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A Review on DNA Vaccines in Pre-Clinical Trials Against SARS-CoV-2

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ABSTRACT

COVID 19 Pandemic is caused by the viral pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Scientific fraternity worldwide swiftly developed various types of vaccines for the prevention and as mitigation measures for curbing the pandemic. Traditional inactivated vaccines, mRNA vaccines (protein subunits such as spike proteins), and viral vector vaccines (non-replicating vectors with protein subunits) have been approved by World Health Organisation (WHO) for emergency use. The emergence of many mutated variants has been a worrying factor in the fight against the pandemic. There has been continuous research in the quest for more therapeutics, especially vaccines to curb and stop the pandemic. According to WHO, there are 194 vaccines in pre-clinical trials belonging to various types out of which sixteen is DNA vaccines. In this review, we have discussed the advantages and disadvantages of the DNA vaccines for Covid - 19. This article tried to explore the available information on DNA vaccines and their current status against Covid – 19 which are in pre-clinical trials.

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1 Introduction

Covid - 19 was first reported from Wuhan, China in December 2019. The severe respiratory syndrome was reported with symptoms of fever, dizziness, and cough. The RNA sequencing was done with the bronchoalveolar lavage fluid sample of the patient revealing it belongs to the family *Coronaviridae* (Wu et al. 2020). Further phylogenetic analysis results revealed that the virus belongs to the order of *Nidovirales*, suborder *cornidovirineae*, family *coronaviridae*, subfamily *orthocoronavirinae*, genus *Betacoronavirus*, and subgenus *Sarbecovirus* and to the species of severe acute respiratory syndrome coronavirus. It was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronaviridae Study Group (CSG), the working group of the International Committee on Taxonomy of Viruses ICTV (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses 2020). As the outbreak spread, worldwide World Health Organisation declared it as a pandemic on 11 March 2020.

2 SARS-COV-2

Severe acute respiratory syndrome coronavirus 2(SARS-COV-2) belongs to the family of *coronaviridae*. There are four groups among this family namely alpha, beta, gamma, and delta. SARS-CoV2 belongs to the beta coronavirus which is known to cause respiratory and gastrointestinal diseases. In the past also, there have been outbreaks from two pathogenic species from this genus namely severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and Middle Eastern respiratory virus (MERS-CoV) in 2012. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a spherical single-strand RNA virus that is in a positive sense (Michael et al. 2021). The genome size of the coronaviruses is largest when compared to other RNA viruses. The genomic length of the SARS-CoV-2 is about 30kb and it has fourteen open reading frames (ORFs) in which genes encode for its structural proteins, accessory proteins, and non-structural proteins (Finkel et al. 2021). ORF1a and ORF1ab encode for the two largest polyproteins which are cleaved to form sixteen non-structural proteins, four structural proteins, and eight accessory proteins. These translated polyproteins are cleaved by a virus-encoded protease. These non-structural proteins play an essential role mainly as enzymes in genome replication and initial transcription regulation. The non-structural proteins included proteases, RNA-dependent polymerase, helicase, exoribonuclease, endonuclease, and methyl transferases (Gordon et al. 2020; Alexandra et al. 2020). Further, the four structural proteins are spike protein (S), an envelope protein (E), membrane (M), and nucleocapsid protein (N). The structural proteins play an important role in the virus entry, pathogenicity, immune evasion, and many more. Spike protein (S) present on the surface is a glycoprotein and it is involved in the viral entry into the host. Spike protein is significant for the binding of the virion with host Human Angiotensin-converting Enzyme -2 (hACE2) which are distributed in the lungs, intestine, heart, and kidney. Spike proteins contain a trimer which is composed of two subunits namely S1 and S2. Among these, the S1 subunit contains the receptor binding domain (RBD) which is responsible for recognizing the receptor of the host hACE2 and binding to it. Once the binding of the virus to the host is completed, host cell protease namely TM protease serine 2 (TMPRSS2) activates the S protein and cleaves it into subunits. S2 subunit contains the Heptad repeat (HR) domain and contains fusion proteins (FP) which are also responsible for the viral fusion with the host hACE-2 cells (Miyuki et al. 2019; Alexandra et al. 2020; Huang et al. 2020). Envelope protein (E) is the smallest structural protein with a size of 8-12 kDa and has three domains namely the N-terminus domain, a transmembrane domain, and C terminal region. The E protein is responsible for assembly, budding of the virions, envelope formation, and pathogenesis (Schoeman and Fielding 2019; Sarkar and Saha 2020). Membrane protein (M) is a glycoprotein with a size of 25–30 kDa and it is the most abundant protein amongst the structural proteins and is composed of three transmembrane domains. These proteins are associated with the other structural proteins in the molecular pathogenesis of the SARS-CoV2 and facilitate the molecular assembly of the virus particles by associating with the nucleocapsid protein (N), thereby assembling the virions in hACE2 cells. M proteins were also found to join the molecules together in the endoplasmic reticulum thereby inducing apoptosis of host hACE2 cells (Yadav et al. 2021; Giuseppina et al. 2020). Nucleocapsid protein (N) is the viral protein coat with the size of 46 kDa and helps in the formation of capsids in coronaviruses. It contains five domains namely the N-terminal domain (NTD), RNA-binding domain (RBD), disordered central linker (LINK), a dimerization domain, and C-terminal domain (CTD). N protein is responsible for recognizing its viral genome and forming a capsid by oligomerization. N protein apart from this important viral life cycle event is also involved in various pathogenic effects such as deregulating its life cycle, inhibiting its cytokinesis, inhibiting its translation machinery, inducing apoptosis, inhibiting interferon, etc. in the host cells (Rota et al. 2003; Surjit and Lal 2009; Cubuk et al. 2021;).

