax	Subunit	NVX-CoV2373	Stable, pre-fusion, full-length S protein made from Novavax's proprietary VLP nanoparticle technology given with Novavax's proprietary saponin-based adjuvant, Matrix-M™	Unestablished	49
Vaxine	Subunit	Covax-19	Used computer modeling and cloud computing techniques to develop vaccine of S subunit that blocks the S from binding to the ACE-2 receptor *in vivo* and administered with Advax™ adjuvant	Unestablished	111
Zydus Cadila	DNA	ZYCoV-D	DNA plasmid vaccine	Unestablished	7
Institute of Medical Biology, Chinese Academy of Medical Sciences	Inactivated	N/A	Inactivated vaccine	Contemporary	7

[a]Legend: blue = publicly released data from journals, green = unpublished publicly announced data, N/A = no answer, company did not report.

RNA stability by decreasing rates of hydrolysis by phosphodiesterases.[103] Another study proved that RNA modification improves RNA secondary structure stability due to increased RNA stacking.[104] Additionally, as mentioned above, the formulated RBD antigen is constructed on a T4 fibritin-derived "foldon" trimerization base, which helps to guide antigen folding into the native trimeric state.[56,105] The T4 trimerization also augments the immunogenicity mainly due to the multivalent display offered in the trimerized state.[19] It is important to note that BioNTech/Pfizer is testing at least four mRNA vaccines in parallel (BNT162a1, BNT162b1, BNT162b2, and BNT162c2) although the prepublished manuscript only contains data from the BNT162b1 candidate.[77] Similar to Moderna, BioNTech/Pfizer has also encapsulated the mRNA within an LNP and administers the vaccine *via* intramuscular injection.[76] The LNP is provided from a partnership with Acuitas Therapeutics. The exact formulation is not specified, but previous publications from Acuitas Therapeutics states that their LNPs are formulated using ionizable cationic lipids, phosphatidylcholine, cholesterol, and polyethylene glycol-lipid with a ratio of 0.05 of RNA to lipid (w/w). Similarly to Moderna, the exact lipids used were

https://dx.doi.org/10.1021/acsnano.0c07197
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not stated.[71] There is no indication that BioNTech/Pfizer utilizes an additional adjuvant with their vaccine although they do mention that the RNA acts as an adjuvant.

Both BioNTech/Pfizer and Moderna released encouraging safety and immunogenicity data.[17,19] Moderna tested a higher range of mRNA (25, 100, and 250 μg), while BioNTech/Pfizer tested 10, 30, and 100 μg. Safety evaluations noted no severe adverse events that warranted the discontinuation of either trial. Some of the more prominent adverse events in the Moderna trial included pain, headache, and chills, while BioNTech/Pfizer's vaccine mainly caused pain, fatigue, and headache (Table 1). Antibody response was also positive in both trials. Moderna tested antibody response through ELISA assays while BioNTech/Pfizer utilized a RBD-binding IgG assay.[17,19] For Moderna, when comparing the response in vaccinated patients to convalescent serum from past SARS-CoV-2 patients, the 250 μg group generated higher S-2P geometric mean titers (GMTs) by day 15 (163,449 vs 142,140 arbitrary units (AU)), while the 25 and 100 μg groups produced higher GMTs by day 36 (391,018 and 781,399 AU, respectively), 7 days after a second boost. BioNTech/Pfizer recorded neutralizing anti-RBD titers much higher than convalescent serum levels. By day 21 (day of the second dose, or 21 days), the 30 μg group had a higher geometric mean concentration (GMC) than convalescent sera (1,536 vs 602 U/mL), while it took until day 28 (7 days after a second dose) for the 10 μg group (4,813 U/mL). The 100 μg group, which only used one dose, had higher GMC levels by day 21 (1,778 U/mL). Both Moderna and BioNTech/Pfizer tested T-cell responses and demonstrated T_H1 skewed T-cell responses with detectable CD4+ and CD8+ response to their respective antigens.[17,20] Neither developer mentioned the production of antibodies other than IgG. It is difficult to directly compare the results between the trials because measurements and data reporting are not standardized, highlighting an opportunity and need to standardize vaccine trials and reporting requirements.

