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Research and Development Steps for an Effective Vaccine, during the COVID-19 Pandemic Situation

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Abstract

Introduction: The COVID-19 vaccine, considered by many as a "game-changer" and therefore the most effective & prophylactic strategy tool for pandemic control and prevention, is being developed by many research, pharmaceutical and scientific institutions. Vaccine development has always been a really long and expensive process, but modern innovative technologies, along with incredible and specific economic and scientific efforts, immensely helped to narrow the development time and progress, in order to have in less than 12 months from official start of the pandemic, more than one approved vaccines and actually make possible the beginning of a massive vaccine worldwide distribution and administration campaign, in record time.

Methodology and objectives: Through the scientific literature review, this article aims to supply for interested healthcare professionals and public, a summarized and detailed picture of the vaccines under development or approved ones, implemented platforms, clinical study trials phases, institutions, developing countries and funders, to explain vaccine options and candidates, against SARS-CoV -2.

Results: There are currently 261 vaccines in development, 78% of which are in the preclinical phase. 56 candidates (22%) from different developers have been admitted to clinical trials,

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of which 44% are in Phase I trials; 30% are in Phase I/II; 7% in Phase II; 21% in Phase II/III and 21% in clinical trials of Phase III. Also, based on the platform used for development, actual data shows that there are around: RNA-based technology 13% of vaccines, DNA 8%, non-replicating viral vector 14%, viral replicating vector 8%, virus like particles 6%, inactivated virus 6%, live virus attenuated 1%, protein fraction 31%, unknown platform 13%. 45% of studies are conducted by the pharmaceutical industry, 28% by public and academic institutions. Among candidates, 11% of them received funding support from public and private grants. The U.S.A. includes around 26.8% of global research, followed by Asia 29% (China, leader with 19.5% globally and 43% of clinical trials). Europe also 29% of developing vaccines with 15 countries involved.

Conclusions: At the end of 2020, first vaccine candidates received important regulatory approvals and began their global use. This is a fantastic news but we are just in the middle of our campaign to fight back against the pandemic. Ongoing & future studies, together with global real-time data, will focus on the determination of SARS COV-2 antibodies and T lymphocyte-mediated immunity, optimizing pharmaceutical product development and vaccine efficacy protection against COVID-19.

Keywords: COVID-19, vaccine, research and development, pandemic

INTRODUCTION

The COVID-19 pandemic is caused by Severe Acute Respiratory Sindrome Coronavirus 2 (SARS-CoV-2), with reports of more than 75 million cases of COVID-19 (January 2021) (1). The daily changing statistics data show over 215 countries and territories affected, resulting in more than 1.9 million deaths and 64.71 million people recovered from the virus, with a global death-case ratio of 2.15 %.

The virus is spread mainly among people during close contact, most often through droplets produced by coughing, sneezing and talking. Virus particles usually fall to the ground or spread through the air. People can also become infected by touching a contaminated surface and then touching their face, introducing the virus particles to their organism (2). The virus is more contagious after infection, about three days before the onset of symptoms, or even through asymptomatic individuals.

Common symptoms include fever, dry cough, fatigue, shortness of breathing, and loss of taste or smell (ageusia/anosmia). Complications may include, among others, pneumonia and acute respiratory distress syndrome (3). The time from exposure to the onset of symptoms is usually about five days but can range from two to fourteen days. There is not yet a specific antiviral treatment; primary treatment is symptomatic and supportive therapy, so the approval of one/more effective vaccine(s) can be a "game-changing" strategy (2).

A. Vaccine development for COVID-19

A pandemic can involve multiple waves of COVID-19 over 1-3 years, and SARS-CoV-2 can become an endemic virus globally. We need to prepare for a worst-case scenario, in which the rapid development and high rates of COVID-19 vaccination are essential to the morbidity, mortality reduction and limitation of economic damages that accompanies a pandemic, from day one of onset till the slow return to the "old normality" (4).

Vaccines are the most effective strategy available to prevent and reduce the impact of a flu outbreak. But although vaccination is the most effective means of prevention, a specific vaccine may not be available during the initial wave(s) of a pandemic.

If SARS-CoV-2 resembles other coronaviruses that infect humans, those who become infected will be immune for months and years (still unknown..), but not their entire lives (5). When most of a population is immune to an infectious disease, this ensures indirect protection or "herd immunity" to those still "susceptible" to the disease.

"Herd immunity", also known as "population immunity" is a form of indirect protection against infectious disease (4). This occurs when a large percentage of a population has become immune to an infection, either through vaccination or previous infections, providing a resultant level of protection for the rest, individuals who are still not immune. The greater the number of immune individuals in a community, the less likely it is

that the rest of non-immune individuals will contact an infectious individual, helping to protect non-immune individuals from infection (4). According to estimations on the COVID-19 infectivity rates, we should need at least 70% of the population to be immune to achieve the "herd immunity" objective.

