REVIEW PAPER

UDK: 616.98:578.834]:615.371

DOI: 10.5937/hraish2001001M

COVID-19 as milestone for the use of new vaccine types

Milan Marković

Institute for Biological Research "Siniša Stanković", National Institute of The Republic of Serbia, Department of Immunology, University of Belgrade, Serbia

Corresponding author: Milan Marković, Institute for Biological Research "Siniša Stanković", National Institute of The Republic of Serbia, Department of Immunology, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia,
ORCID ID: https://orcid.org/0000-0001-8352-9003,
Phone: +381 11 2078 423,
Mob: +381 69 733 037,
E-mail address: milan.markovic@ibiss.bg.ac.rs

Received 09 December 2020
Accepted 29 December 2020

Abstract

COVID-19 is an infectious disease caused by SARS-CoV-2 virus which belongs to the Coronaviridae group. The symptoms of the disease are primarily present in the form of respiratory syndrome similar to the symptoms of other respiratory viruses such as influenza virus but also the very common SARS-CoV-1 and MERS-CoV viruses known from the recent past. However, based on short clinical experience, it was found that apart from lungs COVID-19 affects all organ systems with unclear pathogenesis. Respiratory failure followed by systemic hypoxia and coagulation disorders is hallmarks of severe pathology. In addition, this virus belongs to the group of RNA viruses that share common characteristics with the HIV virus, which makes it very challenging for the therapy of modern medicine. There is currently controversy around the world about how to combat this epidemic. In addition to hygienic measures and measures of safe distance and isolation, there is an intense controversy about prophylactic vaccines. In this review article, the insight into all aspects of vaccination and methods for their design based on technological tools possessed by modern science with special reference to genetic (DNA, RNA) vaccines is provided, and the question arises of whether there is a justification of novel technology urgent application in the event of a COVID-19 pandemic.

Key words: *COVID-19; Vaccines; Live attenuated; VLP; Vector; Protein; DNA; RNA.*

INTRODUCTION

COVID-19 (SARS-CoV-2) infection broke out at the end of the 2019 year in Wuhan, China, and spread all over the world by 11 March of 2020 year when the global epidemic state is declared by the World Health Organization (WHO). According to the WHO statistics, this is one of the 20 infectious diseases which cause epidemic state in the past decade [1]. Many of them are followed by respiratory failures such as H1N1 [2] and MERS [3] so-called the novel coronavirus. In that manner, over the past years, there are a lot of challenges in front of health and science communities concerning the treatment of coronavirus-associated diseases (SARS and MERS) [4] to which is now added the COVID-19 [5]. In that context, one of the main issues which originate earlier than COVID-19 epidemics is the vaccine development for such contagious diseases. Speaking in general, the antiviral vaccines are divided into the next following types: inactive and/or live-attenuated viruses, virus-like particle (VLP), viral vectors, protein-based, DNA-based, and mRNA-based vaccines [6]. All the type of vaccines will be elaborated in detail in this review article.

LIVE ATTENUATED VACCINES

Inactive virus vaccines are made from the whole virus particles inactivated by formaldehyde or gamma irradiation, while the live-attenuated vaccines are developed in recombination with live attenuated viruses as a mutant [7]. In inactive virus/bacteria vaccines the virus or bacteria is functional but has been weakened, so it can replicate in the body several times and generate an immune response without causing the development of the disease. The example for such type of vaccines are chickenpox, measles, mumps and rubella, rotavirus, and shingles vaccine viruses, as well as the BCG vaccine which contains live weakened tuberculosis bacteria. If a live attenuated vaccine does cause disease, e.g., chickenpox disease, it is usually mild than

a disease caught by another person in the community [8]. But if administered to a person with an impaired immune system response, e.g., leukemia or HIV infection, or in those who are taking immunosuppressive medications, may cause severe disease as a result of uncontrolled replication and growth [9]. What is certain, attenuated viruses/bacteria in a form of vaccines when injected into the body induce the immune response, after which the virus particles are eliminated by innate immune mechanism and expelled from the body via kidney or liver, without causing the disease development. Both of these vaccines are in the pre-clinical phase of study for COVID-19 and need for several years to set up clinical use [10]. According to the WHO draft landscape of COVID-19 candidate vaccines, data collected up to the 12 November of 2020, there are 164 candidate vaccines in pre-clinical evaluation, and 48 candidate vaccines in clinical evaluation (**Table 1**). Only 3 study (Acıbadem Labmed Health Services, Serum Institute, and Griffith University all situated in India) deals with the live attenuated virus vaccines and just 4 of all study (one in Vietnam, one in Thailand, and two in Brazil) test a whole virus structure, and each of these trials are in the pre-clinical phase of evaluation (**Figure 1**). This way of developing vaccines has certain advantages such as good preservation of virus particle structure, rapid development, excellence in neutralizing antibody (Ab) induction, formulation with various types of adjuvant, and the most important is excellence in the induction of T and B cells responses [7]. It is known that inactive or live-attenuated vaccines are the most common way of immunization used in the past with great success. Particularly, this offers the best possible simulation to the natural way of pathogen introduction, minimizing the most of the eventual consequences of overall homeostasis disruption in interplay between host and pathogen. Apart from this, the priority in COVID-19 pandemic is given to new technology in vaccines design with only few trials for "old fashion" vaccines. To understand the inferior position of standard approach in the vaccine market, detail evaluation of main features, advantages and risks need to be reviewed.

VIRUS-LIKE PARTICLE VACCINES

Virus-like particles (VLPs) represent structures consisted of several proteins but without genetic material, by which they retain the organization of native viruses [11]. They are formed by the self-assembly of viral capsid proteins, and besides lacking viral genome retain the ability to enter the cells. VLPs are even convenient for fusion with other heterologous antigen epitopes, which makes them good as a platform for multimeric vaccine design [12]. So far, several types of vaccines based on VLP technology are known to be commercialized in the world, such as vaccines for hepatitis B virus as well as human papillomavirus [13]. It is known that different virus capsid protein might be able to efficiently assemble into various cells such as human embryonic kidney cell culture (HEK293), yeast, baculovirus, lentivirus, some bacteria such as *E. coli, Spodoptera frugiperda (sf9)* cells etc [11]. But on the other hand, despite the structure of virus capsid proteins are well preserved when synthesized in all those systems, the main disadvantage is that immunogenic VLPs require the optimum assembly conditions [7]. Furthermore, VLP vaccines designed in a form of multimeric antigen display in various cell cultures are different size ranging from 22 up to 150 nm [14]. Despite good preservation of virus particle structure in those systems, the variety of VLP particles in size is basically a problem when it comes to their immunogenicity. This might be a problem with COVID-19 VLP vaccines as well. Namely, one of the study deals with the VLP vaccine for COVID-19 produced in baculovirus, which is consisted of receptor-binding domain (RDB) with a full-length spike (S) or S1, co-expressing with M and E subunit as a part of virus structure [15]. In parallel, there are 16 studies in pre-clinical phase, and 2 studies in clinical phase according to WHO statistics (**Figure 1**, **Table 1**) dealing with COVID-19 VLPs made from different capsid protein such as envelope virus-like particles (eVLPs), S protein, RDB domain as well as unknown COVID-19 structures (Doherty Institute) (**Table 1**). All those VPL vaccines are produced by various cell cultures, starting from using plant derived VLP (Medicago Inc., Shiraz University), across VLP produced in baculovirus Expression Vector System BEVS (Tampere University), the form of lentivirus and baculovirus vehicles together, than VLP gained from cell cultures associated with hepatitis B virus antigen (HBsAg) (SpyBiotech/Serum Institute of India), or integrated in HIV VLPs (IrsiCaixa AIDS Research/IRTA-CReSA/Barcelona Supercomputing Centre/Grifols) (**Table 1**). In order to prime the immune response, some of them are combined with different adjuvants (Mahidol University/ The Government Pharmaceutical Organization (GPO)/Siriraj Hospital) or navigated to antigen presenting cells using particle-based Dendritic Cell (DC) targeting approach (University of Manitoba). The question is would all those COVID-19 VLPs vaccine be equally effective? All these VLP based vaccines are listed in **Table 1** with the information about the researchers groups and/or organizations involved in their production (data obtained from official website of WHO).

