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Recent challenges and progress of potential vaccines for Coronavirus disease 2019 (COVID-19)

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ABSTRACT

The pandemic of COVID-19 emerged at the end of 2019 and spread rapidly in almost all the countries around the world. This has become a severe global health concern. COVID-19 pandemic is still spreading and reoccurring, so vaccines are urgently needed to control the spreading of this epidemic. Facts have shown that vaccines are the most successful and efficient way to combat and manage infectious diseases. Enormous efforts have been made by government, industry, and academia to develop successful vaccines in a few months span. Some vaccines have been evaluated for efficiency in animal and preliminary clinical trials. This brief review summarizes the approaches used in the vaccines design and focuses on the progress of COVID-19 vaccine development. We have also highlighted the challenges faced in the development of COVID-19 vaccines.

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1. INTRODUCTION

Coronaviruses, genotypically and phenotypically, are a distinct family of viruses. They are enveloped viruses having ssRNA that belong to the family *Coronaviridae* which can cause mild to severe respiratory illness in humans. The virus named as 'Corona' just because of their appearance in electron microscope that they are covered with a crown-like structure. The genome of coronavirus is approximately 26-32 kb that encodes structural and non-structural proteins. The structural proteins play role in virus entry and non-structural prteins play role in viral replication. The structural proteins consist of four major types of proteins such as Spike (S) protein, membrane (M) protein, nucleocapsid (N) protein and envelope (E) protein (1–3). The S protein is a primary antigenic component of the virus making it an attractive target in candidate vaccine designs. It composed of two subunits S1 and S2. S1 consists of a receptor-binding domain and facilitate the binding of virus to the host cell through angiotensin-converting enzyme 2 (ACE2) receptor. S2 consists of fusion machinery and help in the fusion of

host cell membrane. The non-structural proteins encoded by the virus are Papain-like protease (PLpro), RNA-dependent RNA polymerase (RdRp), and coronavirus main protease (3CLpro) (4,5).

Coronavirus disease-2019 (COVID-19) is a global health concern and has been declared as a global pandemic by the World Health Organization (WHO) (6). The increasing symptoms of COVID-19 cases reported from mild to severe, which can ultimately cause death in the co-morbid condition. The COVID-19 symptoms are shortness of breath, cough, fever and pneumonia which generally appears between 2-14 days after exposure to the virus. It was reported that severe symptoms of COVID-19 lead respiratory, neurological, hepatic and gastrointestinal problems that could cause death. The studies reported COVID-19 direct transmission from human-to-human by respiratory droplets or contact with infected people or indirect transmission by infected materials and surface (1,7–9). As of December 29, 2020, COVID-19 infected 79,931,215 people with 1,765,265 deaths globally, and 10,224,303 peoples only in India with 148,153 deaths [WHO, 29 December 2020 update].

Till date, there are three vaccines for COVID-19 which has granted emergency use by the certain national regulatory authorities. But, none of them yet received WHO EUL/PQ authorization. It is expected that, the Pfizer vaccine got assessment very soon and some other candidate vaccines soon afterward (10). Presently, the COVID-19 management focuses on supportive care. The treatment of infected person depends upon self-care and use of antiviral medicine (11). Thus, there is an urgent need to develop more novel vaccine to prevent COVID-19 epidemics. Huge efforts have been made by researchers around the world to develop a potential vaccine for COVID-19. Vaccines are a potential key to deal with the COVID-19 pandemic. To date, more than 200 candidate vaccines are under development using a variety of techniques with the help of bioinformatics and specifically immunoinformatics. Nowadays, artificial intelligence based models, trained on specific molecules have facilitated advance and efficient therapies for human diseases (12,13). So, machine-learning and deep-learning based approaches have also been applied for the design of COVID-19 vaccines (14–16). By December 29, 2020, total 61 candidate vaccines were in the clinical trials (phase I, II or III). In this review, we have emphasized various antigens and vaccine types used to develop for COVID-19, challenges and current progress.

2. VACCINE DEVELOPMENT FOR COVID-19

Vaccines are highly effective and cheap method to reduce the infectious disease burden and morbidity (17,18). The rapid development of a potential vaccine is urgently required for the efficient treatment of COVID-19. Various vaccines are in the process of development by applying different approaches such as whole inactivated virus, subunit vaccines, vectored, and live-attenuated vaccine etc. (19). Immunoinformatics tools and computational approaches play a crucial role in supporting and improving vaccine development (20). Most of the vaccines which are currently entered in to the clinical phase are based on protein subunit, nucleotide, replicated virus, inactivated virus etc (19). Various studies have been performed using immunogenic epitopes for vaccine development (18,21–23). Computational approaches can be used to predict Histocompatibility complex (MHC)-I and MHC-II epitopes with antigenic properties

(24,25). In another approach of immmunoinformatics, conserved T-cell and B-cell epitopes for COVID-19 protein were identified (26).

S protein, M protein, N protein and E protein are the main structural proteins which help into the entry of virus in the host cell. These proteins can enhance the T-cell responses of $CD4^+/CD8^+$ by performing as antigens to neutralize the antibodies [10]. Currently, researchers have implemented a variety of approaches to develop an efficient vaccine for COVID-19 because of its strain variability (27). These approaches are whole virus vaccine, subunit vaccine, antibody vaccine, nucleotide vaccine, and replicating vector [11]. So far, various pharmaceutical companies and Universities worldwide are trying to develop an effective vaccine candidate. In this review, we have summarized about the current outlook on the development of vaccine and also about approaches and challenges in vaccine development for COVID-19.