Supposing that the herd immunity epidemiological state is present and maintained in a population, for an adequate period of time, the disease is going toward elimination, since endemic transmissions no longer occurs. If "this local" elimination is achieved and spread worldwide, with a following number of cases permanently reduced to zero, then disease can be declared officially as "eradicated" (4,5).

The immune system does not distinguish between natural infections and vaccines induction effect, forming an active response to both, so the immunity resulted through vaccination is similar to what would have occurred in a case of recovering from the disease (6).

- ***Introduction to vaccine immunology***

Immunity to COVID-19 remains a quest with many unknown topics, an "enigma" that science and scientists must solve.

The ideal vaccine candidate should be a "safe product, able to produce a strong, consistent, protective immune response, seek as few doses as possible, and be affordable and accessible to all globally"(6).

There are two main types of "memory" immune responses. The first is driven by B lymphocyte cells, which produce antibodies. The second type of cell capable of recalling an infection is T lymphocyte cells. T cells may be sufficient to control infection in the absence of antibodies and act by organizing immune defenses (T-helpers) or by directly killing infected cells, to limit new virus production (cytotoxic T cells) (6,7). It is possible that the Sars-CoV-2 T cells' memory may last longer than antibodies, similar to other coronaviruses cases.

We currently do not have a test to assess the T cell response, but also even the presence of antibodies does not give us a full and clear overview of protection level in patients with COVID-19 (7).

B. How long does a vaccine take from formulation to global distribution?

Developing a new vaccine has always been a long, complicated, challenging process, that requires a great deal of professionalism to analyze, study and resolve possible problems, encountered from the start, up to the moment of approval, before widespread human use (7). Vaccine development requires many stages; under normal conditions the preclinical and clinical phase can last 5-15 years until completion. Following the development of vaccines, an ongoing commitment to post-licensing safety analysis is required. Taking into account post-licensing safety studies, the whole process can take approximately 10-30 years (8).

- ***Challenges of a clinical trial performed during an ongoing pandemic situation***

Recruitment of a sufficient number of patients: In emergency conditions (like a pandemic), it

becomes more challenging to recruit an adequate and required number of patients.

Policymakers face many decisions to make, including:

(a) the selection of the "right" candidates for a study process (limited number of patients and resources, make the candidate selection critical),

(b) choosing the right study model (some study models work best for different situations) (8).

- ***Vaccine approval process in pandemic conditions***

Many countries have implemented alternative authorization procedures that can help, to speed up the availability of vaccines (8). For pandemic vaccines, two main approaches are being used:

- *"Mock-up" procedure*

This procedure allows a vaccine to be developed and authorized before a pandemic occurs, based on the presumption that some viruses have the "potential" to cause a pandemic (9). Once the current type of virus causing the pandemic has been identified, the manufacturer can replace this mutant material in the pre-prepared vaccine (for which regulatory approval has been given previously) and request it to be authorized as a 'final' pandemic vaccine (8,9).

The vaccine will be given a *"Conditional Approval"*, which means that the benefits outweigh its risks, but full data to support its authorization are not yet available, and further studies should provide these after licensing.

- *Emergency Procedure*

Authorization of these pandemic vaccines is faster; the information (M.A. dossier) applied by the manufacturer is evaluated in an accelerated time frame (about 70 days rather than 210 days) (10). Vaccines should submit a complete dossier of information reporting data on developing vaccines as soon as they become available, rather than waiting for a complete set of data to be collected. Once sufficient data has been collected, focused on assessing the risk-benefit ratio, the manufacturer applies to the regulatory authorities (FDA/EMA or other local institutions) for a formal marketing authorization approval (MAA – Marketing Authorization Approval). Regulatory Authorities have to evaluate the submitted dossier and reach a final decision within approximately 25-40 days (10).

METHODS

Type of study: The study methodology is based on the conceptual and critical literature review, conducting detailed scientific analysis, to compare and evaluate a range of perspectives on COVID-19 vaccines development key moments. Grouping articles by concepts, identifying current "understanding" and a photographic overview of "where things are" in the area of research and development processes, for an effective vaccine in the COVID-19 pandemic.

Timeframe: This review was performed periodically from April 2020 - November 2020, focusing on the latest researches and publications.

Inclusion criteria: During publications research have been used the following keywords: "COVID-19; COVID-19 vaccine, COVID-19

vaccine research, COVID-19 vaccine development, COVID-19 vaccine pipeline, COVID-19 vaccine safety" in Pubmed, Google scholar, medical journals with high impact factor such as *New England Journal of Medicine*, *Lancet, Nature*, paper position of international associations of microbiology and infectious diseases (ECCMID), of immunology, clinical immunology (EAAACI),
respiratory (ERS), etc., sites of pharmaceutical companies.

Exclusion criteria: Media articles with no appropiate certified references or any scientific certification.

Data analysis: The data were analyzed and processed, yielding graphical presented results.

