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REVIEW PAPER

COVID-19 therapy approaches and vaccine development: The role of nanotechnology

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) caused an outbreak in Wuhan, China in December 2019, and right after that SARS-COV-2 spreads around the world infecting millions of people worldwide. This virus belongs to wide range virus family and cause moderate to severe signs in patients, the Sars-COV-2, can spread faster than others between humans and leads to severe outbreak. Recently researchers succeed to develop various vaccines including inactivated or attenuated viral vaccines as well as subunit vaccines to prevent SARS-COV-2 infection. Nanotechnology is advantageous for the design of vaccines since nano scale materials could benefit the delivery of antigens, and could be used as adjuvants to potentiate the response to the vaccines. Indeed, among various vaccines entered clinical trials, there are mRNA-based vaccine designed based on lipid nanoparticles. Herein, we summarized SARS-COV-2 structure, pathogenesis, therapeutic approaches and some COVID-19 vaccine candidates and highlighted the role of nanotechnology in developing vaccines against SARS-Cov-2 virus.

Keywords: *RNA, Nanoparticle, SARS-COV-2, Vaccine, Therapy*

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INTRODUCTION

The outbreaks of coronavirus infection by Severe Acute Respiratory Syndrome (SARS) occurred in 2002-2003 which threaten global pandemic along with Middle East Respiratory Syndrome (MERS) in 2011 [2]. A cluster of pneumonia cases of unknown etiology was reported in Wuhan, Hubei Province, China on 31 December 2019. In January 9, 2020, China CDC reported on a novel coronavirus as the cause of this outbreak, which is phylogenetically in the SARS-COV clade [3]. Human coronaviruses constitute a large family of viruses that usually cause mild to moderate upper respiratory illnesses in people similar to common cold [4]. The new virus causing the COVID-19 pandemic is highly transmissible between humans and spread rapidly [5]. Vaccines are essential countermeasures that are urgently needed to control the pandemic [6] and to reduce the mortality and morbidity burden associated with SARS-COV-2 infection [7]. There has been almost 331 candidate vaccines in development worldwide until 4 September 2020 [1]. In June 2020, France, Germany, Italy, and the Netherlands formed the Inclusive Vaccine Alliance to persuade pharmaceutical companies to provide EU member states with affordable COVID-19 vaccines [8].

In the following sections authors aimed at summarizing the recent report on the virus structure, COVID-19 pathogenicity and the most recent therapeutic approaches against this disease. Further, since nanomaterials have been widely used to adjust the immune responses,

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we will discuss vaccines developed by several companies including *Moderna* and *Pfizer* which exploited lipid nanoparticles for vaccine delivery. This technology has definitely made developed vaccines safer, versatile, and more stable.

SARS-COV and SARS-COV-2 similarity

Based on the phylogenetic relationships and genomic structures, the SARS-COV-2 belongs to genera Betacoronavirus (β coronavirus) which has a close sequence similarity to that of severe acute respiratory syndrome-related coronaviruses (SARS-COV). Further, extensive studies reported that the virus uses ACE2 receptor for entry into the cell just like SARS-COV [9]. The genomic sequence of SARS-COV-2 indicates similar but distinct genome composition of SARS-COV and MERS-COV [2]. When comparing the spike protein at 30 ends among coronaviruses, specifically these three betacoronaviruses, the differences are observed in 1273, 21493, and 1270 amino acids, in SARS-COV-2, SARS-COV and MERS-COV, respectively. Genetically, SARS-COV-2 shows greater similarity to SARS-COV (about 79%) than MERS-CoV (about 50%) [10]. Considering the closely related genome of SARS-COV-2 to SARS-COV and MERS-COV and the extensive clinical and experimental data on these viruses, it is somehow predictable that how the host immune system may interact with this particular virus and to what extend the virus may evade such host responses [2]. According to WHO, the mortality rates of SARS and MERS-COV is reported to be 10% and 36%, respectively [11-14]. SARS-COV-2 mortality rate has been reported to be 2%, but only in a few months it's been confirmed that how fast the new virus spreads [15]. As previously indicated by genetic data, SARS-COV-2 is classified as a member of the beta-coronavirus genus which binds to the human angiotensin-converting enzyme 2 receptor [16].