3. SELECTION OF ANTIGEN FOR COVID-19

Antigen-presenting cells are a necessary element to a vaccine for the response of the immune system (28). Several structural proteins of COVID-19 are considered as antigen for the development of effective candidate vaccine. These proteins are whole cell antigens (WCA), S protein, N protein, and M protein (Figure 1). A number of excellent review articles are available that described these proteins in details (19,29,30). Antigens (WCA) include all necessary elements such as lipids, proteins, polysaccharide, and nucleic acids etc. that are essential for the virus. The live-attenuated and whole-cell killed vaccines development has been carried out by using WCA (31). S protein has been anticipated as the potential antigen for the development of COVID-19 vaccine because it plays a significant role in mediating virus entry (32). The complete S protein and its various fragments such as S1 subunit, RBD (receptor binding domain) FP (Fusion Peptide), and NTD (N-terminal domain) have been frequently used for vaccine design (30). But, some studies have been reported that, vaccines developed based on S protein did not give complete protection and also have some safety concern (33,34). N protein is a multi-functional and reported as a highly conserved sequence protein among all form of coronavirus (35). This conservation suggested that N protein is a potential candidate for COVID-19 vaccine design. N protein is more conserved in comparison to S protein with high antigenic properties (36). M protein is a trans-membrane glycoprotein normally present on the surface of coronavirus and participate in the assembly of virus (37). Structural and immunogenic study suggested that trans-membrane domain of this protein has cluster of T-cell epitopes that are capable to induce the cellular response (38). M protein can be considered as a potential antigen as it is also conserved among various species. E protein present in coronavirus with channel activity and having less immunogenicity. In comparison to other discussed proteins, E protein is not found to be appropriate for the design of COVID-19 vaccine (39).

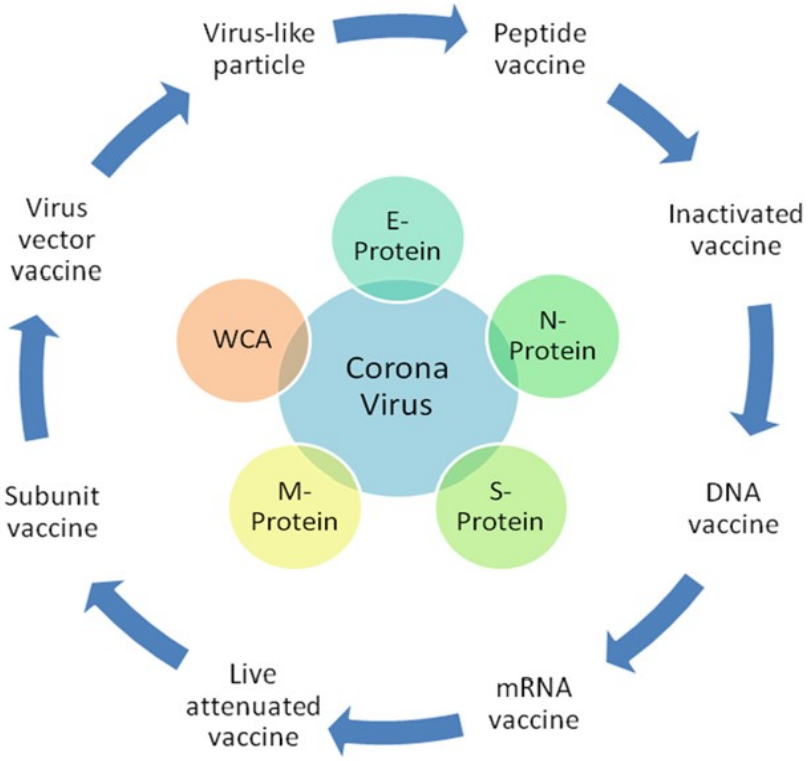

Figure 1. Different antigenic proteins and vaccine types for COVID-19.

4. TYPE OF COVID-19 VACCINES IN THE PIPELINE

As of 29 Dec 2020, total 233 candidate's vaccines were in pipeline developed by various Universities and industries. To develop these vaccines various approaches such as live attenuated, subunit, live vector, mRNA-based, DNA-based, protein-based, epitope-based and inactivated vaccines are used (Figure 2). We have briefly described below about these vaccine types. Various articles are available for detail description of each type of the vaccine (19,30,40).

Protein subunit vaccines have become a popular choice for design of COVID-19 vaccines due to their strong safety, which is especially beneficial for patients with the weak immune system (41). Subunit vaccines have powerful immunogenicity with one or more antigens that have ability to stimulate the immune system of host proficiently. Generally, the production of this type of vaccine is easy and safe. Several vaccine developers are working on subunit vaccine for COVID-19, most of them are using S protein as antigens (42). mRNA vaccine has regained attention due to the growing and mounting of mRNA technology for the modification, synthesis, and delivery. mRNA vaccines are arising as a potential substitute to conventional approach of vaccine design because of its improved immunogenicity to mimic the process of infection (43). DNA vaccine generally contained molecules of DNA plasmid which could encode one or more antigens. The DNA vaccines present more stability in comparison to mRNA vaccines (44). Live attenuated vaccines are live avirulent viruses that communicate with heterologous antigens. It is an established technology and probably come forward as a favorite candidate vaccine for COVID-19 epidemic (45). Although, Live attenuated vaccines have a risk in the virus transfer as they can cause infection in the same tissues as like to wild type pathogen (46).

Vaccines based on peptide or epitopes include only certain fragments of the whole antigen and are generally synthesized chemically. The approach of epitope-based vaccine targets diverse B-cell and T-cell epitopes of antigenic proteins (47). Peptide-based vaccines are the simplest form of vaccines that are easy to design, easy to verify, and quickly produced. These vaccines can be prepared by mixing the peptide and adjuvant, or peptide can be distributed with the help of suitable nanocarrier (48). Inactivated vaccines are formed by heat, chemical treatment or radiation inactivation using bacteria or viruses. These procedures terminate the replication capability of the pathogen, thereby making them more stable and safer (49). Vaccines based on virus vectors are powerful vaccination tools. Their ability to infect cells makes them highly proficient, precise and capable to trigger a powerful immune response. Although their advantages, viral vector have some disadvantages. For example, the use of retroviruses and lentiviruses may lead to the possibility of tumours development in the patients (50). Some viral vectors like adeno-associated viruses may not be cheapest due to their low titer. Ultimately, viral vectors are an effective choice for the development of COVID-19 vaccine (51,52). One of the other approaches of vaccination is Virus-like particles (VLPs) based vaccine. It is a multi-protein structure that can mimic the real natural virus, but do not have the genome of the virus (53,54). VLP is a harmless and useful tool which is widely used to study the viral infection/replication mechanisms and efficiency evaluation of vaccines (55).