Limitations of the study:

- The information is new, recently published, which may lead to lack of proper scientific formatted, even by publishers.
- As there are numerous publications in a short amount of time, few articles can be missed, but all the efforts have been made toward comprehensiveness of the revised literature.

RESULTS

In the conditions of the COVID-19 pandemic, during April-November 2020, there have been a high substantial number of publications describing the unprecedented rapid path taken in the research and development of vaccines candidates for COVID-19. Following the review of more than 38 articles, with respective data analysis, there are the following summarised results:

1. Pharmaceutical companies, medical staff and academic institutions worldwide have been working intensively to discover effective treatments and prevention for COVID-19. In July, there were around 464 studies in development, while in December 2020, this number reached 580 studies. It should be noticed that vaccines in the development process constitute the most significant interest of researchers, with around 44% of researches (11). Other main topic of interest, with respectively 16% of studies (July) and 14% (November) continue to be for monoclonal antibodies and 5% for antiviral drugs. There is an increase in the number of studies for cell-based treatments from 4% to 6%, meanwhile studies for gene therapies (RNA) remain the same and there is a decrease in studies aimed to discover new indications for existing drugs from 5% to 4%. Increased interest has been shown in studies on medical devices. During this period, 9 clinical studies were discontinued. (Figure 1.a) (12).

2. Actually, there are 261 vaccines in different stages of development, 56 more than in July, most of them are in the preclinical phase, but 58 candidates have already entered the clinical phase, 35 more than in July.(Figure 1.b) (12).

3. Among the vaccines that have entered the clinical phase till November 2020, 43% are in the first clinical phase, compared to 48% in July, but there is an increased number of vaccines in the third clinical phase, 20% of all vaccines in the

clinical phase, while in July there were no vaccines in clinical phase III.(Figure 2.a) (11,12,13). Also there are 3 vaccines that have finished their phase III studies, publishing their results and received first approvals.

4. It turns out that from the beginning of the pandemic until November 2020, vaccines with nine different types of platforms are under development, the most used platform continues to be the one with Protein subunit (30%), followed by vaccines with a non-replicating viral vector (12%) in July and (14%) in November, with an RNA-based technology (13%) of vaccines, with DNA about 7%. The less-used platform continues to be vaccines with a live attenuated virus (1%) (12,13,14).

5. Not all developers have commented on how vaccines are administered. From this study results that the most common method of administration is the intramuscular route in 27% of cases, followed by the intranasal route (21%), the oral route (16%), the intradermal route (11%) and other routes such as intravenous, microneedle arrays, oral/nasal, subcutaneous, (Figure 2.b) (15,16,17).

6. Most vaccine studies are being conducted by the pharmaceutical/private sector and industries (45%), followed by 22% from unknown entities, 16% from public institutions, 12% of academic institutions/universities and 5% non-profit organizations. (Figure 2.c) (18).

7. The development of new drugs and vaccines is a very expensive process, which for many companies and institutions is unaffordable, the most expensive stages are related to the clinical testing phases, in large number of individuals. For emerging vaccines with promising results and features, special funds (grants) have been guaranteed from the private or public sector. These go for approximately 11% of vaccines, to reach clinical trials, while the rest of 89% of vaccines are not externally funded (Figure 2.d) (11,12).

8. Since discovering the new coronavirus complete viral genome, research teams globally have been mobilized in an unprecedented race time to seek and develop an effective vaccine. This race is against time and against rivals (rival companies or different countries), which the objective of becoming the new "field leader". The largest number of vaccines is being developed in North American countries, accounting for 33% of researchers. It is followed by regions such as Asia and Europe, each with about 29% of emerging vaccines. In Asia, China is the leading country with 19.5% globally, followed by Japan (2.93%) and other countries. Europe has the largest number of countries involved (15 countries). The U.K. has the highest number of researches, 6.34% of global researches. Russia is the second country with the largest number of researches in Europe, 4.88% of global researches, followed by other European countries each with a percentage of 1-4%. It is worth mentioning the contribution of India, where about 3.9% of research is being conducted. Australia and South America have lower numbers of research studies, than to other

regions, in Australia 2.4% and in South America only 0.98% (Figure 3) (11,12,13,14,15,19).