SARS-COV-2 structure

As mentioned earlier, there are four main subgroups of coronaviruses (α, β, γ, and δ) and the human pathogens including COV-OC43, SARS-COV, and MERS-COV belong to β coronavirus subgroup [17, 18]. Studies have shown that the SARS-COV-2 is also a β coronavirus; the amino acid sequences within the seven conserved domains within the genomic open reading frame 1ab (ORF1ab) are 94.6% identical to that of the original SARS-COV [16]. SARS-COV-2 is a spherical enveloped particle bearing single-stranded (positive-sense) RNA associated with a nucleoprotein within a capsid comprising matrix protein. The envelope contains club-shaped glycoprotein projections. Some coronaviruses also contain a hem agglutinin-esterase protein (HE) [19]. Coronaviruses possess the largest genomes among all known RNA viruses, with G + C contents varying from 32% to 43% [20]. The Spike protein present on the virion's outer surface is in a homo-trimeric state which is a crucial recognition factor for virus attachment and host cellular entry [21, 22]. The Glucose Regulating Protein 78 (GRP78) or Binding immunoglobulin protein (BiP) is the master chaperone protein of the unfolded protein response (when unfolded or misfolded proteins accumulate) [23-26].

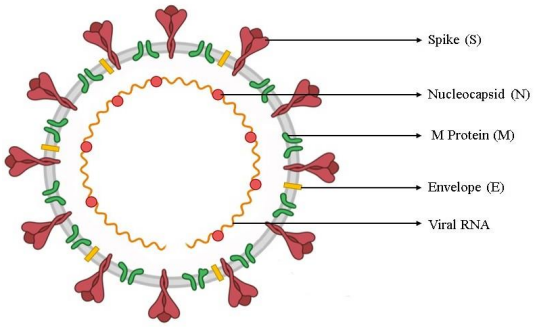

Fig 1. SARS-Cov-2 structure

Pathogenesis of COVID-19

Like SARS-COV, the ACE2 is the main receptor hijacked by SARS-COV-2 for entering the cell [16, 27]. ACE2 is a type I membrane protein which is expressed in lung, heart, kidney and intestine cell and is also reported to be associated with cardiovascular diseases [28]. For host–cell receptor interaction, the receptor-binding domain (RBD) of S1 undergoes hinge-like conformational movements that results in the subsequent hiding or exposing of the determinants of receptor binding [29]. Following virus entry into the host cells and the uncoating stage, the genome is subsequently transcribed and translated. Coronavirus genome replication and transcription occurs at cytoplasmic membranes and involves coordinated processes of both continuous and discontinuous RNA synthesis mediated by the viral replicate, a huge protein complex encoded by the 20-kb replicase gene [30]. Following proteins assembly at cellular membrane and the genomic RNA incorporation, the mature particle is formed by budding from the internal cell

membranes [10]. The rapid viral replication may cause huge epithelial and endothelial cell death and subsequent vascular leakage, triggering the release of exuberant pro-inflammatory cytokines and chemokines. Unfortunately cytokine storm is one of the deadly clinical manifestations of SARS-COV-2 [31].

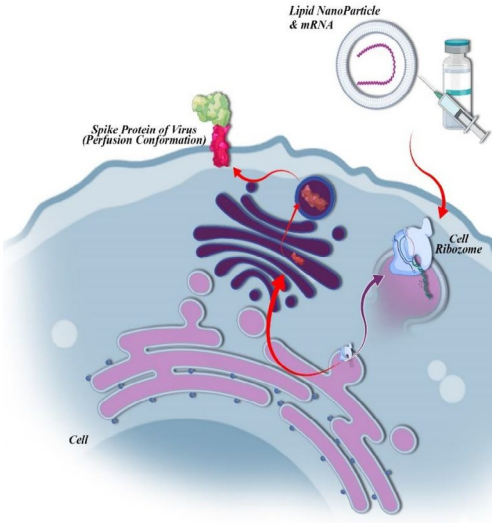

Fig 2. schematic pathogenesis of COVID-19 virus

Therapeutic approaches

Antiviral agents

Remdesivir is known as a promising antiviral drug against a wide array of RNA viruses. Holshue et al. reported on the promising results following treatment of COVID-19 patient with remdesivir [32] and Xiao et al. indicated that remdesivir could effectively control SARS-COV-2 *in vitro* [33]. The report by Spinner et al. demonstrated outstanding results on the potential efficacy of remdesivir in moderate COVID-19 cases for 5-day treatment course compared to the standard care [34]. However, administration of remdesivir COVID-19 is mainly used for severe lower respiratory conditions and, remdesivir efficiency for treatment of low to moderate disease has not been established[35].