5. POTENTIAL VACCINES FOR COVID-19 UNDERDEVELOPMENT

COVID-19 pandemic has given exceptional financial, health, and communal challenge worldwide. The solution to deal with this epidemic is the design of efficient candidate vaccines. A number of COVID-19 candidate vaccines are presently under progress at global level. The efficacy and safety results have been publicaly reported in large studies of five candidate vaccines including Pfizer-BioNTech and AstraZeneca vaccines (10). Successfully developed vaccine would be proficient to keep safe the people from COVIID-19. Researchers from worldwide are using various platforms and approaches to protect the population due to effect of COVID-19. Each type of vaccine development approach has its own benefit and challenges. In this global COVID-19 pandemic, we need more than one effective vaccine to vaccinate global population. Currently, there are 233 candidate vaccines for COVID-19 is in the pipeline of development. Out of these, 172 vaccines are in preclinical stage and 61 candidate vaccines are in different phase of clinical trials (Figure 2). Once vaccines are demonstrated to be safe and afficacious, they must be approved by national regulators, manufactured to rigorous standards, and distributed. Vaccines that reached in clinical trails are tabulated in Table 1. The summary of preclinical study and role of animal models in vaccine testing are discussed in the next section.

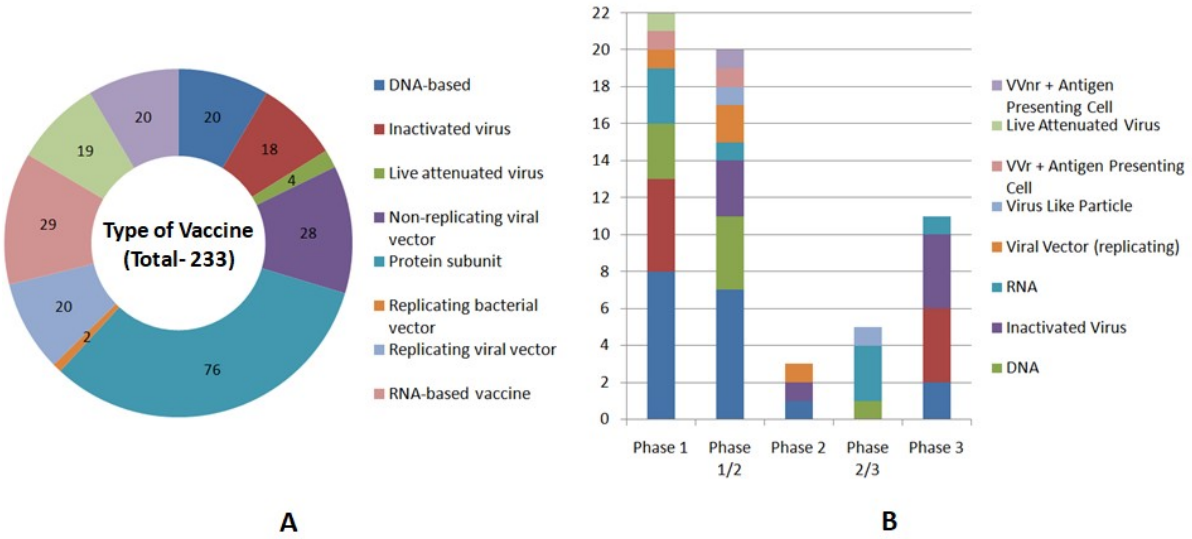

Figure 2: Outlook of under development of COVID-19 vaccines (As of 29 Dec 2020): **(A)** Total 233 undergoing various types of candidate vaccines development by academics and industries from worldwide. **(B)** Total 61 candidate vaccine entered in clinical trials (Table 1). The data of candidate vaccine was retrieved from World Health Organization (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines), and vaccine tracker resource of Milken Institute (https://covid-19tracker.milkeninstitute.org/).

Table 1. Current COVID-19 candidate vaccines in different stage of clinical trials.

Developer	Product category	Clinical stage	Clinical trials registry
Sinovac Research and Development Co., Ltd	Inactivated virus	Phase 3	NCT04383574, NCT04456595
Sinopharm + Wuhan Institute of Biological Products	Inactivated virus	Phase 3	ChiCTR2000031809, ChiCTR2000034780
Sinopharm + Beijing Institute of Biological Products	Inactivated virus	Phase 3	ChiCTR2000032459, ChiCTR2000034780
AstraZeneca + University of Oxford	Viral vector (Non-replicating)	Phase 3	PACTR202005681895696, PACTR202006922165132, NCT04400838, ISRCTN89951424
CanSino Biological Inc./Beijing Institute of Biotechnology	Viral vector (Non-replicating)	Phase 3	ChiCTR2000030906, ChiCTR2000031781, NCT04526990
Gamaleya Research Institute ; Health Ministry of the Russian Federation	Viral vector (Non-replicating)	Phase 3	NCT04436471, NCT04530396
Janssen Pharmaceutical	Viral vector (Non-replicating)	Phase 3	NCT04509947, NCT04436276, EUCTR2020-002584-63-DE, NCT04505722
Novavax	Protein subunit	Phase 3	NCT04368988, NCT04533399, NCT04611802
Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	RNA based vaccine	Phase 3	NCT04283461, NCT04405076, NCT04649151, NCT04470427
BioNTech + Fosun Pharma ; Jiangsu Provincial Center for Disease Prevention and Control + Pfizer	RNA based vaccine	Phase 2/3	NCT04523571, 2020-001038-36, NCT04588480, NCT04368728