The role of the accessory proteins of the SARS-CoV2 in the molecular mechanism and pathogenesis has not been completely understood to an extent but there is a need for continuous research to elucidate more, yet the available studies and research have indicated these accessory proteins do have an important function in molecular mechanism and pathogenesis of the SARS-CoV2. The positive-strand genome encodes for the ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10 accessory proteins of SARS-CoV2 (Narayanan et al.

2008). The accessory proteins along with non-structural proteins are found to be important in the replication, and transcription, in evading host immune responses as well as the viral dissemination. They are also found to inhibit the interferons, inducing apoptosis, inhibiting the translation machinery of the host cells, arresting the host cell cycle, etc., thereby playing an important role in the activation of host cell death pathways and pathogenesis (Silvas et al. 2021).

3 SARS-COV2 and Immune System Response

SARS-CoV2 infection induces natural immune response. In this section, we will discuss the natural immune response as well as the vaccine-induced immune response. SARS – CoV2 infection is known to induce innate and adaptive immune responses in the host. The innate and adaptive immune responses involve the synthesis of pro-inflammatory cytokines which leads to the activation of T cells. As a result of this, CD4 and CD8+ T cells become activated and play a significant role in the viral cycle inhibition and clearance of already infected cells with various molecular mechanisms. It has been found that this innate immune response is activated initially by pathogen recognition receptors (PRRs) 3, 7, and 8 which are present in the immune cells. The innate immune response involves the production of various cells like IFN-ɣ interferon, interleukins such as IL-1β, IL-1RA, IL-7, IL-8, IL-10, monocyte chemoattractant peptide (MCP)-1, macrophage inflammatory protein (MIP) such as 1A and MIP-1B, granulocyte colony-stimulating factor (G-CSF) and tumor necrosis factor-alpha (TNF-α) (Abdurrahman et al. 2020; Shah et al. 2020). These signaling cascades of immune response proteins are very important for recognizing and eliminating the virus so that it is not spreading to the neighboring cells in the host. Interleukin IL-6 is found to be the main factor in the initiation of various pathologic mechanisms thereby leading to acute respiratory distress syndrome in the infected individuals. Increased/exaggerated production of IL-6 has been reported in patients with inflammatory disorders and autoimmune disorders which will lead to endothelial cell damage, cytokine storm, and capillary leak. IL-6 is also known to induce the complement system thereby inducing complex reactive proteins (CRP) (Jordan 2021).