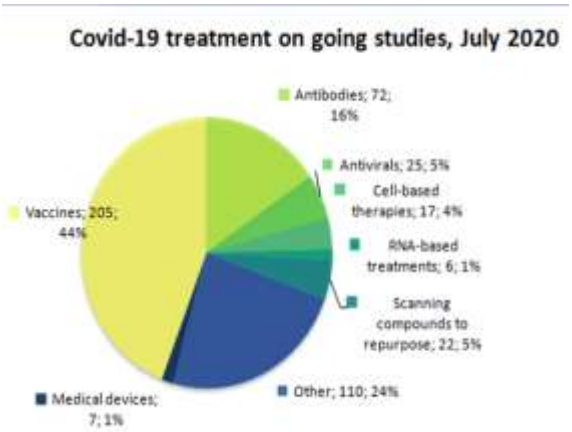

Figure 1a. COVID-19 treatment studies - July 2020

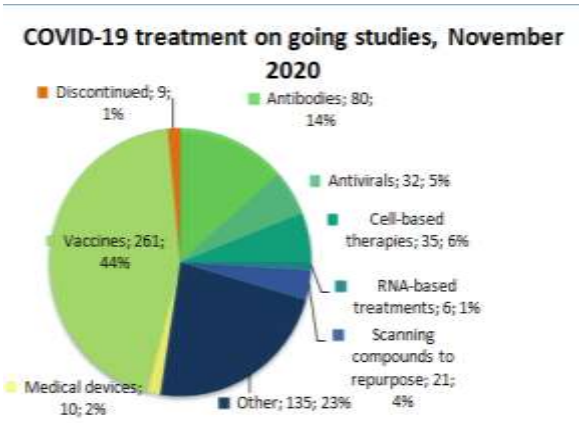

Figure 1.b. COVID-19 treatment studies, November - 2020

Vaccines with virus

- *Live attenuated virus vaccines*

Live attenuated virus vaccines are live viruses passed on to animals or tissue cultures so that the virus loses its virulence.

As a natural infection, the attenuated virus elicits strong immune response, B cells and T cells, longer-term immune responses, but may not be suitable for individuals with compromised immunity. A small possibility of a virus mutation could reverse its virulence and lead to the onset of the disease like the case with SARS 1 vaccines, where a reversal of the virus has been observed. Moreover, such vaccines need a cold chain for community distribution (20).

- *Inactivated virus vaccines*

In an inactivated vaccine, the virus is treated with various methods, such as chemicals (formaldehyde), solar radiation or heat treatment, to inactivate the virus. Such vaccines are safe and cannot cause the disease, but do not cause a high immune response and may need repeated dosing and adjuvants to boost immunization (21).

The advantage of complete virus vaccines is that they include all the natural ingredients of a virus, as proteins, lipids, and nucleic acids, facilitating broad and potent immune responses, unlike other platforms that tend to exceed natural potency.

Protein subunit vaccines

Protein subunit vaccines are based on inducing an immune response against the virus spike protein (protein S), thus preventing them from binding to the ACE-2 receptor.

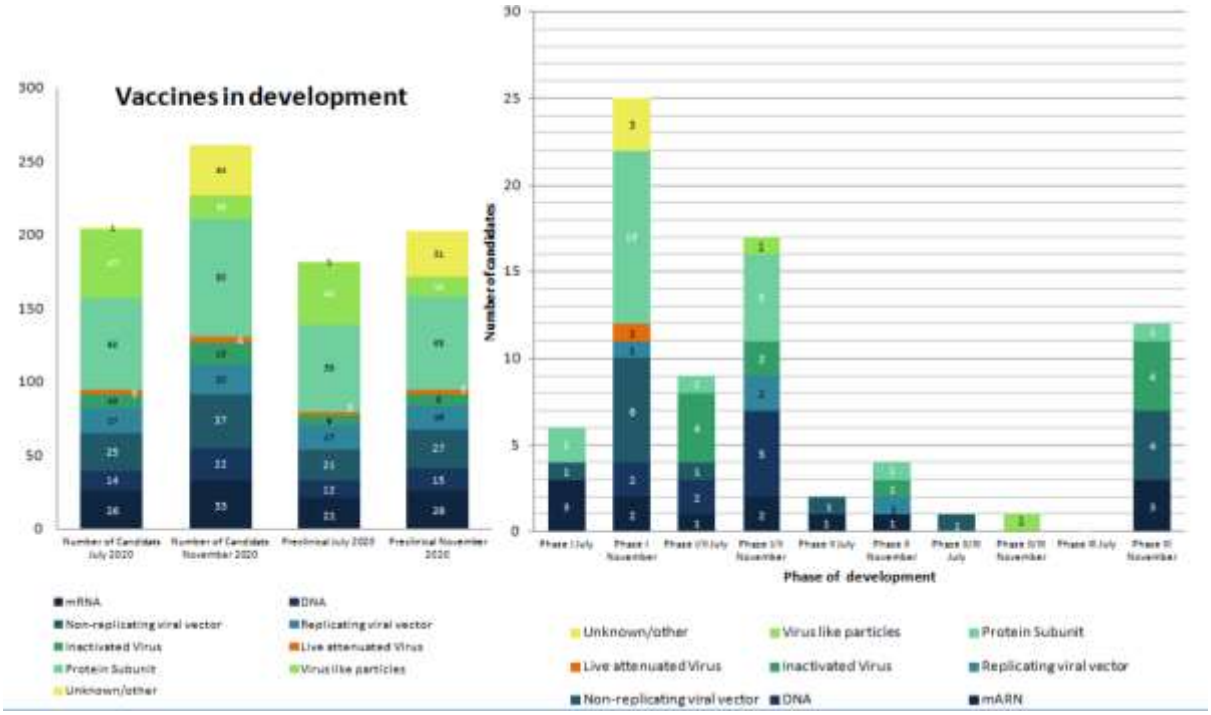

Figure 2.a. COVID-19 vaccines in different stages of development, July vs. November 2020