Anhui Zhifei Longcom Biopharmaceutical + Institute of Microbiology, Chinese Academy of Sciences	Protein subunit	Phase 3	NCT04445194, NCT04550351, NCT04466085, ChiCTR2000040153
CureVac AG	RNA based vaccine	Phase 2/3	NCT04449276, PER-054-20, NCT04515147, NCT04652102, EUCTR2020-004066-19
Institute of Medical Biology + Chinese Academy of Medical Sciences	Inactivated virus	Phase 1/2	NCT04470609, NCT04659239
Research Institute for Biological Safety Problems, Rep of Kazakhstan	Inactivated virus	Phase 1/2	NCT04530357
Inovio Pharmaceuticals + International Vaccine Institute	DNA based vaccine	Phase 2/3	NCT04336410, NCT04447781, ChiCTR2000040146, NCT04642638
AnGes + Takara Bio + Osaka University	DNA based vaccine	Phase 1/2	NCT04463472, NCT04655625
Cadila Healthcare Ltd.	DNA based vaccine	Phase 1/2	CTRI/2020/07/026352
Genexine Consortium	DNA based vaccine	Phase 1/2	NCT04445389
Bharat Biotech International Limited	Inactivated virus	Phase 3	NCT04471519, NCT04641481; CTRI/2020/11/028976
Kentucky Bioprocessing Inc.	Protein subunit	Phase 1/2	NCT04473690
Sanofi Pasteur + GSK	Protein subunit	Phase 1/2	NCT04537208
Arcturus Therapeutics	RNA based vaccine	Phase 1/2	NCT04480957, NCT04668339
Serum Institute of India + Accelagen Pty	Virus like particle	Phase 1/2	ACTRN12620000817943
Shenzhen Kangtai Biological Products Co., Ltd.	Inactivated virus	Phase 2	ChiCTR2000038804, ChiCTR2000039462
ReiThera + Leukocare + Univercells	Viral vector (Non-replicating)	Phase 1	NCT04528641
Vaxart	Viral vector (Non-replicating)	Phase 1	NCT04563702
University of Munich (Ludwig-Maximilians)	Viral vector (Non-replicating)	Phase 1	NCT04569383
Clover Biopharmaceuticals Inc./GSK/Dynavax	Protein subunit	Phase 1	NCT04405908
Vaxine Pty Ltd. + Medytox	Protein subunit	Phase 1	NCT04453852
CSL Ltd. + Seqirus + University of Queensland	Protein subunit	Phase 1	ACTRN12620000674932p
Medigen Vaccine Biologics + Dynavax + National Institute of Allergy and Infectious Diseases (NIAID)	Protein subunit	Phase 1	NCT04487210
Instituto Finlay de Vacunas	Protein subunit	Phase 1/2	RPCEC00000338, RPCEC00000332
Federal Budgetary Research Institution State Research Center of Virology and Biotechnology "Vector"	Protein subunit	Phase 1/2	NCT04527575
West China Hospital + Sichuan University	Protein subunit	Phase 2	ChiCTR2000037518, ChiCTR2000039994
University Hospital Tuebingen	Protein subunit	Phase 1	NCT04546841
COVAXX + United Biomedical Inc	Protein subunit	Phase 1	NCT04545749

Merck & Co. + Themis + Sharp & Dohme + Institute Pasteur + Univeristy of Pittsburgh	Viral vector (Replicating)	Phase 1/2	NCT04497298, CT04498247
Jiangsu Provincial Center for Disease Prevention and Control	Viral vector (Replicating)	Phase 2	ChiCTR2000037782, ChiCTR2000039715
Imperial College London	RNA based vaccine	Phase 1	ISRCTN17072692
Shulan (Hangzhou) Hospital + Center for Disease Control and Prevention of Guangxi Zhuang Autonomous Region	RNA based vaccine	Phase 1	ChiCTR2000034112
Medicago Inc.	Virus like particle	Phase 2/3	NCT04450004, NCT04662697, NCT04636697
Shenzhen Geno-Immune Medical Institute	Viral vector (Replicating) + APC	Phase 1	NCT04299724
Shenzhen Geno-Immune Medical Institute	Viral vector (Non-replicating) + APC	Phase 1/2	NCT04276896
Barbara Carlson, University of Oklahoma	Protein subunit	Phase 1	NCT04523246
Adimmune Corporation	Protein subunit	Phase 1	NCT04522089
Entos Pharmaceuticals Inc.	DNA based vaccine	Phase 1	NCT04591184
Providence Health & Services	DNA based vaccine	Phase 1	NCT04627675
Chulalongkorn University	RNA based vaccine	Phase 1	NCT04566276
Symvivo Corporation	DNA based vaccine	Phase 1	NCT04334980
ImmunityBio, Inc.	Viral vector (Non-replicating)	Phase 1	NCT04591717
City of Hope Medical Center + National Cancer Institute	Viral vector (Non-replicating)	Phase 1	NCT04639466
Israel Institute for Biological Research	Viral vector (Replicating)	Phase 1	NCT04608305
Aivita Biomedical, Inc.	Viral vector (Replicating) + APC	Phase 1/2	NCT04386252
Codagenix/Serum Institute of India	Live attenuated virus	Phase 1	NCT04619628
Center for Genetic Engineering and Biotechnology (CIGB)	Protein subunit	Phase 1/2	RPCEC00000345
Center for Genetic Engineering and Biotechnology (CIGB)	Protein subunit	Phase 1/2	RPCEC00000346
Valneva, National Institute for Health Research, United Kingdom*	Inactivated Virus	Phase 1/2	NCT04671017
Biological ELimited *	Protein subunit	Phase 1/2	CTRI/2020/11/029032
CureVac AG*	RNA based vaccine	Phase 2/3	NCT04652102
Cellid Co., Ltd.*	Viral vector (Replicating)	Phase 1/2	NCT04666012
GeneOne Life Science, Inc.*	DNA based vaccine	Phase 1/2	NCT04673149

6. PRECLINICAL ASSESSMENT IN ANIMAL MODELS: **Role of P**harmacogenomics and Pharmacoproteomics

Preclinical study:

The drug development process is usually divided into three main steps: discovery, preclinical development and clinical trials (56). The overall process from basic research to approved drug is represented in Figure 3.

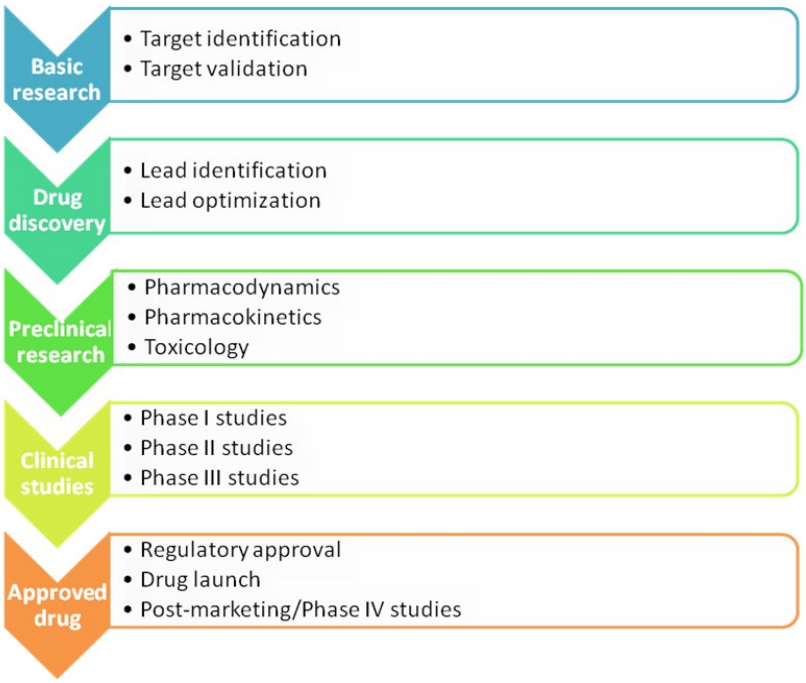

Figure 3: Drug development stages.