The innate immune response which is very fast is followed by the adaptive immune response involving the T and B cells. The adaptive immune response is initiated by the complement pathway activation as well as the presentation of the virus in the epithelium by antigen-presenting cells, MHC-Class II molecules. The affected cells which may be subjected to cell cycle lysis are destroyed by CD8 T cell and Natural Killer (NK) cells with the perforin and enzymes like gran-enzymes they possess. It is followed by the recognition and presentation by dendritic cells to the CD 4 T cell. CD 4 T cell initiates the polyclonal memory cells such as Th1, Th17, and T follicular helper cells. CD4 T cell also helps the plasma cell in the production of virus-specific B cell antibodies such as IgM, IgA, and IgG (Ahmet et al. 2020). The vaccines in clinical use presently are intended to induce the neutralizing B cell antibodies. These clinically approved vaccines are proven to induce more neutralizing antibody titres when compared to the naturally infected antibody titres (Xaquin et al. 2020). The Replicating vector vaccine has been proved to induce the CD4 helper T-cell responses as well as cytolytic CD8 T-cell responses (Corbett et al. 2020). The present clinically used vaccines are also known to induce a balanced humoral and cellular immune response, especially the IgG subclass (van Doremalen et al. 2020). Previous studies also showed that inactivated SARS-CoV-2 vaccine candidate induces high levels of neutralizing antibody (Wang et al. 2020).

4 DNA Vaccines

DNA vaccines involve the introduction of plasmids which contains the gene encoding for the antigen with promoter/terminator to express. DNA vaccines are advantageous since they can induce both B and T cell immune responses involving the T Cytolytic cells, T helper cells, and B cells. The one striking advantage of DNA vaccines is they induce CD8 cytolytic T lymphocytes when compared to the other vaccines. In inactivated virus vaccines and recombinant protein vaccines, the antigen-presenting cells are MHC – class II molecules that are involved in the presentation which may induce T helper cells. In contrast DNA vaccine antigen-presenting cells are MHC – class I molecules are involved in the presentation. These MHC – Class I molecules induce the cytolytic T cells (CTL) (Liu 2003). In a scenario of emerging variants like in the case of Covid- 19, an ideal candidate should be easy to manufacture, have fewer requirements for storage, and cost of manufacturing should be less. DNA vaccines are addressing these issues and are also capable of inducing both humoral, and cellular responses (Ebony and David 2020). DNA vaccine manufacturing does not require the growth of a live virus. Swift up-scale processing can be achieved with the DNA vaccines as they employ the synthetic DNA of the antigen and this will be very impactful in the case of pandemic situations like the one, we have facing right now (Leo et al. 2018).

4.1 Advantages of DNA Vaccines

DNA vaccines have the major advantage of inducing broader immunity when compared to other vaccines. Manufacturing DNA vaccines do not require more expenses as in the case of other vaccines. DNA vaccines do not require specific transportation like RNA vaccines because of their heat stability (Abdulhaqq and Weiner 2008; Stachyra et al. 2014). Some of the common advantages of DNA vaccines are (i) induces both humoral and cellular immune response, (ii) safer when compared to inactivated vaccines which may cause infection, (iii) DNA plasmids are very

simple to create, (iv) have high stability, (v) do not require specific storage conditions like the protein/mRNA vaccines, (vi) genetic manipulation as required can be achieved with DNA plasmids, (vii) genes encoded in DNA vaccines induces stronger immune reaction as the protein expressed from the DNA vaccines are properly conformed, and (viii) manufacturing of DNA vaccines is easy, low cost and easy to transport.

4.2 Disadvantages of DNA Vaccines

Some disadvantage of DNA vaccines are also reported, among these some common are (i) activation of oncogenes, inducing anti-DNA antibodies have been reported in the experimental animals, (ii) adjuvants has to be added to increase the immunogenicity in vivo, (iii) antibody induction may be slower, (iv) lower efficacy is reported in humans, (v) need multiple doses, and (vi) there may be incorporated into the host genome (Abdo Hasson et al. 2015; Kowalczyk and Ertl 1999)

4.3 DNA vaccines for COVID-19 in preclinical trials

According to the World Health Organisation vaccine tracker and landscape as per the WHO, Covid-19 vaccines track there are sixteen DNA vaccines in Phase I/II clinical trials which are presented in Table 1 (Prompetchara et al. 2021; Meyers et al. 2021).

5 DNA vaccines in clinical trials

5.1 DIOS-CoVax2

The synthetic gene encoding the antigen of SARs–CoV2 is constructed with the 3–D model. It is aimed to induce the humoral responses (B cell immunity) as well as the T cell immunity to stop the replication of virus and cytolysis of infected host cells. DIOS-CoVax2 also aimed to reduce the adverse effects by designing the plasmid antigen without the parts of the virus known to induce such reactions in the host. The vaccine is in Phase 1/ 2 trials and found to be effective and it is said to be employing multiple delivery systems for the delivery (News University of Cambridge 2020).