RECOMBINANT VIRAL VECTOR VACCINES

Recombinant viral vector vaccines represent corresponding viral component as a carrier in a form of retrovirus, lentivirus, vaccinia virus, adenovirus, ade-

no-associated virus, cytomegalovirus or sendai virus, transfected by specific antigens (transgene) from a virus of interest. This technology based on vector vaccines are characterized by certain advantages such as high efficiency of gene transduction, specific delivery of genes of interest to the target cells, and the induction of robust specific immune response. On the other hand, depending on the vector used for vaccine design, there are numerous pronounced disadvantages such as generation of replication-competent virus (retrovirus, lentivirus), tumorigenesis risk (retrovirus, lentivirus), limited infection of dividing cells only (retrovirus), pre-existing immunity (vaccinia virus, adenovirus, cytomegalovirus), low titre of antibody production (adeno-associated viruses), as well as the risk of pathogenesis in specific individuals (cytomegalovirus) [16]. Furthermore, by the process being called transgenic transfection the recombinant virus particles might also be detrimental in the context of deletion or inactivation of the transgene of interest itself which may occur either during the vaccine production process or during the immunization procedure [17]. The bright side of viral vaccines is that they do not require adjuvants as it is in case with protein vaccines, but there is concern about the theoretical reduction of desirable immunity and the possibility of carrier-induced infection. Studies evaluating the recombinant adenovirus type-5 (Ad-5) as a vector for HIV-1 vaccine reported increased risk for HIV-1 acquisition among vaccinated men [18-20]. Base on this results Zhu *et al*. express the concern about the usage of Ad-5 as a vector for COVID-19 phase 1 vaccine study [21]. Despite this, there are 5 studies for COVID-19 vaccines based on Adenovirus type-5 all listed in the **table 1**. Furthermore, among all vaccines for COVID-19, viral vectors vaccines occupy a central role with 37 studies which are in pre-clinical phase, and 13 already involved in clinical phase of testing (data obtained from WHO website).

PROTEIN-BASED VACCINES

Protein vaccines are composed of purified or recombinant proteinaceous antigens from a certain pathogen. The first protein-based vaccines originate from the plasma harvested antigens. In that manner, the first hepatitis B protein based vaccine is developed by the purification of viral surface 22-nm lipoprotein particle (HBsAg) from plasma collection of infected individuals [22]. However, limited source of HBsAg in plasma, despite the great tolerability and efficiencies of vaccine, demand more convenient recombinant technology in vaccine design.

The greatest advantage of protein-based vaccines is that they are safe in the context of development of infection, since they are consisted of targeted antigen with epitope responsible for triggering a specific immune response. On the other side, week immune response they are providing needs appropriate adjuvants in order to prime innate immune response [23]. Some of these stabilizers such as aluminium (Al) are used in a form of AlPO4, and this is accepted adjuvant in vaccination practice in many vaccines today starting from 1930 [24]. Usage of Al is supported by the fact that Al enhanced humoral responses priming to MHC class I molecules [25]. However, there are studies pointed out Al side effects indicating relation between Al toxicity and development of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), dementia, Gulf war syndrome and Parkinsonism [26]. Tremendous escalation in development of autism spectrum disorder (ASD) in last decades is brought into tight connection with Al support in vaccines and is a question of serious debate in science today [24, 27-30]. Despite these data available in public, Al is as used as a stabilizer (KM Biologics, Shifa Pharmed) in COVID-19 vaccines [10] and some of them are in phase 3 of clinical trial NCT04560881 sponsored by Laboratorio Elea Phoenix S.A. (data obtained from *ClinicalTrials.gov*). Additionally, protein-based vaccines for corona infection are designed on the basis of full-length Spike, S1, RDB nucleocapsid, formulated with various other adjuvants (**Table 1**). Even the question of adjuvant side-effects, not specifically related to COVID-19 vaccines, is global risk issue and must not be ignored. By the time of writing this article it is registered 70 studies dealing with protein based vaccines, among which 55 are in pre-clinical phase, and 15 are in clinical phase of research all with different adjuvants **(Figure 1, Table 1).**

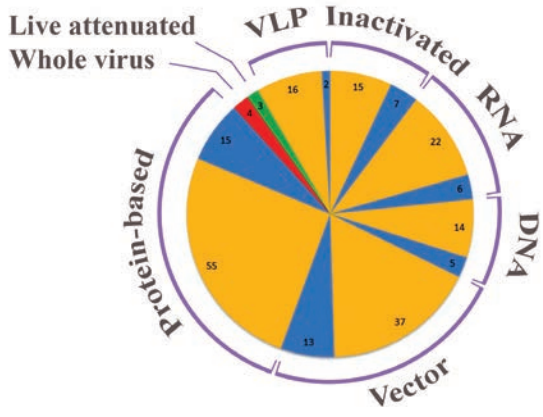

Figure 1. Types of COVID-19 vaccines. The figure shows 8 types of vaccines (inactivated, RNA based, DNA based, produced in vector, protein based, whole virus, live attenuated and VLP) which are in progress. Yellow portion of the figure represents the studies which are in pre-clinical phase and blue portion represents clinical trials. Data were obtained from Draft landscape of COVID-19 candidate vaccines by 12 November 2020 from the official website of WHO.

Table 1. COVID-19 vaccines in pre-clinical/clinical phase of testing. Data obtained from WHO website.

COVID-19 vaccine platform	Type of candidate vaccine	Developer/manufacturer	Current stage of evaluation [10]
Inactivated	Inactivated	Sinovac	Clinical – phase 1/2, phase 3
Inactivated	Inactivated	Wuhan Institute of Biological Products/Sinopharm	Clinical – phase 1/2, phase 3
Inactivated	Inactivated	Beijing Institute of Biological Products/Sinopharm	Clinical – phase 1/2, phase 3
Inactivated	Whole-Virion Inactivated	Bharat Biotech	Clinical – phase 1/2, phase 3
Inactivated	Inactivated	Institute of Medical Biology, Chinese Academy of Medical Sciences	Clinical – phase 1, phase 1/2
Inactivated	Inactivated	Research Institute for Biological Safety Problems, Rep of Kazakhstan	Clinical – phase 1, phase 1/2
Inactivated	Inactivated	Beijing Minhai Biotechnology Co., Ltd.	Clinical – phase 1, phase 1/2
Inactivated	Inactivated + Al	Shifa Pharmed	Pre-clinical
Inactivated	Inactivated	Milad Pharmaceutics Co.	Pre-clinical
Inactivated	Inactivated	Zista Kian Azma Co.	Pre-clinical
Inactivated	Inactivated	Kocak Farma Ilac ve Kimya San. A.S.	Pre-clinical
Inactivated	Egg-based, inactivated, whole chimeric virus	Institute of Vaccines and Medical Biologicals (IVAC; Vietnam) / Dynavax / PATH	Pre-clinical
Inactivated	Egg-based, inactivated, whole chimeric virus	Government Pharmaceutical Organization (GPO; Thailand) / Dynavax / PATH	Pre-clinical
Inactivated	Egg-based, inactivated, whole chimeric virus	Institute Butantan (Brazil) / Dynavax / PATH	Pre-clinical
Inactivated	Inactivated + Al	KM Biologics	Pre-clinical
Inactivated	Inactivated	Selcuk University	Pre-clinical
Inactivated	Inactivated	Erciyes University	Pre-clinical
Inactivated	Inactivated whole virus	National Research Centre, Egypt	Pre-clinical
Inactivated	TBD	Osaka University/ BIKEN/ NIBIOHN	Pre-clinical
Inactivated	Inactivated + CpG 1018	Sinovac/Dynavax	Pre-clinical
Inactivated	Inactivated + CpG 1018	Valneva/Dynavax	Pre-clinical
Live attenuated virus	Codon deoptimized live attenuated vaccines	Mehmet Ali Aydinlar University / Acıbadem Labmed Health Services A.S.	Pre-clinical
Live attenuated virus	Codon deoptimized live attenuated vaccines	Codagenix/Serum Institute of India	Pre-clinical