Preclinical development consists of activities that connect drug discovery in the laboratory with the beginning of human clinical trials. The process of new drug development is time- consuming and expensive as well. In order to increase the probability of successfully completing clinical trails leading to the approval of new drugs, preclinical models are essential. Identifying a safe, effective and successful drug requires thorough preclinical testing, which requires evaluation of pharmacodynamics, pharmacokinetics, and toxicology in *in vitro* and *in vivo* environments (57).

In this aspect, pharmacogenomics and pharmacoproteomics also plays key role in the vaccine design. Pharmacogenomics gives a promising science foundation for vaccine research and development. The aim of pharmacogenomics is to recognize genetic variants that can predict adverse reactions to vaccines, predict abnormal immune responses, promote personalized treatment, and predict sensitivity to diseases and vaccines. Currently, further research is required to completely understand the present strains of coronavirus, their prevention and treatment. Therefore, in the promising field of pharmacogenomics, pharmaceutical science is combined with the analysis of genes and their functions and biomedical research disclose that it may be necessary to change the dose of drug based on individual's genes and/or gene mutations in patients (58). Pharmacoproteomics is the application of proteomic technology in vaccine design and development. In addition to pharmacogenomics, pharmacoproteomics also plays an important role in COVID-19 vaccine design. Proteomic techniques (such as expression

proteomics and functional proteomics) have been useful in the analysis of pharmacology and toxicology. Pharmacoproteomics is a fast developing field, with the advancement of analytical technology. It can handle the complex interactions of a large number of unique proteins and be effectively utilized in clinical trials. Proteomics has very few applications in early clinical development and is limited to a few therapeutic areas. With the development of drugs from discovery, to humans, to patients, to clinical practice and to the community, pharmacoproteomics should play a role in all stages of translational research. (59).

A typical preclinical development plan consists of six major efforts (56):

1. Manufacture of drug substance (DS)/active pharmaceutical ingredient (API);
2. Preformulation and formulation (dosage design);
3. Analytical and bioanalytical methods development and validation;
4. Metabolism and pharmacokinetics;
5. Toxicology, both safety and genetic toxicology and possibly safety pharmacology; and
6. Good manufacturing practice (GMP) manufacture and documentation of drug product for use in clinical trials.

For human, direct clinical trials for extremely pathogenic viruses are not practicable, and without earlier preclinical research, it is not ethically allowed to do so. Therefore, animal research plays a vital role in characterizing the pathogenesis of viruses and evaluating antiviral agents and vaccines for these viruses. The perfect animal models should allow infection and must reproduce the clinical process and pathology observed in humans (60).

Animal model:

The vaccine development for the human is depending on the use of animal's research. Before permitted to enter in clinical phase for human trails, regulatory agencies require that new vaccine candidates undergo preclinical evaluation in animal models. A number of animal models such as mouse, pigs, guinea, hamsters, rabbits, ferrets, rhesus macaques, cats, and marmosets have been evaluated for SARS and MERS corona viruses (42,61). Early attempts were aimed at developing animal models for SARS-CoV, but the specificity of the virus to ACE2 (receptor of SARS-CoV) was the major obstacle to these efforts. Later, by introducing the hACE2 gene into the mouse genome, a SARS-CoV transgenic mouse model was estabilised (62). The first animal model used to develop the MERS-CoV vaccine was rhesus macaques. One more common animal model of MERS-CoV is the common marmoset, in which the virus causes fetal pneumonia (63).

Researchers can use many small and large animal models to study the important characteristics of COVID-19, including pathology, transmission and host responses to SARS-CoV-2, as well as help to establish potential safety and effectiveness for the vaccines. Animal models are desired to evaluate vaccine-related enhanced respiratory disease, and it will be very important to establish a positive control for this disease (64). In comparison to the large animal models, small animals (such as mice and rabbits) are chosen due to their lower cost, ease in manipulation, and easely available efficiency methods (65). Continue to improve and develop animal models of COVID-19 will help the development of vaccines. Major clinical trials are presently in progress to test a

variety of candidate ivaccines in humans (64). The criteria for selecting a good animal models are described below (66).

1. Needs to be closely similar to the disease in humans
2. Access to various immune compartments
3. The response to the same vaccine is similar to that of humans
4. Needs to have multiple readings, including protection, specific immune compartments, etc.

7. CHALLENGES OF COVID-19 VACCINE

A huge amount of money and energy has been devoted in the vaccine development; though, there are lots of challenges that need to be conquer in order to obtain a successful and profitable vaccine. The successful vaccine for COVID-19 will be administered globally; therefore, its safety must be the priority. If the vaccine is permitted for selling, it will not necessarily lead to easy availability. Thus, the selection of platform for the vaccines is a very important concern. A perfect platform would have better supplies everywhere at low cost and minimum supervision. Most of the vaccine formulations need regular refrigeration (19). If the Vaccine is not stored in a temperature range of 2–8 °C, it may seriously affect the efficacy of the vaccine. This will inevitably bring a huge financial and logistic burden to the vaccination program, especially in developing countries. So requirement of continuous refrigeration will make difficulty in global distribution and it is very difficult with tropical climates. According to WHO report, 2.8 million vaccines for various diseases were lost due to crash of cold chain facility. Only near about 10% countries fulfill the WHO criteria for the management of effective vaccine practices (67).