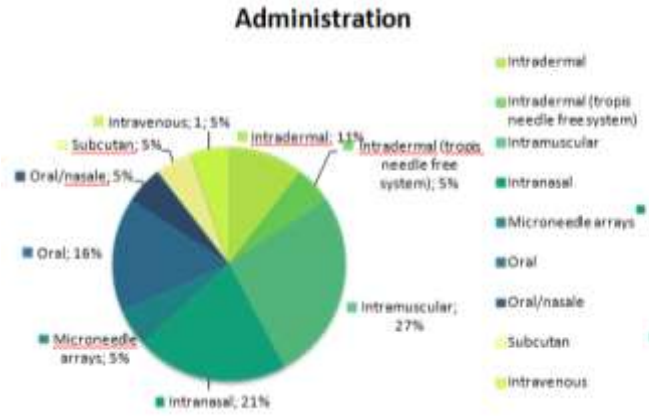

Figure 2.b. Administration methods used in COVID-19 vaccines

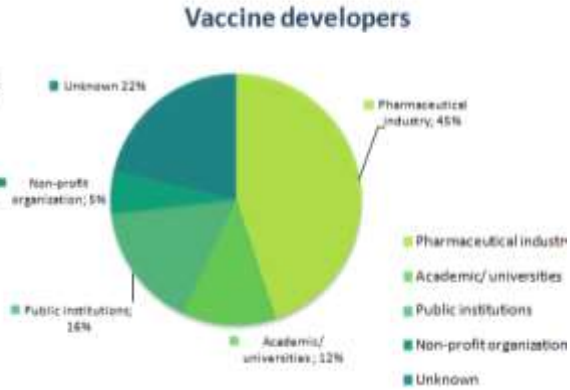

Figure 2.c. COVID-19 Vaccine developers

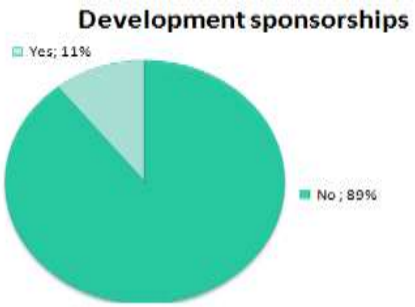

Figure 2.d. Vaccine development – funding

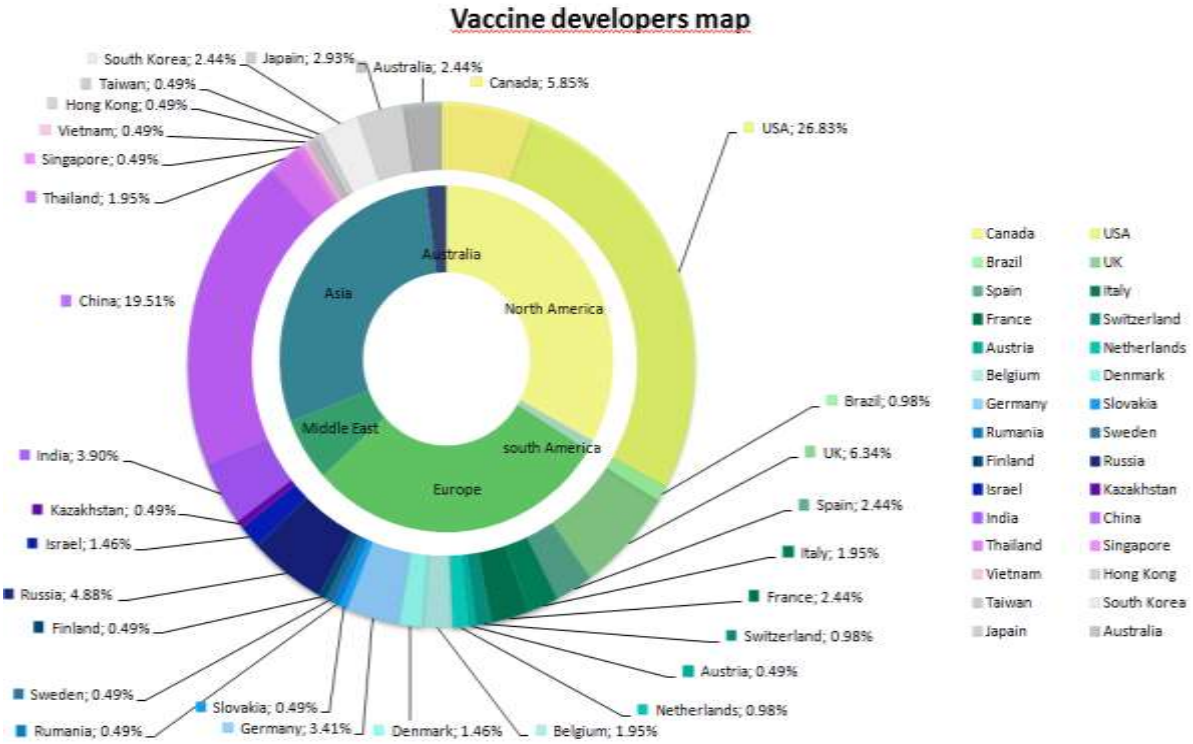

Figure 3. Map of countries - vaccine developers

Spike proteins cause the creation of higher neutralizing antibody titers than any coronavirus antigen. Studies for the development of subunit-based vaccines have reported an increased T cell immune response and the generation of high-titer neutralizing antibodies in vivo (22). These vaccines are very safe and have fewer side effects by boosting the immune system, without introducing infectious viruses.

Virus-like particle vaccine (VLP)

In this case, vaccines are made up of virus particles without genetic material. Such vaccines are safe and provide an adequate immune response, however they are challenging to produce (23). Virus-like particles (VLP) are formed from viral structural proteins, which have an inherent self-structuring property and mimic pathogen's morphology. Unlike "live" viruses, VLPs are non-infectious and non-replicating, as they have no infectious genetic material (24).

Nucleic acid vaccines

- *DNA vaccines*

Nucleic acid vaccines work by inserting DNA or RNA sequences, which encodes a specific disease related antigen in the organism. Once the sequence is translated into the corresponding peptides, an immune response is elicited. Nucleic acid vaccines generally stimulate both humoral and cellular immune responses, unlike conventional vaccines that stimulate only one antibody response (25).

DNA vaccines contain a plasmid with the necessary DNA sequence, which must be inserted into the host cell DNA, to direct the production of antigenic proteins. The production of DNA vaccines is much easier than the one with conventional vaccines. Synthetic DNA is temperature resistant and distribution can be accomplished without cold chain conditions.

The concern is also raised about the possible side effects, from the plasmid interaction with human DNA, disrupting normal transcription. In vitro studies suggest that the rate of mutagenicity is lower than the rate of spontaneous mutations in human cells (26).

- *mRNA vaccines*

Once injected, RNA can be processed by immune cells and directly produce the targeted protein through translation. When the newly produced protein is released from the host cell, the presenting antigen cells will rapidly capture and present to MHC I and MHC II (Major histocompatibility complex) the antigen, located on the surface of the presenting antigen cells. This step is important for the subsequent activation of B cells and T cells, which is the key to the humoral and cytotoxic response (27).

An mRNA vaccine is considered the most promising candidate because it also can be produced very rapidly and save essential time. Meanwhile, the main restrictions and difficulties of development regard distribution and stability issues for RNA degradation, together with safety concerns regarding immunogenicity (28).