Lopinavir/ritonavir is a US Food and Drug Administration (FDA) approved agent for oral combination of HIV treatment. Lopinavir/ritonavir has shown *in vitro* activity against other novel coronaviruses through inhibiting 3-chymotrypsin-like protease [36]. Non randomized cohort studies made it difficult to ensure the direct effect of lopinavir/ritonavir [37]. In hospitalized adult patients infected with severe COVID-19, no advantage was observed following lopinavir–ritonavir therapy beyond standard care. Future trials in patient with severe disease may help to confirm or exclude the possibility of a treatment advantage [38].

Chloroquine and Hydroxychloroquine (CQ/HCQ) have shown to block the virus cellular entry by inhibiting glycosylation of the host receptors, proteolytic processing as well as endosomal acidification [39]. Studies have demonstrated that treatment of hospitalized COVID-19 patients with CQ/HCQ may not completely decrease death risk compared to the standard care, and it is suggested that COVID-19 patients should receive CQ/HCQ therapy only within the context of high quality RCTs [40]. Despite promising clinical outcomes, both agents have shown to cause serious adverse reactions (<10%) including QTc prolongation, hypoglycemia, neuropsychiatric effects, and retinopathy [41, 42].

Convalescent plasma therapy

Convalescent plasma or hyperimmune immunoglobulins is regarded as another potential adjunctive therapy for COVID-19 disease [43]. In a retrospective analysis, it's been shown that convalescent plasma therapy was more efficacious compared to hormonal shock in severe COVID-19 cases, resulting in reduced mortality and shortening hospital stays [44]. It's been demonstrated that patients recovered from COVID-19 bears specific antibodies against SARS-COV-2 within their serum which could prevent re-infection. At the same time, antibodies are capable of limiting viral reproduction in the acute phase of infection. However, the plasma globulin products safety requires further consideration [45].

NANOTECHNOLOGY

Optimally designed and fabricated nanoparticulate delivery vehicles are well suited to penetrate deep into the airway due to their specific physicochemical properties. Hence, nanomedicine has long been exploited for pulmonary delivery of drugs, therapeutic proteins, and mRNAs [46, 47]. Cytokine release syndrome (CRS) is known as one of the main features of COVID-19 disease, resulting from excessive immune responses leading to the severe deterioration of patient health [48-50]. Nanomaterials have been widely used to adjust the immune responses to an optimized level, and such proprieties are further investigated to inhibit

cytokine releases [51]. In the following sections we will discuss vaccines developed by several companies including *Moderna* and *Pfizer* which exploited lipid nanoparticles for vaccine delivery. This technology makes developed vaccines safer, versatile, and more stable [52].

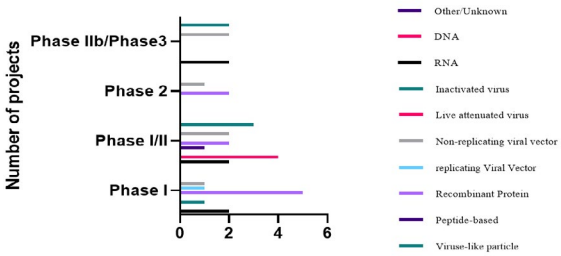

Fig 3. Pipeline of COVID-19 vaccine candidates by technology platform [1]