Thus, the demand for the cold chain is one of the main reason for global inadequate vaccination, becouse it brings major economic and logistical problems to the vaccination program. This problem is perticulary serious in developing countries and remote areas, because they often lack reliable cold chain infrastructure (68,69). Due to these obstacles, it is nessesory to make attempt in the direction of thermally stabilize COVID-19 vaccines. The thermostable vaccines can alleviate bottlenecks in vaccine supply chain, thereby increasing the utilization of vaccines. In the past, researchers have recognized various vaccines that can remain active outside the cold chain and researchers have made great efforts to make thermally stable vaccines. Sun *et al*. is proved that a vaccine with strong thermastability can be produced by linking the epitope of *Mycobacterium tuberculosis* to the self-assembling fibril-forming peptide. Using this method, they proficiently produced a vaccine that can be stored at 45 °C for 7 days without any conformational change (70). Similarly, Beernink's research team designed a mutant antigen of a recombinant meningococcal vaccine that increased its heat tolerance by 21 °C (71,72) as did Campeotto *et al*. introducing 18 mutations to modify it, the heat resistance of malaria-protein vaccine can be increased by 10–15 °C (73). Other researchers have tried to create thermally stable vaccines by modifying viral vectors. In one such study, Stobart *et al*. designed a respiratory syncytial virus (RSV) which is characterized by enhanced expression of pre-F, and has higher immunogenicity and thermal stability than the wild type (74). Vincent Leung *et al.,* tried to develop an enveloped DNA Herpes Simplex Virus type 2 (HSV-2) and an RNA, Influenza A virus (IAV) viral vaccines in a mixture of pullulan and trehalose. Trehalose is a disaccharide that is commonly used as a

cryoprotectant and stabilizing agent, while pullulan is a polysaccharide with good film-forming abilities which is used in the food industry to extend the shelf life of food. Both compounds are FDA approved. The results for this formulation showed that the live-attenuated HSV-2 vaccine can be storage at 40 °C for at least 2 months, while the inactivated influenza vaccine can be storage at 40 °C for at least 3 monthswithout affecting immunogenicity (75). Another common method for thermally stable vaccines is by adding stabilized adjuvants. For example, Pelliccia *et al.* by adding polyethylene glycol (PEG), gold nanoparticles (AuNP) and sucrose (72), they have developed a heat-stable adenovirus vaccine formulation that can maintain immunogenicity for up to 10 days at 37°C (76). Prausntz's group used different stable adjuvant formulations to encapsulate it in microneedle patches which were able to maintain the immunogenicity of the inactivated influenza vaccine during a storage period of 4 months at 60 °C (77,78). Hassett *et al*. performed analysis that showed lyophilized anthrax vaccine maintained its immunogenicity after 16 weeks of storage at 40 °C (79), and lyophilized recombinant ricin toxin A vaccine retained its stability after being stored for 4 weeks at 40 °C (80). Chen *et al*. thermally stabilized formulations of spray-dried recombinant hepatitis B vaccine and meningitis A protein-polysacharide conjugate vaccine. The spray-dried vaccine formulation is stable for 24 months at 37 °C (81). These methods for heat-stable virus vaccines have the potential to solve the cold chain problem.

Therefore, by implementing existing tharmostable formulations, researchers and developers can develop COVID-19 vaccine that do not need strong cold chain facility and help to improve global health by providing vaccines around the world. Government, researchers, manufacturers, and fund providers all hope to get the vaccine by early 2021. Therefore, harmonized efforts between vaccine developers, fund providers, and manufacturers are necessary to make sure that a sufficient number of safe vaccine candidates are successfully produced and evenly distributed globally.

8. CONCLUSION

There was no successful vaccine available for COVIID-19 while we were writing this review. Very recently Pfizer introduced the COVID-19 vaccine with ~93% efficacy and very little side effects. Although, side effect have been seen in many different vaccines made in different ways. This does not appear to be a problem related to a specific type of COVID-19 vaccine. The oxford University-AstraZeneca vaccine, showed advers effect on 60% of recipients in the early phase clinical trial. The Pfizer-BioNTech vaccine also reported side effect. The side effects repoeted by Pfizer-BioNTech vaccine are fatigue, headache, and muscle pain. The most common reported side effect from COVID-19 vaccine is the pain in the area where vaccine is injected. A large number of vaccines is presently being developed worldwide. Currently, vaccines using DNA viral, mRNA, protein subunit, viral vector have been tested in phase I, II, and in phase III clinical trials. Usually, vaccine development requires several years to develop. Scientists are working hard to develop such effective vaccines that give long term protection of the individuals. Various approaches that are used for vaccine developmet require extensive testing for vaccine efficacy and safety. The results achieved from recent clinical trials on the development of candidate vaccine are promising and it is anticipated that in near future safe and sound vaccines will be made available for the global population to manage this pandemic.

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REFERENCES

1. Banerjee A, Kulcsar K, Misra V, Frieman M, Mossman K. Bats and Coronaviruses. Viruses. 2019;11(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6356540/

2. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annual Review of Virology. 2016;3(1):237–61.

3. Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pac J Allergy Immunol. 2020;38(1):10–8.

4. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020 16;181(2):271-280.e8.

5. Du L, He Y, Zhou Y, Liu S, Zheng B-J, Jiang S. The spike protein of SARS-CoV — a target for vaccine and therapeutic development. Nature Reviews Microbiology. 2009;7(3):226–36.

6. Whitworth J. COVID-19: a fast evolving pandemic. Trans R Soc Trop Med Hyg. 2020;114(4):241–8.

7. Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCoV. Viruses [Internet]. 2020 Feb [cited 2020;12(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7077245/

8. Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. International Journal of Antimicrobial Agents. 2020;55(3):105924.

9. Coleman CM, Frieman MB. Coronaviruses: Important Emerging Human Pathogens. J Virol. 2014;88(10):5209–12.

10. Coronavirus disease (COVID-19): Vaccines. Available from: https://www.who.int/news-room/q-a-detail/coronavirus-disease-(covid-19)-vaccines

11. Shamim S, Khan M, Kharaba ZJ, Ijaz M, Murtaza G. Potential strategies for combating COVID-19. Arch Virol. 2020; Available from: https://doi.org/10.1007/s00705-020-04768-3

12. Shukla R, Yadav AK, Singh TR. Application of Deep Learning in Biological Big Data Analysis [Internet]. Large-Scale Data Streaming, Processing, and Blockchain Security. IGI Global; 2021. p. 117–48. Available from: www.igi-global.com/chapter/application-of-deep-learning-in-biological-big-data-analysis/259468

13. Chandra Kaushik A, Raj U. AI-driven drug discovery: A boon against COVID-19? AI Open. 2020;1:1–4.

14. Gupta E, Mishra RK, Niraj RRK. Identification of potential vaccine candidates against SARS-CoV-2, A step forward to fight novel coronavirus 2019-nCoV: A Reverse Vaccinology Approach. bioRxiv. 2020 ;2020.04.13.039198.