Live attenuated virus	Codon deoptimized live attenuated vaccines	Indian Immunologicals Ltd/Griffith University	Pre-clinical
Non-Replicating Viral Vector	Adenovirus Type 5 Vector	CanSino Biological Inc./Beijing Institute of Biotechnology	Clinical – phase 1, phase 2, phase 3
Non-Replicating Viral Vector	Adeno-based (rAd26-S+rAd5-S)	Gamaleya Research Institute	Clinical – phase 1/2, phase 2, phase 3
Non-Replicating Viral Vector	Adenovirus Type 26 vector	Janssen Pharmaceutical Companies	Clinical – phase 1/2, phase 2, phase 3
Replicating Viral Vector	Intranasal flu-based-RBD	Beijing Wantai Biological Pharmacy/ Xiamen University	Clinical – phase 1, phase 2
Replicating Viral Vector	VSV-S	Israel Institute for Biological Research	Clinical – phase 1/2
Non-Replicating Viral Vector	hAd5 S+N 2nd Generation Human Adenovirus Type 5 Vector	ImmunityBio, Inc. & NantKwest Inc.	Clinical – phase 1
Non-Replicating Viral Vector	Replication defective Simian Adenovirus (GRAd) encoding S	ReiThera/LEUKOCARE/Univercells	Clinical – phase 1
Non-Replicating Viral Vector	Ad5-nCoV	CanSino Biological Inc/Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Clinical – phase 1
Non-Replicating Viral Vector	Ad5 adjuvanted Oral Vaccine platform	Vaxart	Clinical – phase 1
Non-Replicating Viral Vector	MVA-SARS-2-S	Ludwig-Maximilians - University of Munich	Clinical – phase 1
Replicating Viral Vector	Replication-competent VSV	Merck Sharp & Dohme/IAVI	Clinical – phase 1
Replicating Viral Vector	Measles-vector based	Institute Pasteur/Themis/Univ. of Pittsburg CVR/Merck Sharp & Dohme	Clinical – phase 1
Non-Replicating Viral Vector	Ad 5 vector for intranasal administration	University of Helsinki & University of Eastern Finland	Pre-clinical
Non-Replicating Viral Vector	Adenovirus Type 5 Vector	Globe Biotech Limited, Bangladesh	Pre-clinical
Non-Replicating Viral Vector	Sendai virus vector	ID Pharma	Pre-clinical
Non-Replicating Viral Vector	Adenovirus-based	Ankara University	Pre-clinical
Non-Replicating Viral Vector	Adeno-associated virus vector (AAV-COVID)	Massachusetts Eye and Ear/Massachusetts General Hospital/AveXis	Pre-clinical
Non-Replicating Viral Vector	MVA encoded VLP	GeoVax/BravoVax	Pre-clinical
Non-Replicating Viral Vector	MVA-S encoded	DZIF – German Center for Infection Research/ IDT Biologika GmbH	Pre-clinical
Non-Replicating Viral Vector	MVA-S	IDIBAPS-Hospital Clinic, Spain	Pre-clinical

Non-Replicating Viral Vector	adenovirus-based NasoVAX expressing SARS2-CoV spike protein	Altimmune	Pre-clinical
Non-Replicating Viral Vector	Adeno5-based	Erciyes University	Pre-clinical
Non-Replicating Viral Vector	Ad5 S (GREVAX™ platform)	Greffex	Pre-clinical
Non-Replicating Viral Vector	Oral Ad5 S	Stabilitech Biopharma Ltd	Pre-clinical
Non-Replicating Viral Vector	adenovirus-based + HLA-matched peptides	Valo Therapeutics Ltd	Pre-clinical
Non-Replicating Viral Vector		Vaxart	Pre-clinical
Non-Replicating Viral Vector	MVA expressing structural proteins	Centro Nacional Biotecnología (CNB-CSIC), Spain	Pre-clinical
Non-Replicating Viral Vector	parainfluenza virus 5 (PIV5)-based vaccine expressing the spike protein	University of Georgia/University of Iowa	Pre-clinical
Non-Replicating Viral Vector	Recombinant deactivated rabies virus containing S1	Bharat Biotech/Thomas Jefferson University	Pre-clinical
Non-Replicating Viral Vector	Influenza A H1N1 vector	National Research Centre, Egypt	Pre-clinical
Non-Replicating Viral Vector	Newcastle disease virus expressing S	Icahn School of Medicine at Mount Sinai	Pre-clinical
Replicating Bacteria Vector	Oral Salmonella enteritidis (3934Vac) based protein expression system of RBD	Farmacológicos Veterinarios SAC (FARVET SAC) / Universidad Peruana Cayetano Heredia (UPCH)	Pre-clinical
Replicating Viral Vector	Intranasal Newcastle disease virus vector (rNDV-FARVET) expressing RBD	Farmacológicos Veterinarios SAC (FARVET SAC) / Universidad Peruana Cayetano Heredia (UPCH)	Pre-clinical
Replicating Viral Vector	YF17D Vector	KU Leuven	Pre-clinical
Replicating Viral Vector	Measles Vector	Cadila Healthcare Limited	Pre-clinical
Replicating Viral Vector	Measles Vector	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Pre-clinical
Replicating Viral Vector	Measles Virus (S, N targets)	DZIF – German Center for Infection Research/ CanVirex AG	Pre-clinical
Replicating Viral Vector	Horsepox vector expressing S protein	Tonix Pharma/Southern Research	Pre-clinical

Replicating Viral Vector	Live viral vectored vaccine based on attenuated influenza virus backbone (intranasal)	BiOCAD and IEM	Pre-clinical
Replicating Viral Vector	Recombinant vaccine based on Influenza A virus, for the prevention of COVID-19 (intranasal)	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Pre-clinical
Replicating Viral Vector	Attenuated Influenza expressing an antigenic portion of the Spike protein	Fundação Oswaldo Cruz and Instituto Butantan	Pre-clinical
Replicating Viral Vector	Influenza vector expressing RBD	University of Hong Kong	Pre-clinical
Replicating Viral Vector	Replicating VSV vector-based DC-targeting	University of Manitoba	Pre-clinical
Replicating Viral Vector	VSV-S	University of Western Ontario	Pre-clinical
Replicating Viral Vector	VSV-S	Aurobindo	Pre-clinical
Replicating Viral Vector	VSV vector	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Pre-clinical
Replicating Viral Vector	M2-deficient single replication (M2SR) influenza vector	UW–Madison/FluGen/Bharat Biotech	Pre-clinical
Replicating Viral Vector	Newcastle disease virus vector (NDV-SARS-CoV-2/Spike)	Intravacc/ Wageningen Bioveterinary Research/ Utrecht Univ.	Pre-clinical
Replicating Viral Vector	Avian paramyxovirus vector (APMV)	The Lancaster University, UK	Pre-clinical
Protein based	Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	Clinical – phase 1/2, phase 2b, phase 3
Protein based	Adjuvanted recombinant protein (RBD-Dimer)	Anhui Zhifei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy of Sciences	Clinical – phase 1, phase 1/2, phase 2
Protein based	RBD-based	Kentucky Bioprocessing, Inc	Clinical – phase 1/2
Protein based	S protein (baculovirus production)	Sanofi Pasteur/GSK	Clinical – phase 1/2
Protein based	Adjuvanted protein subunit (RBD)	Biological E Ltd	Clinical – phase 1/2
Protein based	Native like Trimeric subunit Spike Protein vaccine	Clover Biopharmaceuticals Inc./GSK/Dynavax	Clinical – phase 1