5.2 SN14 vaccine

SN14 vaccine is the product of Joint research and development of Scancell, the University of Nottingham, and Nottingham Trent University. It is aimed to induce a humoral response to neutralize the spike proteins and to induce T cell response against the Spike & Nucleocapsid proteins. It is also aimed to be effective in various mutant strains as the Nucleocapsid portion is found to be conserved in the variants. The SN14 also employs monoclonal AvidiMab™ to increase the immunogenicity and specificity and

Table 1 Covid-19 DNA Vaccines in Clinical Trails

No	DNA Vaccine	Developers
1	DNA - Multiple Delivery system	DIOSynVax Ltd + University of Cambridge
2	DNA vaccine	Ege University
3	DNA plasmid vaccine RBD&N	Scancell/University of Nottingham/ Nottingham Trent University
4	DNA with electroporation	Karolinska Institute / Cobra Biologics (OPENCORONA Project)
5	DNA with electroporation	Chula Vaccine Research Center
6	Plasmid DNA, Needle-Free Delivery	Immunomic Therapeutics, Inc./EpiVax, Inc./PharmaJet
7	DNA plasmid vaccine (S,S1,S2,RBD &N)	National Research Centre, Egypt
8	DNA vaccine	BioNet Asia
9	ms DNA vaccine	MediphageBioceuticals/University of Waterloo
10	DNA vaccine	Entos Pharmaceuticals
11	DNA plasmids containing S-gene	Biosun Pharmed
12	DNA plasmid vaccine	Globe Biotech Limited, Bangladesh
13	Plasmid DNA, nanostructured RBD	National Institute of Chemistry, Slovenia
14	DNA plasmid vaccine encoding RBD	Vaccibody, Oslo Research Park, Norway
15	DNA Immunostimulatory sequences	Inserm
16	The 3 regions of SARS-Cov-2 Spike-protein: NTD, RBD, and HR1-HR2 inserted into the plasmid of PcDNA3.1.	Center of Genomics and Bioinformatics of Academy of Science of the Republic of Uzbekistan

soon it is expected to go for clinical trials (FDA news 2020; Scancell Holdings plc 2020).

5.3 Vaccine developed by Karolinska Institute and Cobra Biologics

The OPENCORONA vaccine consortium synthesized the code for N protein and other structural proteins to incorporate into the plasmid. This DNA vaccine employs a synthetic whole virus gene and electroporation technique to deliver. It is expected to undergo clinical trials in 2021 (CovidVax 2020a; Gustaf et al. 2020; Mohamadian et al. 2021).

5.4 Vaccine developed by Chula Vaccine Research Center

Researched and developed by Chula Vaccine Research Center, National Research Council of Thailand, and BioNet-Asia using electroporation for the delivery. It is constructed with synthetic DNA encoding various regions of pike protein and it is inserted into the pCMVkan expression vector. Phase 1/2 clinical trials are being conducted for this candidate (Covidvax 2020b).

5.5 EPV-CoV19 vaccine

Developed by ImmunomicTx, EpiVax, and PharmaJet, it is named EPV-CoV19. This vaccine employs synthetic peptides of T-cell epitopes from spike, membrane, and envelope aimed to induce the T cell response when vaccinated. This vaccine is expected to be effective against all strains distributed worldwide and has long-lasting T cell memory. The vaccine is expected to undergo trial sooner (CovidVax 2021a; Gustaf et al. 2020).

5.6 Mini string DNA Vaccine

It is researched and developed by Mediphage Bioceuticals with the University of Waterloo. In this DNA vaccine, ms DNA is constructed to encode the Virus-Like Particle (VLP) derived from the SARS-CoV-2 genome. This vaccine is developed to present via the nasal spray and aimed to induce both cell-mediated and humoral responses. Soon it may enter the clinical trials (CovidVax 2021b; Press Release MediphageBioceuticals, March 31st, 2020)

5.7 Covigenix VAX-001

Researched and developed by Entos Pharma and Cytiva it is named Covigenix VAX-001. This vaccine is constructed with the DNA encoding for the spike glycoprotein of the SARS – CoV2 with two genetic adjuvants. It is aimed to induce both innate and adaptive immune responses. The vaccine employs the Fusogenix delivery system for better intracellular delivery. The vaccine has completed the Phase 1 trials and has entered the Phase 2 clinical trials (CovidVax 2021c; Press Release Entos 2021; U.S. National Library of Medicine 2021).