The vaccination schedule in the Phase III trials by both Moderna and BioNTech/Pfizer will not deviate from their Phase II setups.[106,107] Moderna will continue to boost on day 29 after an initial injection, and BioNTech/Pfizer will boost at day 21. However, Phase III trials will only evaluate one dose. In Moderna's case, the midlevel dose led to higher immunogenicity than the highest dose while BioNTech/Pfizer demonstrated no substantial differences between their mid- and high-level doses.[17,19] Therefore, Moderna and BioNTech/Pfizer both chose to move forward with their midlevel doses (100 μg and 30 μg, respectively). For phase III, Moderna and BioNTech/Pfizer will also vaccinate much larger populations of 30,000 participants each.

RESULTS-NONREPLICATING VIRAL VECTOR VACCINES: OXFORD/ASTRAZENECA AND CANSINO

One of the most explored viral vector options is the Ad, which is currently being used by both CanSino and Oxford/Astrazenca (Table 2). Ads are common cold causing viruses that have a double-stranded DNA genome. Specifically, CanSino is utilizing Ad type 5 (Ad5), giving the vaccine the name Ad5-nCoV.[21] Oxford/Astrazeneca is employing a different viral vector, an Ad derived from the chimpanzee (the use of the chimpanzee vector minimizes possible interaction with prevalent antibodies against Ads), which was subsequently named AZD1222.[23] Ad5-nCoV specifically encodes for the full-length S protein of SARS-CoV-2, unlike both Moderna and BioNTech/Pfizer, which both encoded subunits of the S protein. The gene was derived from the Wuhan-Hu-1 sequence for SARS-CoV-2 and, along with a tissue plasminogen activator signal peptide, was cloned into an E1 and E3 deleted Ad5 vector.[21] Deletion of E1 inactivates the replication potential of the vaccine while deletion of E3 allows for the insertion of larger genes up to 8 kb.[113] The CanSino vectors were solubilized and administered intramuscularly into the arms of patients. Each shot contained 5×10^{10} viral particles per dose, and patients being tested with higher doses would receive multiple shots allowing for administration of multiples of 5×10^{10} particles.

AZD1222 is designed similarly to Ad5-nCoV, with deletion of E1 to block replication and deletion of E3 to enable incorporation of larger genetic cargo into the viral vector.[23,113] The added sequence encodes for the full-length S protein with a tissue plasminogen activator leader sequence, and the S protein sequence is codon-optimized. Key differences between the two viral vector platforms is discussed in more detail within the discussion section. Neither vaccine manufacturer mentions the use of an adjuvant, so these vectors most likely depend on immune recognition of the nonreplicating virus, perhaps through the DNA they carry which can activate TLR9 within endosomes.[114] Another possibility is recognition of the viral capsid, which can occur through both TLR-dependent and TLR-independent mechanisms.[115] The intracellular adaptor protein MyD88 has been shown to play a prominent role in invoking TLR-mediated immunogenicity with viral vectors being able to engage multiple MyD88 signaling pathways.[115]

The data from the phase I/II trials are summarized in Table 1. CanSino has already completed and published results for both their Phase I and II vaccines; the following will only present data from their Phase II trials. The biggest difference between the two trials is that CanSino removed the highest dose from their Phase I trials due to greater adverse events such as severe fever, fatigue, and muscle and joint pain.[21] Therefore, CanSino's Phase II trial only tested the low and medium concentrations at 1×10^{11} and 5×10^{10} viral particles per dose in 253 and 129 patients, respectively.[22] By day 28, the 1×10^{11} group saw RBD-specific antibody GMT levels peak around 656.5 AU while the 5×10^{10} group peaked at around 571 AU. RBD-specific antibody seroconversion occurred in 96% and 97% of patients within the 1×10^{11} and 5×10^{10} groups, respectively. Seroconversion of NAbs occurred in fewer patients at 59 and 47% in the 1×10^{11} and 5×10^{10} groups, respectively. Antibodies were measured using ELISA assays. Neutralization was measured *in vitro* using a live SARS-CoV-2 virus as well as a pseudovirus.