Protein based	Recombinant spike protein with Advax™ adjuvant	Vaxine Pty Ltd/Medytox	Clinical – phase 1
Protein based	Molecular clamp stabilized Spike protein with MF59 adjuvant	University of Queensland/CSL/Seqirus	Clinical – phase 1
Protein based	S-2P protein + CpG 1018	Medigen Vaccine Biologics Corporation/NIAID/ Dynavax	Clinical – phase 1
Protein based	rRBD produced in CHO-cell chemically conjugate to tetanus toxoid	Instituto Finlay de Vacunas, Cuba	Clinical – phase 1
Protein based	RBD + Adjuvant	Instituto Finlay de Vacunas, Cuba	Clinical – phase 1
Protein based	Peptide	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Clinical – phase 1
Protein based	RBD (baculovirus production expressed in Sf9 cells)	West China Hospital, Sichuan University	Clinical – phase 1
Protein based	SARS-CoV-2 HLA-DR peptides	University Hospital Tuebingen	Clinical – phase 1
Protein based	Multitope peptide-based S1-RBD-protein vaccine	COVAXX / United Biomedical Inc. Asia	Clinical – phase 1
Protein based	Recombinant spike with adjuvant	Iran	Pre-clinical
Protein based	Recombinant S protein produced in BEVS	Tampere University	Pre-clinical
Protein based	RBD protein delivered in mannose-conjugated chitosan nanoparticle	Ohio State University / Kazakh National Agrarian University	Pre-clinical
Protein based	Recombinant spike protein with Essai O/W 1849101 adjuvant	Kazakh National Agrarian University	Pre-clinical
Protein based	Peptides	Neo7Logic	Pre-clinical
Protein based	Recombinant spike protein with Essai O/W 1849101 adjuvant	Kazakh National Agrarian University, Kazakhstan / National Scientific Center for Especially Dangerous Infections	Pre-clinical
Protein based	Recombinant S protein	Max-Planck-Institute of Colloids and Interfaces	Pre-clinical
Protein based	RBD protein (baculovirus production) + FAR-Squalene adjuvant	Farmacológicos Veterinarios SAC (FARVET SAC) / Universidad Peruana Cayetano Heredia (UPCH)	Pre-clinical
Protein based	Protein Subunit	Research Institute for Biological Safety Problems, Rep of Kazakhstan	Pre-clinical
Protein based	RBD-protein	Mynvax	Pre-clinical

Protein based	Recombinant S protein	Izmir Biomedicine and Genome Center	Pre-clinical
Protein based	Peptide + novel adjuvant	Bogazici University	Pre-clinical
Protein based	S subunit intra-nasal liposomal formulation with GLA/3M052 adjs.	University of Virginia	Pre-clinical
Protein based	S-Protein (Subunit) + Adjuvant, E coli based Expression	Helix Biogen Consult, Ogbomoso & Trinity Im-monoefficient Laboratory, Ogbomoso, Oyo State, Nigeria.	Pre-clinical
Protein based	Protein Subunit S,N,M&S1 protein	National Research Centre, Egypt	Pre-clinical
Protein based	Protein Subunit	University of San Martin and CONICET, Argen-tina	Pre-clinical
Protein based	RBD protein fused with Fc of IgG + Adj.	Chulalongkorn University/GPO, Thailand	Pre-clinical
Protein based	Capsid-like Particle	AdaptVac (PREVENT-nCoV consortium)	Pre-clinical
Protein based	Drosophila S2 insect cell expression sys-tem VLPs	ExpreS2ion	Pre-clinical
Protein based	Peptide antigens formulated in LNP	IMV Inc	Pre-clinical
Protein based	S protein	WRAIR/USAMRIID	Pre-clinical
Protein based	S protein +Adjuvant	National Institute of Infectious Disease, Japan/ Shionogi/UMN Pharma	Pre-clinical
Protein based	VLP-recombinant protein + Adjuvant	Osaka University/ BIKEN/ National Institutes of Biomedical Innovation, Japan	Pre-clinical
Protein based	microneedle arrays S1 subunit	Univ. of Pittsburgh	Pre-clinical
Protein based	Peptide	Vaxil Bio	Pre-clinical
Protein based	Peptide	Flow Pharma Inc	Pre-clinical
Protein based	S protein	AJ Vaccines	Pre-clinical
Protein based	Ii-Key peptide	Generex/EpiVax	Pre-clinical
Protein based	S protein	EpiVax/Univ. of Georgia	Pre-clinical
Protein based	Protein Subunit EPV-CoV-19	EpiVax	Pre-clinical
Protein based	gp-96 backbone	Heat Biologics/Univ. Of Miami	Pre-clinical
Protein based	Subunit vaccine	FBRI SRC VB VECTOR, Rospotrebnadzor, Kolt-sovo	Pre-clinical
Protein based	S1 or RBD protein	Baylor College of Medicine	Pre-clinical
Protein based	Subunit protein, plant produced	iBio/CC-Pharming	Pre-clinical

Protein based	Recombinant protein, nanoparticles (based on S-protein and other epitopes)	Saint-Petersburg scientific research institute of vaccines and serums	Pre-clinical
Protein based	COVID-19 XWG-03 truncated S (spike) proteins	Innovax/Xiamen Univ./GSK	Pre-clinical
Protein based	Adjuvanted microsphere peptide	VIDO-InterVac, University of Saskatchewan	Pre-clinical
Protein based	Synthetic Long Peptide Vaccine candidate for S and M proteins	OncoGen	Pre-clinical
Protein based	Oral E. coli-based protein expression system of S and N proteins	MIGAL Galilee Research Institute	Pre-clinical
Protein based	Nanoparticle vaccine	LakePharma, Inc.	Pre-clinical
Protein based	Plant-based subunit (RBD-Fc + Adjuvant)	Baiya Phytopharm/ Chula Vaccine Research Center	Pre-clinical
Protein based	OMV-based vaccine	Quadram Institute Biosciences	Pre-clinical
Protein based	structurally modified spherical particles of the tobacco mosaic virus (TMV)	Lomonosov Moscow State University	Pre-clinical
Protein based	Spike-based	University of Alberta	Pre-clinical
Protein based	Recombinant S1-Fc fusion protein	AnyGo Technology	Pre-clinical
Protein based	Recombinant protein	Yisheng Biopharma	Pre-clinical
Protein based	Recombinant S protein in IC-BEVS	Vabiotech	Pre-clinical
Protein based	Orally delivered, heat stable subunit	Applied Biotechnology Institute, Inc.	Pre-clinical
Protein based	Peptides derived from Spike protein	Axon Neuroscience SE	Pre-clinical
Protein based	Protein Subunit	MOGAM Institute for Biomedical Research, GC Pharma	Pre-clinical
Protein based	RBD-based	Neovii/Tel Aviv University	Pre-clinical
Protein based	Outer Membrane Vesicle (OMV)-subunit	Intravacc/Epivax	Pre-clinical
Protein based	Outer Membrane Vesicle(OMV)-peptide	Intravacc/Epivax	Pre-clinical