15. Crossman LC. Leveraging Deep Learning to Simulate Coronavirus Spike proteins has the potential to predict future Zoonotic sequences. bioRxiv. 2020;2020.04.20.046920.

16. Keshavarzi Arshadi A, Webb J, Salem M, Cruz E, Calad-Thomson S, Ghadirian N, et al. Artificial Intelligence for COVID-19 Drug Discovery and Vaccine Development. Front Artif Intell. 2020;3. Available from: https://www.frontiersin.org/articles/10.3389/frai.2020.00065/full

17. Rémy V, Zöllner Y, Heckmann U. Vaccination: the cornerstone of an efficient healthcare system. J Mark Access Health Policy. 2015;3. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4802703/

18. Sagar M, Yadav AK. Computer-aided vaccine design for liver cancer using epitopes of HBx protein isolates from HBV substrains. Int J Bioinform Res Appl. 2011;7(3):299–316.

19. Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, et al. COVID-19 vaccine development and a potential nanomaterial path forward. Nature Nanotechnology. 2020;15(8):646–55.

20. Lundstrom K. Coronavirus Pandemic—Therapy and Vaccines. Biomedicines. 2020;8(5). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277397/

21. Naz A, Shahid F, Butt TT, Awan FM, Ali A, Malik A. Designing Multi-Epitope Vaccines to Combat Emerging Coronavirus Disease 2019 (COVID-19) by Employing Immuno-Informatics Approach. Front Immunol. 2020;11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.01663/full

22. De Groot AS, Moise L, Terry F, Gutierrez AH, Hindocha P, Richard G, et al. Better Epitope Discovery, Precision Immune Engineering, and Accelerated Vaccine Design Using Immunoinformatics Tools. Front Immunol. 2020;11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7154102/

23. Chandra S, Singh TR. Linear B cell epitope prediction for epitope vaccine design against meningococcal disease and their computational validations through physicochemical properties. Netw Model Anal Health Inform Bioinforma. 2012;1(4):153–9.

24. Gupta A, Chaukiker D, Singh TR. Comparative analysis of epitope predictions: proposed library of putative vaccine candidates for HIV. Bioinformation. 2011;5(9):386–9.

25. Chandra S, Singh D, Singh TR. Prediction and characterization of T-cell epitopes for epitope vaccine design from outer membrane protein of Neisseria meningitidis serogroup B. Bioinformation. 2010;5(4):155–61.

26. Baruah V, Bose S. Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. Journal of Medical Virology. 2020;92(5):495–500.

27. Calina D, Docea AO, Petrakis D, Egorov AM, Ishmukhametov AA, Gabibov AG, et al. Towards effective COVID-19 vaccines: Updates, perspectives and challenges (Review). Int J Mol Med. 2020;46(1):3–16.

28. van Riel D, de Wit E. Next-generation vaccine platforms for COVID-19. Nature Materials. 2020;19(8):810–2.

29. Siu YL, Teoh KT, Lo J, Chan CM, Kien F, Escriou N, et al. The M, E, and N Structural Proteins of the Severe Acute Respiratory Syndrome Coronavirus Are Required for Efficient Assembly, Trafficking, and Release of Virus-Like Particles. Journal of Virology. 2008;82(22):11318–30.

30. Zhang J, Zeng H, Gu J, Li H, Zheng L, Zou Q. Progress and Prospects on Vaccine Development against SARS-CoV-2. Vaccines (Basel). 2020;8(2).

31. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virology Journal. 2019;16(1):69.

32. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020 13;367(6483):1260–3.

33. Roper RL, Rehm KE. SARS vaccines: where are we? Expert Rev Vaccines. 2009;8(7):887–98.

34. 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.

35. McBride R, van Zyl M, Fielding BC. The Coronavirus Nucleocapsid Is a Multifunctional Protein. Viruses. 2014;6(8):2991–3018.

36. Leung DTM, Chi Hang TF, Chun Hung M, Sheung Chan PK, Cheung JLK, Niu H, et al. Antibody Response of Patients with Severe Acute Respiratory Syndrome (SARS) Targets the Viral Nucleocapsid. J Infect Dis. 2004;190(2):379–86.

37. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 201;174(1):11–22.

38. Liu J, Sun Y, Qi J, Chu F, Wu H, Gao F, et al. The membrane protein of severe acute respiratory syndrome coronavirus acts as a dominant immunogen revealed by a clustering region of novel functionally and structurally defined cytotoxic T-lymphocyte epitopes. J Infect Dis. 2010;202(8):1171–80.

39. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Fernandez-Delgado R, et al. Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. PLOS Pathogens. 2014;10(5):e1004077.

40. Amanat F, Krammer F. SARS-CoV-2 Vaccines: Status Report. Immunity. 2020;52(4):583–9.

41. Vartak A, Sucheck SJ. Recent Advances in Subunit Vaccine Carriers. Vaccines. 2016;4(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4931629/

42. Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, et al. COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. Hum Vaccin Immunother. 2020;1–7.

43. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261–79.

44. Liu MA. A Comparison of Plasmid DNA and mRNA as Vaccine Technologies. Vaccines. 2019;7(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6631684/

45. Gonzalez-Nicolini V, Sanchez-Bustamante CD, Hartenbach S, Fussenegger M. Adenoviral vector platform for transduction of constitutive and regulated tricistronic or triple-transcript transgene expression in mammalian cells and microtissues. J Gene Med. 2006;8(10):1208–22.

46. Bull JJ, Smithson MW, Nuismer SL. Transmissible Viral Vaccines. Trends Microbiol. 2018;26(1):6–15.

47. Azmi F, Ahmad Fuaad AAH, Skwarczynski M, Toth I. Recent progress in adjuvant discovery for peptide-based subunit vaccines. Hum Vaccin Immunother. 2014;10(3):778–96.

48. Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide Vaccine: Progress and Challenges. Vaccines (Basel). 2014;2(3):515–36.

49. Delrue I, Verzele D, Madder A, Nauwynck HJ. Inactivated virus vaccines from chemistry to prophylaxis: merits, risks and challenges. Expert Rev Vaccines. 2012;11(6):695–719.

50. Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet. 2004 ;364(9452):2181–7.