Viral vector vaccines

The genetic material from the pathogen SARS-CoV-2, is used to create viral vectors, containing the virus' genetic material. Some vectors used in vaccines are capable of reproducing in the body, meanwhile, other candidates use a non-reproducible vector.

The most commonly used viral vector are adenoviruses. The only reason why these vaccines can be ineffective is if the recipient already has some form of immunity to the generated vector, making it impossible for the virus to enter our cells (27).

C. Comparison of the most promising vaccines for COVID-19

The following table has included summarized information and comparisons; between the most promising vaccine products. These candidates during December 2020 and January 2021 have received the first marketing authorization approvals from FDA and EMA, followed by massive vaccination campaigns in specific countries.

Table 1. The most promising vaccines for COVID-19, November 2020 (16,17,26,28,37,38)

	Pfizer and BioNTech (*BNT162b2*)	**Moderna (mRNA-1273)**	**AstraZeneca & University of Oxford (AZD1222)**	**J&J (Ad26.COV2.S)**
Platform used:	LNP-mRNA	LNP-encapsulated mRNA	Non-Replicating Viral Vector	Non-Replicating Viral Vector
Clinical study starting date	April 29, 2020	March 16, 2020	April 23, 2020	August 10, 2020
Participants:	43,998 participants, 12-85 years.	30 000 US participants, 18+ years	40051 participants, 18+ years	A target of 60,000 adult participants 18+ years
Study design:	Multiple locations in different countries (150 clinical trials sites with racially and ethnically diverse backgrounds) Participants have a 50% chance of receiving either the vaccine candidate or a placebo (randomized, placebo-controlled). The study is also "observer-blinded" . Masking is Triple (Participant, Care Provider, Investigator)	Randomized, Stratified, Observer-Blind, Placebo-Controlled Study. The randomization is in a blinded manner using a centralized Interactive Response Technology (IRT), according to pre-generated randomization schedules. Randomization was stratified based on age and, if they are < 65 years of age, based on the presence or absence of risk factors for severe illness from COVID-19 based on CDC.	Randomized. Participants are assigned to one of two or more groups in parallel for the duration of the study. Masking is Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Double-Blind, placebo controlled. (Two or more parties are unaware of the intervention assignment.)	Randomized, double-blind, placebo-controlled clinical trial. Participants will be randomized in parallel in a 1:1 ratio to receive intramuscular (IM) injections of Ad26.COV2.S or placebo . The study will consist of: a screening phase (up to 28 days), and a long-term follow-up period (1 additional year)
Dosage and administration:	Two doses are required (separated by 21 days) IM administration	2 doses of mRNA-1273 on Day 1 and on Day 29. IM administration	2 doses AZD1222 4 weeks apart IM administration	Single dose IM administration