Protein based	Spike-based (epitope screening)	ImmunoPrecise/LiteVax BV	Pre-clinical
VLP	RBD-HBsAg VLPs	SpyBiotech/Serum Institute of India	Clinical – phase 1/2
VLP	Plant-derived VLP adjuvanted with GSK or Dynavax adjs.	Medicago Inc.	Clinical – phase 1
VLP	Plant derived VLP	Shiraz University	Pre-clinical
VLP	VLPs produced in BEVS	Tampere University	Pre-clinical
VLP	VLP	Max Planck Institute for Dynamics of Complex Technical Systems	Pre-clinical
VLP	Virus-like particle-based Dendritic Cell(DC)-targeting vaccine	University of Manitoba	Pre-clinical
VLP	VLP	Bezmialem Vakif University	Pre-clinical
VLP	VLP	Middle East Technical University	Pre-clinical
VLP	Enveloped Virus-Like Particle (eVLP)	VBI Vaccines Inc.	Pre-clinical
VLP	S protein integrated in HIV VLPs	IrsiCaixa AIDS Research/IRTA-CReSA/Barcelona Supercomputing Centre/Grifols	Pre-clinical
VLP	VLP + Adjuvant	Mahidol University/ The Government Pharmaceutical Organization (GPO)/Siriraj Hospital	Pre-clinical
VLP	Virus-like particles, lentivirus and baculovirus vehicles	Navarrabiomed, Oncoimmunology group	Pre-clinical
VLP	Virus-like particle, based on RBD displayed on virus-like particles	Saiba GmbH	Pre-clinical
VLP	ADDomerTM multi-epitope display	Imophoron Ltd and Bristol University's Max Planck Centre	Pre-clinical
VLP	Unknown	Doherty Institute	Pre-clinical
VLP	VLP	OSIVAX	Pre-clinical
VLP	eVLP	ARTES Biotechnology	Pre-clinical
VLP	VLPs peptides/ whole virus	Univ. of Sao Paulo	Pre-clinical
DNA	DNA plasmid vaccine with electroporation	Inovio Pharmaceuticals/ International Vaccine Institute	Clinical – phase 1/2
DNA	DNA plasmid vaccine + Adjuvant	Osaka University/ AnGes/ Takara Bio	Clinical – phase 1/2
DNA	DNA plasmid vaccine	Cadila Healthcare Limited	Clinical – phase 1/2
DNA	DNA Vaccine (GX-19)	Genexine Consortium	Clinical – phase 1/2
DNA	bacTRL-Spike	Symvivo	Clinical – phase 1

DNA	DNA plasmids containing S-gene	Biosun Pharmed	Pre-clinical
DNA	DNA plasmid vaccine	Globe Biotech Limited, Bangladesh	Pre-clinical
DNA	Plasmid DNA, nanostructured RBD	National institute of Chemistry, Slovenia	Pre-clinical
DNA	DNA, engineered vaccine inserts compatible with multiple delivery systems	DIOSynVax Ltd / University of Cambridge	Pre-clinical
DNA	DNA vaccine	Ege University	Pre-clinical
DNA	DNA plasmid vaccine RBD&N	Scancell/University of Nottingham/ Nottingham Trent University	Pre-clinical
DNA	DNA plasmid vaccine S,S1,S2,RBD &N	National Research Centre, Egypt	Pre-clinical
DNA	DNA with electroporation	Karolinska Institute / Cobra Biologics (OPENCORONA Project)	Pre-clinical
DNA	DNA with electroporation	Chula Vaccine Research Center	Pre-clinical
DNA	DNA	Takis/Applied DNA Sciences/Evvivax	Pre-clinical
DNA	Plasmid DNA, Needle-Free Delivery	Immunomic Therapeutics, Inc./EpiVax, Inc./ PharmaJet	Pre-clinical
DNA	DNA vaccine	BioNet Asia	Pre-clinical
DNA	msDNA vaccine	Mediphage Bioceuticals/University of Waterloo	Pre-clinical
DNA	DNA vaccine	Entos Pharmaceuticals	Pre-clinical
RNA	LNP-encapsulated mRNA	Moderna/NIAID	Clinical – phase 1, phase 2, phase 3
RNA	3 LNP-mRNAs	BioNTech/Fosun Pharma/Pfizer	Clinical – phase 1, phase 1/2
RNA	mRNA	Curevac	Clinical – phase 1, phase 2
RNA	mRNA	Arcturus/Duke-NUS	Clinical – phase 1/2
RNA	LNP-nCoVsaRNA	Imperial College London	Clinical – phase 1
RNA	mRNA	People's Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech.	Clinical – phase 1
RNA	mRNA	Providence Therapeutics	Pre-clinical
RNA	mRNA	Cell Tech Pharmed	Pre-clinical
RNA	mRNA	ReNAP Co.	Pre-clinical
RNA	D614G variant LNP-encapsulated mRNA	Globe Biotech Ltd	Pre-clinical
RNA	saRNA formulated in a NLC	Infectious Disease Research Institute/ Amyris, Inc.	Pre-clinical
RNA	LNP-encapsulated mRNA encoding S	Max-Planck-Institute of Colloids and Interfaces	Pre-clinical
RNA	Self-amplifying RNA	Gennova	Pre-clinical
RNA	mRNA	Selcuk University	Pre-clinical

RNA	LNP-mRNA	Translate Bio/Sanofi Pasteur	Pre-clinical
RNA	LNP-mRNA	CanSino Biologics/Precision NanoSystems	Pre-clinical
RNA	LNP-encapsulated mRNA cocktail encoding VLP	Fudan University/ Shanghai JiaoTong University/RNACure Biopharma	Pre-clinical
RNA	LNP-encapsulated mRNA encoding RBD	Fudan University/ Shanghai JiaoTong University/RNACure Biopharma	Pre-clinical
RNA	Replicating Defective SARS-CoV-2 derived RNAs	Centro Nacional Biotecnología (CNB-CSIC), Spain	Pre-clinical
RNA	LNP-encapsulated mRNA	University of Tokyo/ Daiichi-Sankyo	Pre-clinical
RNA	Liposome-encapsulated mRNA	BIOCAD	Pre-clinical
RNA	Several mRNA candidates	RNAimmune, Inc.	Pre-clinical
RNA	mRNA	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Pre-clinical
RNA	mRNA	China CDC/Tongji University/Stermina	Pre-clinical
RNA	LNP-mRNA	Chula Vaccine Research Center/University of Pennsylvania	Pre-clinical
RNA	mRNA in an intranasal delivery system	eTheRNA	Pre-clinical
RNA	mRNA	Greenlight Biosciences	Pre-clinical
RNA	mRNA	IDIBAPS-Hospital Clinic, Spain	Pre-clinical
T-cell based	CD8 T cell peptide targeting (S, M, N) and (NSPs) SARS-CoV-2 proteins	OSE immunotherapeutics	Pre-clinical

DNA VACCINES

One of the mostly examined vaccines in the last decade is DNA vaccine. The DNA vaccine is based on the principle of synthesis of the DNA sequence in the form of a plasmid encoding a protein antigen of the virus. Under optimal conditions, when this sequence reaches the nucleus, this information is transcribed into mRNA, followed by translation into a protein form, which further leads to activation of the immune system and the formation of antibodies of interest, which would provide a specific immunity [31]. However, this sequence of events has a number of obstacles that need to be solved prior to their application. By the synthesis of the appropriate DNA sequence for vaccine, several items should be enabled: successful transport into cells, resistance to the enzymatic degradation in the cytosolic medium, protection from the nucleus entry defend system while preventing DNA vaccine incorporation within the host DNA [32]. Apart from mentioned, limited ability to control immune response triggered by this kind of vaccines must be accounted.

In spite of the production of DNA vaccines is very fast, easy to design and manipulate with, as well as that they do not require continuous cold storage [33], the very efficient and sophisticated delivery system to the cells is required, and for that purpose around 37 various stabilizers are used for plasmid DNA vaccine stability [34]. But nevertheless, the stabilizers used in these vaccines are not fully evaluated in terms of their toxicity. This is one of two main problems when it comes to the DNA vaccines. Namely, it is already discussed about the risks related to usage of various adjuvants such as monophosphoryl lipid A, soap-based molecule QS21, squalene oil substance MF59, cyto-