Oxford/Astrazeneca only tested one dose at 5×10^{10} viral particles per dose ($n = 533$) but in a few patients ($n = 10$) also tested a booster of the same dose 28 days after the first.[23] Some of the patients were also prophylactically given a common anti-inflammatory drug, acetaminophen, and in those patients, there were fewer adverse events. Antibody concentrations against the SARS-CoV-2 S protein peaked at day 28 in the single-dose patients (157 AU), while the extra dose further improved response (639 AU within 56 days). The acetaminophen did not block generation of the antibody response. Depending on the neutralization assay used, the vaccine induced neutralizing titers from 62 to 100% of patients in the single-dose group and 100% in the double-dose patients. Both trials also documented moderate increases in T-cell responses as measured by ELISpot assays.[22,23]

https://dx.doi.org/10.1021/acsnano.0c07197
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Adverse events from both the Oxford/Astrazeneca and CanSino vaccines were varied from mild to moderate and did not warrant termination of either trial. The most prominent adverse effects in both the Oxford/Astrazeneca and CanSino trials were pain, fatigue, and headache (Table 1). Oxford/Astrazeneca did not test for other antibodies outside of IgG.[23] CanSino did test for IgM antibodies, but only against the nucleocapsid of SARS-CoV-2 to ensure that participants did not have previous exposure to the virus.[21] For advancement into Phase III clinical trials, CanSino noted that their lowest dose (5×10^{10} particles) demonstrated similar immunogenicity compared to their middle dose (1×10^{11} particles) while also reducing adverse events.[22] Thus, their Phase III trial will move forward at the lowest dose. They are set to begin testing in countries outside of China such as Saudi Arabia, where they already have an agreement to vaccinate 5,000 participants.[116] The company is looking to increase the number of participants by setting up clinical trials in other countries such as Russia, Brazil, and Chile.[116] Oxford/Astrazeneca will continue to test at its dose of $5 \times 10,^{10}$ but in its Brazil trials, will not test a prime boost.[117] This is most likely due to the similar immunogenicity and neutralization capability observed between the single dosed and double dosed groups.[23] They will also test in multiple countries with Phase III trials ongoing in Brazil, South Africa, and the United Kingdom.[23] Oxford/Astrazeneca will continue providing prophylactic acetaminophen for pain management.[117]

DISCUSSION

SARS-CoV-2 has obligated global cooperation and teamwork as well as competition in fighting against the disease. The current vaccination efforts exemplify how the discovery and manufacturing of these vaccines is truly a worldwide effort that occurs within a context of commercial competition. BioNTech is based in Germany, Moderna and Pfizer are in the United States, CanSino is headquartered in China, Oxford is located in the United Kingdom, and Astrazeneca is headquartered in both the United Kingdom and Sweden. There are additional companies involved in clinical trials such as Bharat Biotech, Genexine, and Anges that are headquartered in India, South Korea, and Japan, respectively.[118−120] This is not an exhaustive list of all the countries working on a coronavirus vaccine, as there are other vaccine development efforts spanning the world (Table S1).

The need for vaccines against COVID-19 is so pressing that it is likely that unless one vaccine is much more effective than any other, multiple vaccine candidates will obtain approval in different locations. Generation of huge numbers of necessary doses will also favor production and sale by multiple companies. Multiple vaccine candidates from numerous countries will allow for wider and more rapid distribution to generate global "herd immunity". Most or all of the vaccines are likely to be initially purchased by their countries' government for distribution. Less developed countries are not involved in the vaccine effort, although they can be locations for vaccine testing if COVID-19 is prevalent. This is already evidenced by the Phase III clinical testing of CanSino and Oxford/Astrazeneca in Brazil and other countries. Distribution to developing countries may be highly influenced by vaccine design, price, and scalability. For instance, vaccines that require cold chain distribution or administration by health care professionals involve greater logistical challenges, especially in countries with limited resources.[116,117]