Conclusion

With the scenario of emerging SARS-CoV2 variants and reported immune evasion by the variants, it is important to look upon the new therapeutics to curb the pandemic situation. At present, inactivated virus vaccines, mRNA vaccines, and RNA-based replicating vector vaccines are in clinical use. Even though these vaccines have been developed and approved rapidly we are facing the issue of the emergence of variants that are reported to possess immune evading properties. DNA vaccines provide various advantages such as inducing the cytolytic T cell response apart from the humoral B cell responses which cumulatively be effective in arresting viral life cycle, cytolysis of infected cells, and neutralizing antibodies. Apart from these therapeutic advantages, DNA vaccines also have the advantage of storage conditions, rapid upscaling process, and rapid manufacturing, etc., In this review, we have discussed the general properties of DNA vaccines and the candidates from this category in pre-clinical trials. We may have various DNA vaccines against Covid - 19 shortly.

References

Abdo Hasson, S.S.A., Al-Busaidi, J.K.Z., & Sallam, T.A. (2015). The past, current and future trends in DNA vaccine immunisations. *Asian Pacific Journal of Tropical Biomedicine*, *5*(5), 344-353. https://doi.org/10.1016/S2221-1691(15)30366-X.

Abdulhaqq, S.A., & Weiner, D.B. (2008). DNA vaccines: developing new strategies to enhance immune responses. *Immunologic Research*, *42*, 219. https://doi.org/10.1007/s12026-008-8076-3.

Abdurrahman, T., Aslihan A. G., & Marco M.C. (2020). COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. *Turkish Journal of Medical Sciences*, *50*, 620-632. doi:10.3906/sag-2004-168.

Ahmet, K.A., Mübeccel A., Dilek, A., et al. (2020). Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *European Journal of Allergy and clinical immunology*, *75*(7), 1564-1581. https://doi.org/10.1111/all.14364.

Alexandra, C.W., Young-Jun, P., Alejandra T. M., et al. (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, *181*(2), 281-292. https://doi.org/ 10.1016/j.cell.2020.02.058.

Corbett, K.S., Flynn, B., Foulds, K.E., et al. (2020). Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. *New England Journal of Medicine*, *383*(16), 1544-1555. doi:10.1056/NEJMoa2024671.

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. (2020). The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Natural Microbiology*, *5*, 536-544. https://doi.org/10.1038 /s41564-020-0695-z.

Covidvax. (2020b). December, Retrieved from https://covidvax.org/covid19-vaccine/ Chula VRC/DNA-with-electroporation-Chula-Vaccine-Research-Center-National-Research-Council -of-Thailand-BioNet.

Covidvax. (2021a). December, Retrieved from https:// covidvax.org/covid19-vaccine/ ImmunomicEpivax/DNA-plasmid-needle-free-Immunomic-Tx-EpiVax-PharmaJet.

Covidvax. (2021b). October, Retrieved from https://covidvax.org/ covid19-vaccine/ Waterloo Uni/DN Mediphage-Bioceuticals-University-of-Waterloo-Lambton-College

CovidVax. (2020a) December Retrieved from https://covidvax.org/covid19-vaccine/Karolinska/DNA-with-electroporation-Karolinska-Institute-OpenCorona-EU-project-Folkh-lsomyndigheten-Igea-Cobra-.

CovidVax. (2021c). September update, Retrieved fromhttps://covidvax.org/covid19-vaccine/ EntosPharma/DNA-Entos-Pharma-Cytiva.

Cubuk, J., Alston, J.J., Incicco, J.J. et al. (2021). The SARS-CoV-2 nucleocapsid protein is dynamic, disordered, and phase separates with RNA. *Nature Communication*, *12*, 1936. https:// doi.org/10.1038/s41467-021-21953-3.

Ebony, N., & David, B.W. (2020). DNA vaccines: prime time is now. *Current Opinion in Immunology*, *65*, 21-27. https://doi.org/10.1016/j.coi.2020.01.006.

FDA news. (2020). UK Pharma Company Scancell Selects COVID-19 Vaccine for Clinical Trials. December 2020, Retrieved from https://www.fdanews.com/articles/200556-uk-pharma-company-scancell-selects-covid-19-vaccine-for-clinical-trials.