sine-guanin pair CpG, and preservatives like Thimerosal which contain a mercury (Hg), as well as stabilizers such as Al [34]. The second is that there are obvious problems when it comes to the incorporation of the foreign sequence of plasmid DNA into the host DNA. There are approximately 1,000 to 4,000 copies per μg of DNA which persist 6 weeks, and 200 to 400 copies per μg of DNA which persist 6 months after vaccination [35]. This alteration (transfection) must not be neglected because it might affect the normal upstream and/or downstream DNA transcription. Moreover, the fact that DNA tests were mostly done on animals, and very little is known about their effects on humans, as well as obvious concerns about possible integration of plasmid DNA into human genome puts to the fore the ethical question about clinical trials on humans [31]. Furthermore, inadequate immunogenicity of DNA vaccines, pointed out that these vaccines could not be a proper alternative to the conventional [36]. Namely, due to the immune system is slightly but continuously stimulated by the plasmid DNA vaccine to produce antibodies, the outcome might be serious side effects in a form of chronic stimulation of immune response and subsequent persistent inflammation [31]. On the other hand there are studies explaining that DNA vaccines are more efficacious than vaccines based on recombinant proteins [37] and recombinant viruses [38]. Taking all into account, DNA vaccines are weak inducers of immune responses when compared to the inactive or live-attenuated virus vaccines, and various strategies are being developed to improve their poor efficacy [36]. Another serious concern about usage of DNA vaccine is that they could be triggering button for autoimmune responses, and activation of cancer-causing genes [31]. Moreover, there are literatures data indicating that carriers of the latent infections, where the host co-habituated with the pathogen without conflict (disease tolerance) can be the critical for this kind of vaccination, causing the severe forms of disease upon DNA vaccine application [39]. Namely, Taylor *et al*. explain that DNA form of tuberculosis vaccine may be completely safe when it comes to naive individuals, but if this vaccine is administrated among the people with latent infection it can activate infection leading to severe clinical manifestations [39]. Since the human body lives in symbiosis with various bacteria and viruses, some people achieve disease tolerance with strains that are generally pathogenic, which makes the vaccination as the approach for the development of protection among this population questionable. This is especially referred to the vaccination with prolonged stimulation of immune response, such the DNA vaccines are. Except from basic scientific and ethical controversies related to DNA vaccines even more doubtful is their massive use in order to eradicate the disease. Be that as it may, vaccines in a form of DNA have been more and more discussed as alternatives to conventional vaccine approaches [40]. DNA vaccine is also in the phase of testing for COVID-19, and it is based on sequence which decode the full-length spike (S) or S1 subunit, involved in infection initiation [41]. Furthermore, according to the WHO statistics there are currently 19 types of DNA vaccines for COVID-19 among which 14 are in pre-clinical, and 5 are already in clinical phase of testing (**Figure 1**) [10]. Detailed list is given in **Table 1**.

RNA VACCINES

The new form of genetic vaccines is mRNA vaccine. The principle of mRNA-based vaccines relies on their ability to encode antigen (pathogen) into the protein forms using the host ribosomal machinery. Such a protein is supposed to activate a specific immune response without causing the disease development. The first usage of mRNA as a tool for delivering information for protein production was tested on mice 1990s [42]. Only three years later the injection of influenza mRNA was used to induce immune response which resulted in cytotoxic T lymphocyte generation in mice, suggesting that mRNA might be good alternative for vaccine design [43]. mRNA technology is intensively assessed in drug development and several RNA-based drugs were approved by Food and Drug Administration (FDA) for clinical use [44]. Kim *et al* in 2020 implicated that RNA-based drugs are very easy to design and they possess long-lasting effects, but the main disadvantage is that RNA drugs can only target to the liver efficiently but not into other organs [44]. When it comes to the application of this technology to vaccine design, translation from animal to humans introduced the researchers with a lot of side effects, making a serious concern about their usage in development of immune protection [45]. They are mostly connected with mRNA instability, high innate immunogenicity, and inefficient delivery *in vivo* [46]. To overcome instability of RNA, design of self-amplifying RNA is forced [46]. Conventional mRNA-based vaccines encoded the protein of interest become rapidly digested by the cell enzymes in cytoplasm which goes in terms of their poor specific immunogenicity, similarly to DNA vaccine. On the other hand, self-amplifying mRNAs besides target antigen encode the proteins involved in viral replication, enabling their intracellular amplification [47]. From this point, the risks bound to DNA vaccine entering the nucleus to express the gen of interest, are overcome with mRNA vaccines approaches [48]. On the other hand, it is known that endogenous retroviruses (ERVs) derived from outside retroviruses particles comprise up to 5–8% of the human genome [49, 50]. These alterations were inactivated by mutations or the repair DNA mechanisms which are still unknown, protecting the host from the development of cancer.

Moreover, with advances in science in the field of genetics, it has been shown that much of the DNA (98%) although it does not decode protein sequences is not as dysfunctional as previously claimed [51, 52], but it is discovered that within those 98% DNA over 15% encode functional RNA molecules among which 58648 genes were identified as long non-coding RNAs (lncRNAs) parties [53, 54]. At the same time when the era of RNA technologies and their application in disease treatment started, the first data illustrating regulatory role of RNAs in different biological domains were reported. Only few decades ago, RNAs were treated as basically inert macromolecules that exclusively serve to the protein synthesis [55, 56]. Under these circumstances, the ideal concept of mRNA vaccine as a template for the synthesis of relevant fragment with the aim to promote specific immune reaction, without serious side interaction is expected. However, with the discovery of the network of noncoding RNAs and their active principle in regulation of gene expression through RNA/mRNA interactions, cellular homeostasis maintenance and host defence against viral infection, the spectrum of doubts about possible interactions of introduced sequence with this network is opened [57]. Namely, after the infection with RNA of SARS-CoV in mice, more than 1000 lncRNAs were shown to be expressed activating IFN response [58]. Some studies pointing out that to overcome this high innate immunity, in terms to improve the mRNA vaccine, the early type of IFN I must be reduced [59]. To solve these problems two approaches of mRNA design are used to lower degradation of mRNA and to ensure its translation. First is pseudo nucleotides (nucleosid-modified) mRNA, and the second is the vaccine based on optimization of the nucleotide sequence with unmodified nucleoside [60]. But, there are still no data pointing out which vaccine is better for prophylactic use in humans, modified or unmodified mRNA. Additionally, it is well known that viral RNA interacts with host micro (mi)RNAs leading to its post-translational repression avoiding replication in tissue compartments, which is the method of eliciting host immune response. Thus viral RNA accumulates into the nucleus and uses host transfer (t)RNAs as a primer for replication. Such a produced nuclear (nc)RNAs further regulates host cellular function [61]. Based on that, viral RNAs possess mechanisms to manipulate and take over the host lncRNAs function [62]. Furthermore, there is no doubt that there are certain homologies between viral and human RNAs which can result in proteins homolog generation. Such proteins might be inhibitors for intracellular signalling pathways, causing the depletion of physiological statements and impairing the state of metabolic diseases such as diabetes, which is seen on the example of enterovirus RNA [63-65]. These are the risks which must be examined in detail in order to clinical use of such a type of vaccine.

All this underlined possible interactions in intracellular pool of RNA, which were totally unknown half of the century ago and accordingly, excluded from evaluation at the moment of accelerated research in the area of RNA technology in disease treatment. Bypassing these facts, some review articles highlight the commercial aspect of genetic vaccines [66]. Apart from all concerns, mRNA vaccines for COVID-19 are developing very fast, shortening the period of testing [67-76]. In that manner, the references list pointing to COVID-19 mRNA vaccine development is growing (**Table 1**). By now 28 types of COVID-19 vaccines based on mRNA technology are in preparation, among which 22 are in pre-clinical and 6 in clinical phase of testing (**Figure 1**).

In terms of genetic vaccines certain *pros* is that the DNA/RNA fragments do not cause COVID-19 infection, but the *cons* are unpredictable interactions at the level of the genome, RNA regulatory network as well as inability to control dynamic, intensity and lasting of immune response. Attached to the last, exaggerated risks of genetic vaccination for the latent asymptomatic carriers of the virus should be taken into account [35, 77-79].

CONCLUSION

Very soon after the coronavirus was detected in December 2019, genetic sequencing of the COVID-19 genome was performed a month later, which led to an urgent international response for the rapid development of a vaccine. Even that public's confidence to the vaccination is built on the standard "old fashion" procedures in protective immunity development, absolute supremacy in designing and placing COVID-19 vaccines has been attained by new technologies such as those based on mRNA and DNA concepts. So far, both approaches are multiply tested against different viral diseases but neither of them passed the route from clinical trials to application, regarding to not trivial obstacles that have occurred on their way to the market and are not related only to satisfactory efficiency in acquiring the desired immunity. The controversies related to the design as well as potential application of both types of vaccines, independently from the development of immune protection, implied the entry into the field of complex and under-known interactions within the cell, not only just at the genome level as is most commonly speculated. Furthermore, the compensatory mechanisms for maintaining the homeostasis inside human genome, as well as their involvement into many pathological processes are still a mysterious, which demands a lot of investigation before the usage of genetic tools in terms of immunisation.