Vaccine is a well potential rout

Vaccine development is a key strategy to prevent widespread viral infections and reduce the morbidity and mortality rates [53, 54], however, a minimum of 12 to 18 months is required before widespread vaccine deployment [42]. Antiviral vaccines include two broad categories. First, gene-based vaccines which deliver gene sequences encoding protein antigens to be produced by the host cells. These include live virus vaccines, recombinant vaccine vectors, or nucleic acid vaccines. Protein-based vaccines on the other hand include whole-inactivated virus, individual viral proteins or subdomains, or viral proteins assembled as particles [55]. The current status of COVID-19 vaccine development includes three phases including 1) vaccine candidates, 2) preclinical vaccine candidates, and 3) research-stage vaccine candidates. Most of the mentioned vaccines are based on the S antigen either as inactivated vaccines, subunit vaccines, viral vectored vaccines, and nucleic acid-based DNA or mRNA vaccines [56]. For instance, *Moderna* started clinical investigation of mRNA-1273 vaccine just 2 months following virus sequence identification. Viral vectors-based vaccines offer a high level of protein expression and long-term stability and are capable of inducing strong immune responses [1]. Early data are emerging for the most advanced clinical vaccines which induce antibody and T cell responses.

Although the COVID-19 vaccines advanced to clinical development at considerable speed, many uncertainties remain due to the lack of the strong clinical data. Surplus, considering the unusual circumstances associated with developing a vaccine during the pandemic, possibility of success benchmarks for developing traditional vaccine are likely to underrepresent the risks associated with delivering a licensed vaccine for COVID-19 disease [1].

Promising vaccines for SARS-COV-2

mRNA-based Vaccines

Messenger RNAs or mRNAs are types of RNAs found largely in the cytoplasm. During the translation process for the production of protein, mRNAs are translated by ribosomes. mRNA vaccines are robust and highly efficient vaccines that are now being studied in a wide variety of viruses including Zika, influenza, and Ebola viruses. In recent years mRNA engineering and optimization of their formulations made them safe and non-infectious accompanied with validated immunogenicity [57].

Adenovirus type-5 vectored nCoV-19 vaccine

Modern's mRNA COVID-19 vaccine and *CanSino's* non-replicating adenovirus type-5 (Ad5) vectored COVID-19 vaccine is among the vaccines that entered phase 1 clinical trials on March 16, 2020. Preliminary assessment of *CanSino's* non-replicating Ad5 (Adenovirus Type 5) vectored COVID-19 vaccine at 28 days' post-vaccination demonstrated safety, tolerability, and immunogenicity in healthy adults. The Ad5 vectored COVID-19 vaccine has been developed by *Beijing Institute of Biotechnology* (Beijing, China) *and CanSino Biologics* (Tianjin, China). This is a replication defective Ad5 vectored vaccine which express the spike glycoprotein of SARS-COV-2. In the first reported phase I trial, the human adenovirus 5-vectored COVID-19 vaccine showed to induce both pseudovirus-neutralizing and live-virus-neutralizing antibody titers in a similar range in healthy adults at 28 days' post-vaccination. The Ad5 vectored COVID-19 vaccine has been shown to be well tolerated in healthy adults in all three doses groups. The most common adverse effects were mild to moderate fever, fatigue, headache, and muscle pain and there was no difference in the incidence of adverse reactions across the groups. The Onset of detectable immune responses following Ad5 vectored COVID-19 vaccine administration was fast, with T-cell responses peaked at day 14 following vaccination and antibodies peaked at day 28. The antibody responses to the vaccine in the high dose group

was slightly greater compared to that in the middle dose or low dose groups [58].

Moderna N Biotech vaccine demonstrated 94.5% effectiveness of its mRNA-1237 vaccine in phase 3 clinical trial. The trial carried out in collaboration with the National Institute of Allergy and Infectious. This vaccine can be kept for 6 months at -20 and for 30 days at 4-8 which facilitate its use in the world especially in the low-income countries. Further, no outstanding safety concerns were reported and the vaccine are well tolerated. However, efficiency and transmission prevention of mRNA-1273 are not well studied [59].

BNT162b1

BNT162b1 is a lipid-nanoparticle-formulated, nucleoside-modified mRNA vaccine which translate the trimerized receptor-binding domain (RBD) of the spike glycoprotein of SARS-CoV-2 virus. It's been investigated for two dosages separated by 21 days in which the first dose was 10, 30 or 100 µg of BNT162b1. Due to the lack of required immunogenicity following 100 µg doses compared to the 30-µg dose, the second vaccination with 100 µg dose was not performed. There has been an increase in RBD-binding IgG concentrations and SARS-CoV-2 neutralizing titers in sera following augmenting the first dose quantity or after the second dose. This vaccine showed tolerability and safety consistent with those previously observed for mRNA-based vaccines. [60].