Finkel, Y., Mizrahi, O., Nachshon, A., et al. (2021). The coding capacity of SARS-CoV-2. *Nature*, *589*, 125-130. https://doi.org/ 10.1038/s41586-020-2739-1

Giuseppina, M., Farthing, R.J., Lale-Farjat, S.L.M., & Bergeron J.R.C. (2020). Structural Characterization of SARS-CoV-2: Where We Are, and Where We Need to Be. *Frontiers in Molecular Biosciences*, *7*, 605236. https://doi.org/10.3389/fmolb.2020.605236.

Gordon, D.E., Jang, G.M., Bouhaddou, M., et al. (2020). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*, *583*, 459-468. https://doi.org/10.1038/s 41586-020-2286-9.

Gustaf, A., Lars, F., Negin. N., et al. (2020). The SARS-CoV-2 N Protein is a Good Component in a Vaccine. *Journal of Virology*, *94*(18), e01279-20. https://doi.org/10.1128 /JVI.01279-20.

Huang, Y., Yang, C., Xu, X.F., et al. (2020). Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacologica Sinica*, *41*, 1141-1149. https://doi.org/10.1038/s41401-020-0485-4.

International Code of Virus Classification and Nomenclature. (2020) Retrieved from https://talk.ictvonline.org/information/w/ictv-information/383/ictv-code

Jordan, S.C. (2021). Innate and adaptive immune responses to SARS-CoV-2 in humans: relevance to acquired immunity and vaccine responses. *Clinical and Experimental Immunology*, *204*(3), 310–320. https://doi.org/10.1111/cei.13582.

Kowalczyk, D.W., & Ertl, H.C.J. (1999). Immune responses to DNA vaccines. *Cellular and Molecular Life Sciences CMLS*, *55*, 751-770 (1999). https://doi.org/10.1007/s000180050330.

Leo Y.Y.L., Leonard, I., & Aeron, C. H. (2018). A Review of DNA Vaccines Against Influenza. *Frontiers in Immunology*, *9*, 1568. https://doi.org/10.3389/fimmu.2018.01568.

Liu, M.A. (2003), DNA vaccines: a review. *Journal of Internal Medicine*, *253*, 402-410. https://doi.org/10.1046/j.1365-2796.2003.01140.x.

Mohamadian, M., Chiti, H., Shoghli, A., Biglari, S., Parsamanesh, N., & Esmaeilzadeh, A. (2021). COVID-19: Virology, biology and novel laboratory diagnosis. *The journal of gene medicine*, *23*(2), e3303. https://doi.org/10.1002/jgm.3303

Meyers, L. M., Gutiérrez, A. H., Boyle, C. M., Terry, F., et al. (2021). Highly conserved, non-human-like, and cross-reactive SARS-CoV-2 T cell epitopes for COVID-19 vaccine design and validation. *NPJ vaccines*, *6*(1), 71. https://doi.org/10.1038/s41541-021-00331-6

Michael, M., Geraldine O. H. Catherine, H., et al. (2021). Severe Acute Respiratory Syndrome Coronavirus 2. *Reference Module in Biomedical Sciences*. Doi: 10.1016/B978-0-08-102723-3.00139-6.

Miyuki, K., Michiyo, K., Kazuya, S., & Shutoku, M. (2019). Biochemical Analysis of Coronavirus Spike Glycoprotein Conformational Intermediates during Membrane Fusion. *Journal of Virology*, *93*(19), e00785-19. https://doi.org/10.1128/JVI.00785-19.

Narayanan, K., Huang, C., & Makino, S. (2008). SARS coronavirus accessory proteins. *Virus Research*, *133* (1), 133-121. https://doi.org/10.1016/j.virusres.2007.10.009.

New, University of Cambridge, August. (2020). University of Cambridge to trial Covid-19 vaccine candidate, Clinical trials arena. Retrieved from https://www.clinicaltrialsarena.com /news/cambridge-uni-covid-vaccine/.

Press Release, (March 31st, 2020). MediphageBioceuticals, Press Release 2020, March 31 MediphageBioceuticals, Inc. Initiates a COVID-19 Vaccine Development Program, Retrieved from https://static1.squarespace.com/static/5cab96df2727be742dcf64ac/t /5efdf1b534e269 1d9e3ef002/1593700790604/COVID19+Press +Release%2805.15.2020%29.V2+%28New +L ayout%29-2.pdf.