ACKNOWLEDGMENT

Many thanks to dr Sanja Mijatovic and dr Danijela Maksimovic-Ivanic for assistance in summarizing the facts and highlighting the issues of this article. This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (451-03-68/2020-14/200007).

REFERENCES

1. Disease outbreaks by year 2020 [Internet]. World Health Organization. 2020. Available from: https://www.who.int/csr/don/archive/year/en/.
2. Balkhair A. The Struggle Against Pandemic Influenza A (H1N1) 2009. Sultan Qaboos Univ Med J 2009; 9(3):257-60.
3. Balkhair A, Al Maamari K, Alawi FB. The Struggle Against MERS-CoV (The Novel Coronavirus). Oman Med J 2013; 28(4):226-7.
4. De Wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol 2016; 14(8):523-34.
5. Coronaviridae Study Group of the International Committee on Taxonomy Of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020; 5(4):536-44.
6. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ et al. Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases. ACS Cent Sci 2020; 6(3):315-31.
7. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol 2020; 38(1):1-9.
8. Varicella (Chickenpox) Vaccine: What You Need to Know [Internet]. Centers for Disease Control and Prevention; [cited 2019 Aug 15]. Available from: https://www.cdc.gov/vaccines/hcp/vis/vis-statements/varicella.html.
9. HIV Infection and Adult Vaccination [Internet]. Center for Disease Control and Prevention; 2020 [cited 2020]. Available from: https://www.cdc.gov/vaccines/adults/rec-vac/health-conditions/hiv.html.
10. Draft landscape of COVID-19 candidate vaccines [Internet]. World Health Organization; 2020 [cited 2020]. Available from: https://www.who.int/publications/m/item/draft-landscape-of-covid19-candidate-vaccines.
11. Syomin BV, Ilyin YV. Virus-Like Particles as an Instrument of Vaccine Production. Mol Biol 2019; 53(3):323-34.
12. Plummer EM, Manchester M. Viral nanoparticles and virus-like particles: platforms for contemporary vaccine design. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2011;3(2):174-96.
13. Roldao A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines 2010; 9(10):1149-76.
14. Grgacic EV, Anderson DA. Virus-like particles: passport to immune recognition. Methods 2006; 40(1):60-5.
15. Zha L, Zhao H, O. Mohsen M, Hong L, Zhou Y, Li Z et al. Development of a COVID-19 vaccine based on the receptor binding domain displayed on virus-like particles. BioRxiv 2020; DOI: 10.1101/2020.05.06.079830.
16. Ura T, Okuda K, Shimada M. Developments in Viral Vector-Based Vaccines. Vaccines (Basel) 2014; 2(3):624-41.
17. Bull JJ, Nuismer SL, Antia R. Recombinant vector vaccine evolution. PLoS Comput Biol 2019; 15(7):e1006857.
18. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008; 372(9653):1881-93.
19. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis 2011; 11(7):507-15.
20. Moodie Z, Metch B, Bekker LG, Churchyard G, Nchabeleng M, Mlisana K et al. Continued Follow-Up of Phambili Phase 2b Randomized HIV-1 Vaccine Trial Participants Supports Increased HIV-1 Acquisition among Vaccinated Men. PLoS One 2015; 10(9):e0137666.
21. Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020; 395(10240):1845-54.
22. Ho JK, Jeevan-Raj B, Netter HJ. Hepatitis B Virus (HBV) Subviral Particles as Protective Vaccines and Vaccine Platforms. Viruses 2020; 12(2):126.
23. Skwarczynski M, Toth I. Peptide-based synthetic vaccines. Chem Sci 2016; 7(2):842-54.
24. Salimian Rizi B, Achreja A, Nagrath D. Nitric Oxide: The Forgotten Child of Tumor Metabolism. Trends Cancer 2017; 3(9):659-72.
25. Kwissa M, Lindblad EB, Schirmbeck R, Reimann J. Co-delivery of a DNA vaccine and a protein vaccine with aluminum phosphate stimulates a potent and multivalent immune response. J Mol Med (Berl) 2003; 81(8):502-10.
26. Maya S, Prakash T, Madhu KD, Goli D. Multifaceted effects of aluminium in neurodegenerative diseases: A review. Biomed Pharmacother 2016; 83:746-54.
27. Data & Statistics on Autism Spectrum Disorder [Internet]. Center for Disease Control and Prevention; 2020 [cited 2020]. Available from: https://www.cdc.gov/ncbddd/autism/data.html.
28. Lukiw WJ, Kruck TPA, Percy ME, Pogue AI, Alexandrov PN, Walsh WJ et al. Aluminum in neurological disease - a 36 year multicenter study. J Alzheimers Dis Parkinsonism 2019; 8(6):457.
29. Rice CE, Rosanoff M, Dawson G, Durkin MS, Croen LA, Singer A et al. Evaluating Changes in the Prevalence of the Autism Spectrum Disorders (ASDs). Public Health Rev 2012; 34(2):1-22.
30. Tomljenovic L, Shaw CA. Do aluminum vaccine adjuvants contribute to the rising prevalence of autism? J Inorg Biochem 2011; 105(11):1489-99.
31. Zhang A. DNA Vaccines: Scientific and Ethical Barriers to the Vaccines of the Future [Internet]. 2011 [cited 2011 Nov 15]. Available from: https://www.hcs.harvard.edu/hghr/online/dna-vaccines/.
32. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. Expert Rev Vaccines 2016; 15(3):313-29.
33. Gorecki DC, Simons JP. The dangers of DNA vaccination. Nat Med 1999; 5(2):126.