ChAdOx1 nCoV-19 vaccine (AZD-1222)

Adenovirus-vectored vaccine ChAdOx1 nCoV-19 developed by the University of Oxford, UK, and *AstraZeneca* encoding the spike protein of SARS-COV-2 has shown to be immunogenic in mice through eliciting a robust humoral and cell-mediated response. A single vaccination with ChAdOx1 nCoV-19 induced humoral as well as cellular immune responses in rhesus macaques with significantly reduced viral loads in broncho alveolar lavage fluid and respiratory tract tissues of vaccinated animals challenged with SARS-COV-2 compared with control animals. Investigations on animal models indicated a significant upregulation of IFN-γ at 1 DPI in ChAdOx1 nCoV-19 vaccinated animals compared to control animals. ChAdOx1 nCoV-19 was also shown to be effective in preventing damage to the lungs following high dose challenge with SARS-COV-2 [61].

In clinical context, the *Oxford* Company vaccine showed to elicit a specific antibody response to the SARS-COV-2 spike glycoprotein and the RBD at 28 days following a single dose administration across all groups including people aged 70 years and older. Further, the booster dose clearly affects the antibody titers at day 56 post vaccination [62]. The safety, tolerability, and immunogenicity of SARS-COV-2 vaccines was also investigated in older populations. The adenovirus 5 vector-based vaccine showed to decrease the reactogenicity in adults aged 55 years and older compared to 18–54 age group after receiving a single dose of vaccine; although the immunogenicity was concurrently decreased in this older aged group [63].

Newcastle disease virus (NDV)

Newcastle disease virus (NDV) vector vaccines translate the spike protein of SARS-COV-2 virus in wild type of spike or in a membrane-anchored format in the absence of the polybasic cleavage site. All NDV vector vaccines raised antibody titers that have shown to be neutralizing when the vaccine was given intramuscularly. Lack of viral antigens in the lung tissue of mice demonstrated the protection of COVID-19 vaccines against mouse-adapted SARS-COV-2 challenge [64].

DNA vaccine

DNA vaccines development is regarded as an innovative approach resulting in the induction of humoral as well as cell-mediated antigen-specific immune responses in systemic and mucosal compartments [65]. Unlike conventional protein-based vaccines, DNA vaccines are based on bacterial plasmids encoding vaccine antigens driven by efficient eukaryotic promoters. The first DNA vaccines investigated in 1990 include injecting RNA or DNA vectors expressing chloramphenicol acetyltransferase, luciferase, and beta-galactosidase into mouse skeletal muscle [66]. DNA vaccines are potentially immunogenic through eliciting both cellular and humoral immunity with favorable stability for molecular reproduction. There are reports demonstrating an effective yet simple way of administering DNA vaccines via the intranasal route in rats to provoke the mucosal immunity through the development of IgA, IgG immunoglobulins and bronchus-associated lymphoid tissue [67]. Yu et al. generated a series of prototype DNA vaccines expressing various S immunogens and

Table 1. Covid-19 virus vaccine characteristics [60, 61, 64, 71, 72]

Vaccine	Vaccine characteristics	Type of vaccine	Pharmaceutical Company
Ad5-nCoV	Adenovirus type 5 vector that expresses S protein	Vector	*CanSino Biologicals*
mRNA-1273	LNP-encapsulated mRNA vaccine encoding S protein	RNA	*Moderna*
INO-4800	DNA plasmid encoding S protein	DNA	*CELLECTRA*
AZD-1222	Replication-deficient adenovirus vector, include the full-length codon-optimized spike protein coding sequence of SARS-Cov-2	Vector	*University of Oxford and AstraZeneca*
BNT162b1	Nucleoside-modified mRNA vaccine	RNA	*BioNTech and Pfizer*
COVAXIN	Inactivated virus-based SARS-Cov-2 vaccine	Inactivated virus	*Bharat Biotech*
CoronaVac	Inactivated virus-based SARS-Cov-2 vaccine	Inactivated virus	*Sinovac Life Sciences*

assessed protective efficacy against intranasal and intratracheal challenge with SARS-COV-2 in rhesus macaques. They demonstrated vaccine protection with substantial (>3.1 and >3.7 log10) reduction in median viral loads in BAL and NS, respectively, and in S immunized animals compared to control. Protection however was not sterilizing but instead appeared to be mediated by rapid immunologic control following challenge [68].