The race for a vaccine is also improved by the fact that there are many different vaccination nanotechnologies at play. Both BioNTech/Pfizer and Moderna are employing vaccination technologies in nucleic acid based vaccines that have not yet progressed beyond clinical trials for previous diseases. However, a key advantage of mRNA vaccines is their quick deployment and generation. In fact, Moderna was the first company to enter into clinical trials for COVID-19 vaccines in the United States and dosed the first patient in its Phase I study within 63 days of sequence selection.[8] The first batch of the vaccine was created within an impressive 25 days of sequence selection. In principle, mRNA vaccines allow an indefinite number of boosting regimens because the vaccine does not carry the antigen which could be blocked by an established neutralization response in seropositive individuals.[121] Furthermore, due to the antigen expression within the host, any *in vivo* post-translational modifications that occur are representative of the native antigen within the body. This is especially crucial for the S protein, which has up to 22 glycosylation sites within each protomer.[122] Areas of glycosylation can negate the neutralization capabilities of antibodies rendering vaccines ineffective even though high antibody titers are measured. Therefore, glycosylation is an important feature to consider when subunit vaccines are produced through heterologous expression. Nucleic acid based vaccines also activate the host's immune system using multiple mechanisms eliciting both B and T-cell responses.[67] RNA can induce immune responses by activating both TLR3 and 7/8 for CD8+ T-cell activation as well as promote antibody production and CD4+ T-cell activation.[72] BioNTech/Pfizer explicitly mentions this in their manuscript. However, their RNA modification also reduces the immunogenicity of their RNA, which may dampen the adjuvant qualities of the RNA. It remains to be seen whether the increased translation capabilities from the RNA modification outweighs the decrease in immunogenicity. Compared to DNA vaccines, RNA vaccines do not require nuclear localization for antigen production allowing for activity once the RNA gains entry into the cell's cytoplasm.[74] On the flip side, this also means that the expression is generally shorter lived and may require boosts while DNA vaccine immunogenicity can persist and may not require multiple dosing.[123,124] A potential way to boost the immune response would be the use of self-amplifying RNA that can replicate *in vivo* using replication machinery that is encoded into the vectors.[125] In fact, Imperial College London in a collaboration with Morningside Ventures has employed this approach for SARS-CoV-2 and is currently in Phase I/II of testing.[126] Furthermore, the half-life of unmodified RNA is very short due to rapid degradation within the body.[121] To help overcome this challenge, there are nucleotide modifications that can increase stability. Complexing the RNA with protamine also reduces RNase degradation while improving TLR-mediated adjuvant activity.[127,128] Both Moderna and BioNTech encapsulate their RNA within LNPs to help protect against degradation as well as employ chemical modifications to improve RNA stability.[17,19]

The nonreplicating viral vector vaccines have also made rapid progress in SARS-CoV-2 vaccination efforts. Both Oxford/Astrazeneca and CanSino have demonstrated safe but immunogenic vaccines warranting advancement into Phase III trials. These viral vectors have been investigated for many years, and technological advances have improved vector generation and large-scale production. Viral vector vaccines

https://dx.doi.org/10.1021/acsnano.0c07197
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also possess inherent immunostimulatory profiles through both TLR-dependent and TLR-independent mechanisms.[62,68] In some instances, they can impart humoral responses as well as promote CD4+ and CD8+ T-cell responses even without the use of another adjuvant;[70] ELISpot assays demonstrated significant T-cell response activation in both the Oxford/Astrazeneca and CanSino trials.[22,23] Another benefit is that viral vectors can enter into dendritic cells (DCs) leading to enhanced antigen presentation and immune cell activation. They can also function after entry into both actively dividing and quiescent cells leading to wide tissue tropism.[129] However, this tropism can also induce more severe side effects.