Study completion date:	January 29, 2023 Participants will be monitored for two years after they receiving second dose of the vaccine candidate or placebo.	October 27, 2022	October 25, 2022	March 10, 2023 The duration of individual participation, including screening, will be maximum 2 years and 1 month. The end-of-study is considered as the completion of the last visit for the last participant in the study.
Phase III Study Results:	Efficacy was consistent across age, gender, race and ethnicity demographics. Based on an analysis of 170 cases of COVID-19 in trial participants, the vaccine efficacy rate was 95%, 162 cases of COVID-19 were observed in the placebo group versus 8 cases in the vaccine group. No serious safety concerns observed; the only Grade 3 adverse event more significan than 2% in frequency was fatigue at 3.8% and headache at 2.0%	Vaccine promotes the creation of neutralizing antibodies in older adults at comparable rates to younger adults It is 94.5% effective. The analysis was based on 95 covid-19 cases, of which 90 were observed in the placebo group and five were reported in the vaccine grup The majority of adverse events were mild or moderate in severity. Grade 3 (severe) events more significan than or equal to 2% in frequency after the first dose included injection site pain (2.7%), and after the second dose included fatigue (9.7%), myalgia (8.9%), arthralgia (5.2%), headache (4.5%), pain (4.1%) and erythema/redness at the injection site (2.0%)	The vaccine induces potent antibody and T cell immune responses across all age groups, including older adults. Phase 3 interim analysis including 131 Covid-19 cases indicates that the vaccine is 70.4% effective when combining data from two dosing regimens In the two different dose regimens vaccine efficacy was 90% in one and 62% in the other Higher efficacy regime used a halved first dose and standard second dose, half dose regime may be more effective because it “better mimics” a real infection. There were no hospitalized or severe cases in anyone who received the vaccine The vaccine was on hold after two UK participants developed a severe and unexplained illness. The participants developed a serious neurological condition and after investigation, clinical trials continued.	Phase III results are not published yet. Johnson & Johnson's COVID-19 vaccine trial was on hold after an unexplained illness in a volunteer, but after investigation, the unidentified illness was not related to the vaccine.

Authorization application:	On **November 20**, a submitted request to the U.S. Food and Drug Administration for an Emergency Use Authorisation. **FDA approval on 11 December 2020** **EMA approval on 21 December 2020**	**On November 16** Moderna announced that the trial had met the statistical criteria pre-specified in the study protocol for efficacy. **FDA approval on 18 December 2020** **EMA approval on 6 January 2021**	**On 12 January 2021**, EMA announced the submission for conditional marketing authorisation At end of January 2021 is expected an opinion on marketing authorization Approved in UK on **3 December 2020**	In January- February 2021
Manufacturing and distribution:	Vaccine will have to be chilled to -70°C. The vaccine is estimated to cost $19.50 a dose. The companies expect to produce globally up to 50 million vaccine doses in 2020 and up to 2 billion doses by the end of 2021	Vaccine will have to be chilled -20°C for 6 months, 2°-8°C/ for up to 30 days, Room temperature: 12 hours post thaw The vaccine is estimated to cost $35 a dose It is expected to produce 20 million doses by the end of the year and up to three billion doses of the vaccine in 2021.	The vaccine is estimated to cost around $4 per dose The vaccine can be stored at fridge temperature (2-8°C). It is expected more than 300 million doses will be available by the end of the first quarter of 2021. At peak manufacturing capacity, 100 to 200 million doses of the vaccine can be produced in a month. Large scale manufacturing ongoing in over 10 countries to support equitable global access	The vaccine can be stored at fridge temperature (2-8°C). The vaccine is estimated to cost $10 a dose The pharmaceutical company can supply 100 million doses of its candidate, in a month.

DISCUSSION

❖ ***Vaccine efficacy and mechanism of action***

Although many companies announced previously to approval that the COVID-19 vaccine was almost ready, this process has many complications and even after approval requires further evaluations. The vaccine should be effective, stimulating the synthesis of specific antibodies, at a certain concentration (titer) and providing protection for a reasonable time, together with appropiate availability to widely globally distribution.