Press Release. (1st September, 2021). Entos Pharma, Entos' COVID-19 DNA Vaccine Candidate Approved for Phase 2 Clinical Trial in South Africa, Retrieved from https://www.entospharma. com/news/entos-covid-19-dna-vaccine-candidate-approved-for-phase-2-clinical-trial-in-south -africa.

Prompetchara, E., Ketloy, C., Tharakhet, K., Kaewpang, P., et al. (2021). DNA vaccine candidate encoding SARS-CoV-2 spike proteins elicited potent humoral and Th1 cell-mediated immune responses in mice. *PloS one*, *16*(3), e0248007. https://doi.org/10.1371/journal.pone.0248007.

Rota, P. A., Oberste, M. S., Monroe, S. S., Nix, W. A., et al. (2003). Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science (New York, N.Y.)*, *300* (5624), 1394–1399. https://doi.org/10.1126/science.1085952.

Sarkar, M., & Saha, S. (2020). Structural insight into the role of novel SARS-CoV-2 E protein: A potential target for vaccine development and other therapeutic strategies. *PloS one*, *15*(8), e0237300. https://doi.org/10.1371/journal.pone.0237300.

Scancell Holdings plc. (2020), Scancell announces selection of COVID-19 vaccine candidate, SN14 Retrieved from https://www.scancell.co.uk/Data/Sites/1/media/ publications/ rns/covid-19-vaccine-candidate-selected.pdf). updated in Covidvax, December 2020, https://covidvax .org/covid19-vaccine/Scancell/DNA-Scancell-University-of-Nottingha m-Nottingham-Trent-University.

Schoeman, D., & Fielding, B. C. (2019). Coronavirus envelope protein: current knowledge. *Virology journal*, *16*(1), 69. https://doi.org/10.1186/s12985-019-1182-0

Shah, V. K., Firmal, P., Alam, A., Ganguly, D., & Chattopadhyay, S. (2020). Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. *Frontiers in immunology*, *11*, 1949. https://doi.org/10.3389/fimmu.2020.01949.

Silvas, J. A., Vasquez, D. M., Park, J. G., Chiem, K., et al. (2021). Contribution of SARS-CoV-2 Accessory Proteins to Viral Pathogenicity in K18 Human ACE2 Transgenic Mice. *Journal of virology*, *95*(17), e0040221. https://doi.org/10.1128/JVI.00402-21.

Stachyra, A., Góra-Sochacka, A., & Sirko, A. (2014). DNA vaccines against influenza. *Acta Biochimica Polonica*, *61*(3), 515-522.

Surjit, M., & Lal, S. K. (2009). The Nucleocapsid Protein of the SARS Coronavirus: Structure, Function and Therapeutic Potential. *Molecular Biology of the SARS-Coronavirus*, 129–151. https://doi.org/10.1007/978-3-642-03683-5_9.

U.S. National Library of Medicine. (2021). A Clinical Trial of a Prophylactic Plasmid DNA Vaccine for COVID-19 [Covigenix VAX-001] in adults. September 14, Retrieved from https://clinicaltrials.gov/ct2/show/NCT04591184).

van Doremalen, N., Lambe, T., Spencer, A., Belij-Rammerstorfer, S., et al. (2020). ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature*, *586*(7830), 578–582. https://doi.org/10.1038/s41586-020-2608-y.

Wang, H., Zhang, Y., Huang, B., Deng, W., et al. (2020). Development of an Inactivated Vaccine Candidate, BBIBP-CorV, with Potent Protection against SARS-CoV-2. *Cell*, *182*(3), 713–721.e9. https://doi.org/10.1016/j.cell.2020.06.008.

Wu, F., Zhao, S., Yu, B., Chen, Y. M., et al. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, *579* (7798), 265–269. https://doi.org/10.1038/ s41586-020-2008-3.

Xaquin, C. D., Sebastian, O., Karin, L., & Gunilla, B. K. H. (2020). Immunity to SARS-CoV-2 induced by infection or vaccination. *Journal of Internal Medicine*, *291*(1), 32-50. https://doi.org/10.1111/joim.13372.

Yadav, R., Chaudhary, J. K., Jain, N., Chaudhary, P. K., et al. (2021). Role of Structural and Non-Structural Proteins and Therapeutic Targets of SARS-CoV-2 for COVID-19. *Cells*, *10*(4), 821. https://doi.org/10.3390/cells10040821.