Humans have been widely exposed to human Ads leading to pre-existing immunity against some Ad vectors, which may impact the effectiveness of the vaccines. Indeed, CanSino noted that 52% of their participants had high pre-existing immunity to Ad5.[22] The 48% of participants that had low pre-existing immunity generated two times the levels of NAbs- and RBD-specific antibodies than the higher pre-existing immunity group, suggesting that pre-existing immunity to the viral vector impairs response to the vaccine. Oxford/Astrazeneca circumvents this issue by using a chimpanzee Ad, where seroprevalence rates are much lower in humans. Oxford/Astrazenca only detected NAbs against the chimpanzee viral vector in one of 98 patients.[23] There are other ways to thwart the issue of pre-existing immunity. For instance, much of the pre-existing immunity generated from Ad is derived from the hypervariable regions of its hexon protein.[130] Genetic modifications of the hypervariable regions as well as to another structural protein, the fiber knob domain, can reduce neutralization.[70] Lastly, Ads infect a wide range of animals from birds and reptiles to bats, and similarly to Oxford/Astrazeneca utilizing less prevalent chimpanzee Ad vectors, one can imagine the repurposing of other Ad viral vectors.[129] Other nonhuman Ad vectors that have been investigated in the past include porcine, bovine, canine, ovine, and fowl viral vectors.[131−133] All of the viral vectors noted above are nonpathogenic toward humans, but can infect mammalian cells, and some—such as the bovine viral vector—are naturally replication deficient imparting high safety.[134−137]

Other Companies in Advanced Clinical Trials. Outside the four major companies discussed above, there are a small number of other companies that have moved beyond initial clinical trials and are now investigating more extensive clinical trials either in Phase II or III. These companies have not published their data but have announced positive results in their trials with press releases and statements. Some of the companies are currently preparing the data for publication. The following information is summarized in both Table 1 and Table 2. Sinovac Biotech and Sinopharm are two of those companies, and they both employ an inactivated form of the virus.[138,139] Inactivated vaccines are well-established and are produced through chemical or heat treatment leading to replication-deficient vaccines.[62] Sinopharm's vaccine has already received emergency authorization in China for employees in state-owned businesses that require global traveling.[139] The company has two products in clinical testing developed by either the Wuhan Institute of Biological Products or the Beijing Institute of Biological Products. The Institute of Medical Biology, Chinese Academy of Medical Sciences is developing another inactivated vaccine and has progressed into Phase II clinical trials, but there is no public information about the clinical trials or the vaccine.[140] Two other companies, Inovio Pharmaceuticals and Zydus Cadila, have recently completed their Phase I testing citing favorable safety results.[82,141] Inovio additionally announced induction of both T-cell and humoral responses.[82] Inovio and Zydus Cadila produce nucleic-acid based vaccines, but unlike Moderna and BioNTech/Pfizer, utilize DNA rather than RNA. Anhui Zhifei Longcom Biopharmaceutical and the Institute of Microbiology of the Chinese Academy of Sciences are codeveloping a subunit vaccine that has moved into Phase II testing, but there have been no public announcements of the trial.[91] The vaccine consists of an RBD dimer from SARS-CoV-2 administered with an adjuvant.[7] Novavax is another subunit vaccine developer that has recently released positive data from their Phase I/II trial, which also had a reassuring safety profile.[18] They employ a full-length S protein subunit vaccine administered with their patented saponin-based Matrix-M adjuvant. The recombinant S protein is generated with mutations at the S1 and S2 cleavage site to protect against protease degradation and additional proline substitutions at the heptad repeat1/central helix to keep the protein in its prefusion conformation. The S protein and Matrix-M adjuvant are mixed together right before injection.[142] The vaccine led to antibody neutralization titers in all patients both against the S protein (after one dose) as well as to the wild-type virus (after two doses). Vaxine announced positive safety data as the latest subunit vaccine that has entered into advanced clinical trials.[94] The vaccine was developed using computer modeling to identify epitopes that may block the S protein from binding to the ACE2 receptor.[112]