34. Zeng Y, Ramsey JD, King R, Leviten M, Mcguire R, Volkin DB et al. Identifying stabilizers of plasmid DNA for pharmaceutical use. J Pharm Sci 2011; 100(3):904-14.
35. Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG et al. Plasmid DNA vaccines: investigation of integration into host cellular DNA following intramuscular injection in mice. Intervirology 2000; 43(4-6):258-72.
36. Leitner WW, Ying H, Restifo NP. DNA and RNA-based vaccines: principles, progress and prospects. Vaccine 1999; 18(9-10):765-77.
37. Davis HL, Demeneix BA, Quantin B, Coulombe J, Whalen RG. Plasmid DNA is superior to viral vectors for direct gene transfer into adult mouse skeletal muscle. Hum Gene Ther 1993; 4(6):733-40.
38. Hurpin C, Rotarioa C, Bisceglia H, Chevalier M, Tartaglia J, Erdile L. The mode of presentation and route of administration are critical for the induction of immune responses to p53 and antitumor immunity. Vaccine 1998; 16(2-3):208-15.
39. Taylor JL, Turner OC, Basaraba RJ, Belisle JT, Huygen K, Orme IM. Pulmonary necrosis resulting from DNA vaccination against tuberculosis. Infect Immun 2003; 71(4):2192-8.
40. Liu MA. DNA vaccines: an historical perspective and view to the future. Immunol Rev 2011; 239(1):62-84.
41. Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J et al. Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun 2020; 11(1):2601.
42. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A et al. Direct gene transfer into mouse muscle in vivo. Science 1990; 247(4949 Pt1):1465-8.
43. Martinon F, Krishnan S, Lenzen G, Magne R, Gomard E, Guillet JG et al. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. Eur J Immunol 1993; 23(7):1719-22.
44. Kim YK. RNA Therapy: Current Status and Future Potential. Chonnam Med J 2020; 56(2):87-93.
45. Wadman M. Public needs to prep for vaccine side effects. Science 2020; 370(6520):1022.
46. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov 2018; 17(4):261-79.
47. Geall AJ, Verma A, Otten GR, Shaw CA, Hekele A, Banerjee K et al. Nonviral delivery of self-amplifying RNA vaccines. Proc Natl Acad Sci U S A 2012; 109(36):14604-9.
48. Fuller DH, Berglund P. Amplifying RNA Vaccine Development. N Engl J Med 2020; 382(25):2469-71.
49. Nelson PN, Hooley P, Roden D, Davari Ejtehadi H, Rylance P, Warren P et al. Human endogenous retroviruses: transposable elements with potential? Clin Exp Immunol 2004; 138(1):1-9.
50. Belshaw R, Pereira V, Katzourakis A, Talbot G, Paces J, Burt A et al. Long-term reinfection of the human genome by endogenous retroviruses. Proc Natl Acad Sci USA 2004; 101(14):4894-9.
51. Ehret CF, De Haller G. Origin, Development and Maturation of Organelles and Organelle Systems of the Cell Surface in Paramecium. J Ultrastruct Res 1963; 23:SUPPL6:1-42.
52. Elgar G, Vavouri T. Tuning in to the signals: noncoding sequence conservation in vertebrate genomes. Trends Genet 2008; 24(7):344-52.
53. Graur D. An Upper Limit on the Functional Fraction of the Human Genome. Genome Biol Evol 2017; 9(7):1880-85.
54. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet 2015; 47(3):199-208.
55. Delihas N. Discovery and characterization of the first non-coding RNA that regulates gene expression, micF RNA: A historical perspective. World J Biol Chem 2015; 6(4):272-80.
56. Fu XD. Non-coding RNA: a new frontier in regulatory biology. Natl Sci Rev 2014; 1(2):190-204.
57. Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W. Emerging roles of lncRNA in cancer and therapeutic opportunities. Am J Cancer Res 2019; 9(7):1354-66.
58. Peng X, Gralinski L, Armour CD, Ferris MT, Thomas MJ, Proll S et al. Unique signatures of long noncoding RNA expression in response to virus infection and altered innate immune signaling. mBio 2010; 1(5):e00206-10.
59. Pepini T, Pulichino AM, Carsillo T, Carlson AL, Sari-Sarraf F, Ramsauer K et al. Induction of an IFN-Mediated Antiviral Response by a Self-Amplifying RNA Vaccine: Implications for Vaccine Design. J Immunol 2017; 198(10):4012-24.
60. Rauch S, Jasny E, Schmidt KE, Petsch B. New Vaccine Technologies to Combat Outbreak Situations. Front Immunol 2018; 9:1963.
61. Damas ND, Fossat N, Scheel TKH. Functional Interplay between RNA Viruses and Non-Coding RNA in Mammals. Noncoding RNA 2019; 5(1):7.
62. Wang P, Xu J, Wang Y, Cao X. An interferon-independent lncRNA promotes viral replication by modulating cellular metabolism. Science 2017; 358(6366):1051-55.
63. Craig ME, Howard NJ, Silink M, Rawlinson WD. Reduced frequency of HLA DRB1*03-DQB1*02 in children with type 1 diabetes associated with enterovirus RNA. J Infect Dis 2003; 187(10):1562-70.
64. Yin H, Berg AK, Tuvemo T, Frisk G. Enterovirus RNA is found in peripheral blood mononuclear cells in a majority of type 1 diabetic children at onset. Diabetes 2002; 51(6):1964-71.
65. Oikarinen S, Martiskainen M, Tauriainen S, Huhtala H, Ilonen J, Veijola R et al. Enterovirus RNA in blood is linked to the development of type 1 diabetes. Diabetes 2011; 60(1):276-9.
66. Mascola JR, Fauci AS. Novel vaccine technologies for the 21st century. Nat Rev Immunol 2020; 20(2):87-88.
67. Wehling M. Calling for an exponential escalation scheme in vaccine development for COVID-19. Eur J Clin Pharmacol 2020.
68. Schaefer GO, Tam CC, Savulescu J, Voo TC. COVID-19 vaccine development: Time to consider SARS-CoV-2 challenge studies? Vaccine 2020; 38(33):5085-88.
69. Iserson KV. SARS-CoV-2 (COVID-19) Vaccine Development and Production: An Ethical Way Forward. Camb Q Healthc Ethics 2021; 30(1):59-68.
70. Arora NK, Das MK. COVID-19 vaccine development and the way forward. Indian J Public Health 2020; 64(Supplement):S108-S11.
71. Lee N, McGeer A. The starting line for COVID-19 vaccine development. Lancet 2020; 395(10240):1815-16.
72. Sharpe HR, Gilbride C, Allen E, Belij-Rammerstorfer S, Bissett C, Ewer K et al. The early landscape of COVID-19 vaccine development in the UK and rest of the world. Immunology 2020; 160(3):223-232.

73. Graham BS. Rapid COVID-19 vaccine development. Science 2020; 368(6494):945-46.
74. Yang L, Tian D, Liu W. Strategies for vaccine development of COVID-19. Sheng Wu Gong Cheng Xue Bao 2020; 36(4):593-604.
75. Wu SC. Progress and Concept for COVID-19 Vaccine Development. Biotechnol J 2020; 15(6):e2000147.
76. Thanh Le T, Andreadakis Z, Kumar A, Gomez Roman R, Tollefsen S, Saville M et al. The COVID-19 vaccine development landscape. Nat Rev Drug Discov 2020; 19(5):305-06.
77. From adenoviruses to RNA: the pros and cons of different COVID vaccine technologies [Internet]. The Conversation, Academic rigour. 2020 [cited 2020 Sep 17]. Available from: https://theconversation.com/from-adenoviruses-to-rna-the-pros-and-cons-of-different-covid-vaccine-technologies-145454.
78. Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG et al. Plasmid DNA vaccines: assay for integration into host genomic DNA. Dev Biol (Basel) 2000; 104:33-43.
79. Manam S, Ledwith BJ, Barnum AB, Troilo PJ, Pauley CJ, Harper LB et al. Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA. Intervirology 2000; 43(4-6):273-81.

COVID-19 kao prekretnica za upotrebu novih vrsta vakcina

Milan Marković

Institut za biološka istraživanja „Siniša Stanković", Institut od nacionalnog značaja za Republiku Srbiju, Odeljenje za imunologiju, Univerzitet u Beogradu, Srbija

Kratak sadržaj

COVID-19 je infektivna bolest izazvana virusom SARS-CoV-2 koji pripada grupi Coronaviridae. Simptomi bolesti su prvenstveno prisutni u obliku respiratornog sindroma sličnog simptomima do kojih dovode drugi respiratorni virusi poput virusa gripa, kao i virusa koji dovode do oboljenja poznatih pod imenom SARS i MERS. Međutim, na osnovu kratkog kliničkog iskustva, pokazalo se da COVID-19 utiče na sve organske sisteme ali je patogeneza bolesti i dalje nejasna. Blokada u razmeni gasova, sistemska hipoksija i poremećaji u koagulaciji prate najtežu kliničku sliku. Pored toga, ovaj virus pripada grupi RNA virusa koji dele zajedničke karakteristike sa virusom HIV-a, što ga čini veoma izazovnim u pogledu terapije savremenim medicinskim pristupima. Trenutno se širom sveta vode polemike o tome kako se boriti protiv ove epidemije. Pored higijenskih mera i mera bezbednog rastojanja i izolacije, postoji važna polemika oko profilaktičkih vakcina. U ovom preglednom članku pruža se uvid u sve aspekte vakcinacije i metode za njihov dizajn zasnovane na tehnologijama koje poseduje savremena nauka sa posebnim osvrtom na genetske (DNK, RNK) vakcine, i postavlja pitanje da li postoji opravdanost njihove urgentne primene u slučaju pandemije COVID-19.

Ključne reči: *COVID-19; Vakcine; Oslabljeni virusi; VLP; Vektor; Protein; DNK; RNK.*