In an investigation, promising results were observed in individuals who received dose-enhancing intramuscular GLS-5300 DNA vaccine against MERS coronavirus. In this study, 75 adults aged 18–50 years in the USA received 0·67, 2, or 6 mg GLS-5300 intramuscularly at baseline, week 4, and 12 followed immediately by a co-localized intramuscular electroporation. The primary outcome of the study was safety concern, which was assessed during the vaccination period for up to 48 weeks following the dose 3 [69]. Plasmid DNA vaccine omit the need for using live viruses and shows a better safety profile. Generation of DNA plasmid vaccine is relatively straightforward, and the double-strand DNA molecules are more stable than the main virus, protein, and mRNA, and can be freeze-dried for long-term storage. The main limitation of plasmid vaccine is low transfection efficacy which requires transfection modalities. For instance, the Inovio's COVID-19 vaccine candidate, INO-4800, uses a handheld electroporation device, CELLECTRA [70].

The engineered plasmid DNA construct, INO-4800, has been designed against SARS-COV-2 S protein by the US *biotech company Inovio Pharmaceuticals.* This vaccine showed to induce cellular and humoral host immune responses in mice and guinea pigs within days following a single immunization, including cross-reactive responses against SARS-COV-2. The data on the immunogenicity of this COVID-19 synthetic DNA vaccine candidate targeting the SARS-COV-2 S protein, supports studies to further develop this candidate in response to the current global health crisis. The induction of T cell responses against SARS-COV-2 as early as day 7 post-vaccine delivery was reported in BALB/C mice. Rapid cellular responses indicates the potential of lowering the viral loads which could further reduce the spread of SARS-COV-2 and the associated COVID-19 illness [71].

Inactivated vaccine

Inactivated vaccines are traditional vaccines consisting of particles of viruses that have lost their ability to induce disease but they can still trigger immunogenicity. Three biotech companies are currently studying inactivated vaccines including *Sinovac*, *Sinopharm*, and *Bharat*. The CoronaVac is a formaldehyde inactivated vaccine produced by Chinese company *Sinovac*. The CoronaVac unlike *Moderna* and *Pfizer* could be stored at 2-8°C for longer than a year. *In vivo* experimental studies in mice, rats and monkeys have shown promising results. The CoronaVac vaccine has shown promising results in phase 1 and 2 clinical trials and showed the capability to induce immunogenicity in 92 and 98% of participants following 3 and 6μg dose administration [72, 73]. In July 2020, phase III clinical trial was conducted to investigate the safety, efficiency, and robustness of CoronaVac

in Brazil [74]. In case of COVAXIN-produced by Bharat- and *Sinopharm*, these vaccines received approval in phase I and II clinical trials and now have entered phase III clinical trial [75]. Some of the features of these vaccine are summarized in Table 1.

Current challenges and future perspective

As mentioned earlier several vaccine candidates have shown to be effective with no significant observed adverse effects. However, the first issue to be considered is how these vaccines work in high-risk populations including cancer patients, diabetes individuals, and older people. Second, clear understanding of the mechanism of protection of each individual vaccine against COVID-19 is required and finally it should be investigated that to what extent vaccines prevent the transmission of the virus to other people. For the first time in the world, regulatory agencies confirmed vaccines faster to speed up the production of COVID-19 vaccines. Indeed, transparency, commitment, and positive collaborations between companies and agencies could assist with decreasing probable safety issues. Although synthetic mRNA vaccines showed promising results, their storage and shipment have been a great concern for developing and poor countries. While the *Pfizer* vaccine needs to be stored at -80 degrees, *Moderna* claims that mRNA 1273 is stable for 6 months at -20 degrees. It seems that genetic engineers of these two companies try to develop vaccine to be stable in higher temperatures. On the other hand, the governments have to cope with challenges with individuals who are against vaccination policy. The cost of vaccines is also a major challenge to poor countries. Reports shows that richest countries in the world have already pre-ordered nearly four billion doses, so policymakers suggested that they may help finance access for poorer nations. In conclusion, for reaching global immunity we need complete cooperation between countries, pharmaceutical companies, and people to pass pandemic [70, 76-78].

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