The Gamaleya Research Institute based in Russia has been developing nonreplicating Ad vector vaccines.[7] The vaccine consists of two vectors, Ad5 and Ad26.[89,90,143] The vaccine became the first "registered" vaccine in the world, although reports show that only Phase II clinical trials have been completed on a few hundred participants.[111,144] The company has coordinated with the Russian government to skip standard phase III clinical trials, and instead, phase III trials will be held in parallel with broad vaccination efforts. To the best of our knowledge, the results from the phase II clinical trials have not been made public. Even without any public information, Russian officials have stated that they have garnered interest from 20 outside countries with preorders of over a billion doses.[145] Russia plans on mass-producing the vaccine for immediate countrywide vaccination of frontline medical workers and teachers followed by vaccination of the general public in the fall.

Adjuvant Use. Many of the aforementioned vaccines may require the use of an adjuvant to stimulate response against the antigen; however, discussion of the adjuvant is often overlooked. Of the companies addressed in this review, only Anhui Zhifei Longcom, Novavax, Sinovac, and Vaxine explicitly mention the use of an adjuvant.[7,18] Novavax's adjuvant, Matrix-M, boosts vaccine immunogenicity by recruiting APCs to the site of injection thereby increasing antigen presentation to T-cells within draining lymph nodes.[18] The Advax adjuvant used by Vaxine is a polysaccharide microparticle derived from polyfructofuranosyl-D-glucose, and unlike many other adjuvants, Advax boosts the intrinsic immunostimulatory nature of the antigen.[146] In an influenza split vaccine with a Th2 antigen, Advax acted as a Th2 adjuvant, while in an influenza inactivated antigen with a Th1 antigen, it acted as a Th1 adjuvant. To the best of our

https://dx.doi.org/10.1021/acsnano.0c07197
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knowledge, there is no public information on the adjuvant used by Anhui Zhifei Longcom.

BioNTech/Pfizer and Moderna do not explicitly state the use of an adjuvant within their vaccines, but RNA already contains immunostimulatory properties and signals through pathogen recognition receptors.[72] It remains to be seen whether the immunostimulation from RNA is strong enough to confer full protection against SARS-CoV-2. There is also a possibility that the LNP carriers they utilize confer adjuvant properties themselves. Since the discovery that liposomes incorporating dicetyl phosphate provided greater protection against diphtheria toxoid than unmodified liposomes, the study of using lipid carriers also as adjuvants has grown considerably.[101] Unilamellar phospholipid membrane nanoparticles encapsulating viral antigens (otherwise known as virosomes) with adjuvant properties against influenza and hepatitis A are currently already used in the clinic.[147]

Other companies such as GlaxoSmithKline (GSK) and Dynavax have offered their own adjuvants to be tested in combination with different vaccines.[7] GSK's adjuvant system (AS03) consists of α-tocopherol, squalene, and polysorbate 80 in an oil-in-water emulsion and helps boost antigen-specific antibody production by increasing antigen uptake and presentation within lymph nodes.[148] Its adjuvant was utilized previously in the 2009 H1N1 pandemic. Currently, GSK is offering AS03 in collaboration with Clover Biopharmaceuticals[149] and Medicago,[150] which are both in Phase I clinical testing, and Sanofi[151] and Innovax,[152] which remain in preclinical trials. GSK has additionally offered its adjuvant technology to the Coalition for Epidemic Preparedness Innovation, which helped fund companies such as Moderna and Inovio.[153] The Dynavax adjuvant (CpG 1018) is composed of a 22-mer oligonucleotide sequence with the ability to stimulate the TLR9 pathogen recognition receptor for improved CD4+ and CD8+ responses as well as formation of B and T-cell memory.[154] This adjuvant has been utilized with its hepatitis B vaccine for improved antibody response. Dynavax has also partnered with Clover[149] and Medicago[150] as well as Medigen Vaccine,[155] which just entered into Phase I clinical testing. Additionally, Dynavax has offered its adjuvant to Sinovac[156] and Valneva,[157] which remain in the preclinical stage.