51. Ura T, Okuda K, Shimada M. Developments in Viral Vector-Based Vaccines. Vaccines (Basel). 2014 29;2(3):624–41.

52. Draft landscape of COVID-19 candidate vaccines. 2020. Available from: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

53. Noranate N, Takeda N, Chetanachan P, Sittisaman P, A-nuegoonpipat A, Anantapreecha S. Characterization of Chikungunya Virus-Like Particles. PLOS ONE. 2014;9(9):e108169.

54. Changotra H, Vij A. Rotavirus virus-like particles (RV-VLPs) vaccines: An update. Rev Med Virol. 2017;27(6).

55. Dong H, Guo H-C, Sun S-Q. Virus-like particles in picornavirus vaccine development. Appl Microbiol Biotechnol. 2014;98(10):4321–9.

56. Steinmetz KL, Spack EG. The basics of preclinical drug development for neurodegenerative disease indications. BMC Neurol. 2009;9(Suppl 1):S2.

57. Honek J. Preclinical research in drug development. MEW. 2017;26:5–8.

58. Poland GA, Ovsyannikova IG, Jacobson RM. Application of pharmacogenomics to vaccines. Pharmacogenomics. 2009;10(5):837–52.

59. Nandal S, Burt T. Integrating Pharmacoproteomics into Early-Phase Clinical Development: State-of-the-Art, Challenges, and Recommendations. Int J Mol Sci. 2017;18(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343982/

60. Sutton TC, Subbarao K. Development of animal models against emerging coronaviruses: From SARS to MERS coronavirus. Virology. 2015;479–480:247–58.

61. Gretebeck LM, Subbarao K. Animal models for SARS and MERS coronaviruses. Curr Opin Virol. 2015;13:123–9.

62. Yang X-H, Deng W, Tong Z, Liu Y-X, Zhang L-F, Zhu H, et al. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. Comp Med. 2007;57(5):450–9.

63. Falzarano D, Wit E de, Feldmann F, Rasmussen AL, Okumura A, Peng X, et al. Infection with MERS-CoV Causes Lethal Pneumonia in the Common Marmoset. PLOS Pathogens. 2014;10(8):e1004250.

64. Muñoz-Fontela C, Dowling WE, Funnell SGP, Gsell P-S, Riveros-Balta AX, Albrecht RA, et al. Animal models for COVID-19. Nature. 2020;586(7830):509–15.

65. Yong CY, Ong HK, Yeap SK, Ho KL, Tan WS. Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus. Front Microbiol. 2020;10. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01781/full

66. Gerdts V, Wilson HL, Meurens F, van Drunen Littel-van den Hurk S, Wilson D, Walker S, et al. Large animal models for vaccine development and testing. ILAR J. 2015;56(1):53–62.

67. Kristensen DD, Lorenson T, Bartholomew K, Villadiego S. Can thermostable vaccines help address cold-chain challenges? Results from stakeholder interviews in six low- and middle-income countries. Vaccine. 2016;34(7):899–904.

68. Luzze H, Badiane O, Mamadou Ndiaye EH, Ndiaye AS, Atuhaire B, Atuhebwe P, et al. Understanding the policy environment for immunization supply chains: Lessons learned from landscape analyses in Uganda and Senegal. Vaccine. 2017;35(17):2141–7.

69. Azimi T, Franzel L, Probst N. Seizing market shaping opportunities for vaccine cold chain equipment. Vaccine. 2017;35(17):2260–4.

70. Sun T, Han H, Hudalla GA, Wen Y, Pompano RR, Collier JH. Thermal stability of self-assembled peptide vaccine materials. Acta Biomater. 2016;30:62–71.

71. Konar M, Pajon R, Beernink PT. A meningococcal vaccine antigen engineered to increase thermal stability and stabilize protective epitopes. Proc Natl Acad Sci U S A. 2015;112(48):14823–8.

72. Rossi R, Konar M, Beernink PT. Meningococcal Factor H Binding Protein Vaccine Antigens with Increased Thermal Stability and Decreased Binding of Human Factor H. Infect Immun. 2016;84(6):1735–42.

73. Campeotto I, Goldenzweig A, Davey J, Barfod L, Marshall JM, Silk SE, et al. One-step design of a stable variant of the malaria invasion protein RH5 for use as a vaccine immunogen. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(5):998–1002.

74. Stobart CC, Rostad CA, Ke Z, Dillard RS, Hampton CM, Strauss JD, et al. A live RSV vaccine with engineered thermostability is immunogenic in cotton rats despite high attenuation. Nat Commun. 2016;7:13916.

75. Leung V, Mapletoft J, Zhang A, Lee A, Vahedi F, Chew M, et al. Thermal Stabilization of Viral Vaccines in Low-Cost Sugar Films. Scientific Reports. 2019;9(1):7631.

76. Pelliccia M, Andreozzi P, Paulose J, D'Alicarnasso M, Cagno V, Donalisio M, et al. Additives for vaccine storage to improve thermal stability of adenoviruses from hours to months. Nature Communications. 2016;7(1):13520.

77. Chu LY, Ye L, Dong K, Compans RW, Yang C, Prausnitz MR. Enhanced Stability of Inactivated Influenza Vaccine Encapsulated in Dissolving Microneedle Patches. Pharm Res. 2016;33(4):868–78.

78. Mistilis MJ, Joyce JC, Esser ES, Skountzou I, Compans RW, Bommarius AS, et al. Long-term stability of influenza vaccine in a dissolving microneedle patch. Drug Deliv Transl Res. 2017;7(2):195–205.

79. Hassett KJ, Vance DJ, Jain NK, Sahni N, Rabia LA, Cousins MC, et al. Glassy-state stabilization of a dominant negative inhibitor anthrax vaccine containing aluminum hydroxide and glycopyranoside lipid A adjuvants. J Pharm Sci. 2015;104(2):627–39.

80. Hassett KJ, Cousins MC, Rabia LA, Chadwick CM, O'Hara JM, Nandi P, et al. Stabilization of a recombinant ricin toxin A subunit vaccine through lyophilization. European Journal of Pharmaceutics and Biopharmaceutics. 2013;85(2):279–86.

81. Chen D, Kapre S, Goel A, Suresh K, Beri S, Hickling J, et al. Thermostable formulations of a hepatitis B vaccine and a meningitis A polysaccharide conjugate vaccine produced by a spray drying method. Vaccine. 2010 12;28(31):5093–9.