As discussed in an earlier section, nanoparticles offer opportunities for codelivery of antigen and adjuvant to target lymph nodes and APCs, and an increasing body of data suggests that codelivery of antigen and adjuvant improves potency of the vaccines at lower doses and reduces side effects.[52,53] BioNTech/Pfizer claims that the mRNA within their vaccines acts as its own adjuvant enabling codelivery of antigen and adjuvant.[19] The viral vectors by Oxford/Astrazeneca and CanSino as well as the viral DNA may act as adjuvants as well.[114,115] However, neither mRNA nor viral vector vaccines have successfully translated into the clinic, and it is clear that more research is needed to fully understand the potential and limitations of the various nanotechnology platforms in vaccine development.

CONCLUSION

There are over a hundred vaccines being developed throughout the world, and the race to be the first effective vaccine has fueled the rapid development of both preclinical and currently utilized vaccine approaches. There is no "one-size-fits-all" solution as each vaccine strategy has both advantages and disadvantages. Of the leading companies, Moderna, BioNTech/Pfizer, and Inovio are producing nucleic acid based vaccines, and early studies from Moderna and BioNTech/Pfizer generated strong antibody. Ad-based vaccines remain early frontrunners as well, with Oxford/Astrazeneca and CanSino likewise publishing favorable early data. Other state-run institutions and companies such as the Gamaleya Research Institute and Sinopharm have advanced further into their respective Phases of clinical trials and have been granted emergency use by their respective countries. Due to the generation of so many vaccines within many different countries, there will potentially be more than one effective vaccine, and it is crucial that effective vaccines are distributed to all parts of the world to generate global herd immunity. Regardless of the first developer, it is of truly noteworthy importance that the global effort to develop, test, produce, and distribute effective vaccines is being done so rapidly. Success in this effort will not only lead to an end to the pandemic, but what is learned will aid in future research and development efforts as well as the implementation of previously unused vaccine types for future diseases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.0c07197.

Data table summarizing COVID-19 vaccines and their type, developer, and status (Table S1) (PDF)

AUTHOR INFORMATION

Corresponding Authors

Steven N. Fiering − *Geisel School of Medicine, Dartmouth College, Hanover, New Hampshire 03755, United States; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire 03766, United States*; Email: steven.n.fiering@dartmouth.edu

Nicole F. Steinmetz − *Department of Bioengineering, Department of NanoEngineering, Department of Radiology, Moores Cancer Center, and Center for Nano-ImmunoEngineering, University of California San Diego, California 92093, United States*; orcid.org/0000-0002-0130-0481; Email: nsteinmetz@ucsd.edu

Authors

Young Hun Chung − *Department of Bioengineering, University of California San Diego, California 92093, United States*; orcid.org/0000-0002-6578-8076

Veronique Beiss − *Department of NanoEngineering, University of California San Diego, California 92093, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsnano.0c07197

Notes

The authors declare the following competing financial interest(s): Drs. Fiering and Steinmetz are co-founders of and have a financial interest in Mosaic Immunoengineering Inc. The other authors declare no potential conflict of interest.

ACKNOWLEDGMENTS

Dr. Soo Khim Chan is thanked for help in compiling the data for Table S1.This work is supported by a grant from the National Science Foundation NSF CMMI-2027668 (to NFS).

VOCABULARY

vaccine: a substance that produces an immune response in the host protecting the host against pathogens
nucleic acid vaccine: vaccine type utilizing either mRNA or DNA that encode viral antigens
viral vector vaccine: vaccine type with DNA encoding the viral antigens of the target pathogen, viral vectors can be nonreplicating or replicating
inactivated vaccine: well-established vaccine type where the native virus is inactivated with heat or chemical treatment
subunit vaccine: vaccine type utilizing portions of the virus as antigens to produce an immune response
adjuvant: an additional immunostimulatory reagent administered alongside the antigen to boost immune responses

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https://dx.doi.org/10.1021/acsnano.0c07197
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