However, vaccines do not create immunity in all vaccinated people. The causes are complicated, extending from genetic and immunological components to the quality of the vaccines or methods of administration (8).

Assuming that the vaccine elicits an effective immune response in a significant number of vaccinated individuals, the timing of vaccine protection for them is debatable. It takes a long time to check the viability after vaccination of anti-COVID antibodies. Efficacy also depends significantly on the type of vaccine, technology or platform used.

Another important aspect to be considered, is the internal ability and presence of infrastructures, which will enable companies to produce vaccines on a large scale, to achieve rapid global distribution. The proposed new platforms can generate billions of doses in a few months and optimistically, this capabilities will increase in coming months.

There are still many unknown patterns about coronavirus immunity, which makes immunization with a vaccine difficult. Present and future mutations in SARS-COV-2 (U.K./South Africa mutations, etc) can occur at any time, which automatically implies a moderate/high-risk level of impact in efficacy, for any vaccine that is being developed and/or approved (29).

Another crucial aspect of impact is age. Several studies on influenza vaccines have shown that aging of the immune system dramatically reduces vaccination effectiveness, wich impacts vaccine efficacy in eldery as a high risk category. Therefore, for all anti-SARS-COV-2 vaccine, all of the above aspects should be evaluated, and critical immunization failures minimized through dose adjustment or the number of administrations (8).

❖ ***Vaccine safety profile.***

Vaccination safety includes various elements such as: production, product quality control, evaluation of its effectiveness and guarantee of safety, transport and distribution, implementation of good practices for the use and administration, and a vigilant surveillance system, after distribution and use (29).

Most of vaccines under development are using the same technology, as in the production of seasonal flu vaccine, but also are present new technologies or new adjuvants. This leads to the need of the implementation of additional multiple control steps, to ensure their safety and

effectiveness, preventing unknown side/adverse effects (8).

❖ ***Antibody-dependent enhancement, ADE***

The immunological objective of candidate vaccines is to induce neutralizing antibodies that inhibit the CoV entry into cells. Antibodies play a crucial role in controlling viral infections, including blocking virus binding to cells and activating complement system cascades.

A serious concern for vaccine developers is the risk of "antibody-dependent enhancement" (ADE), which is a phenomenon in which antibodies generated against an infection or from a previous vaccination may worsen the pathogenesis of a subsequent infection, by the same virus or similar (8).

ADE occurs when non-neutralizing antibodies facilitate the entry of the virus into the host cells. Antibodies bind to antibody receptors on the plasma membrane of cells, and the virus binds to the other end of the antibody where the antigen binds.

The ADE's mechanism is not yet fully understood and is thought to vary according to pathogen and patient. This *"Trojan Horse"* strategy allows the virus to infect a cell by escaping from its endosome and trigger damage to the immune system, causing a more aggressive pathogenesis (30).

❖ ***The risk for ADE from vaccine in COVID-19 disease***

It is still unknown whether antibodies generated by the vaccine may increase the severity of disease caused by SARS-CoV-2 infection or mediate harmful immune responses. However, this potential risk needs to be considered, during future possible waves, after massive vaccitation campains.

In the current COVID-19 pandemic state, there are several pending questions about the nature of SARS-CoV-2. Suspicions have been raised that patients with COVID-19 suffering from severe symptoms may have been preceded by one or more previous coronavirus exposures and are experiencing ADE's effects (30). However, this cannot be confirmed because although current clinical evidence suggests that this is just as a possibility, the host molecular and immune response to SARS-CoV-2 infection has not yet been fully elucidated to prove that ADE is occurring.

ADE can occur in COVID-19 infection, but at this moment, it is not known and proved that ADE is occurring in COVID-19 cases, so both antigen treatment and vaccine developments will need to consider this phenomenon and monitor possible presentations (31).

❖ ***Specificity selection***

Most likely, SARS-CoV-2 infection results in a combination of neutralizing and strengthening antibodies, the amount and concentration of which form the patient's clinical response. Therefore, attention must be paid to the nature of the immune response that a vaccine is intended to elicit.

The primary determinant of whether an antibody is protective or strengthening is the epitope for which it was created (30). Given the key role of

the "spike" protein in mediating virus entry into the cell and the immune responses, it induces, almost all SARS-CoV-2 vaccines aim to target it. However, spike protein has also been shown to be a major mediator of antibody-dependent stimulation (ADE).

❖ ***Avoidance of side effects from ADE.***

Some examples of "in vitro" studies have shown that several spike protein epitopes tend to be more "dangerous" than others, for the types of antibodies that are allocated to them. The most "neutralizing" region of the spike protein is the binding site (RBD-receptor binding domain) within the S1 subunit, making it a target of choice for specific and effective vaccines.

An alternative target may be the nucleocapsid (N) protein since protein N is not a virus surface protein, consequently, vaccine-induced antibodies that target protein N will not facilitate virus entry, generating a humoral and cellular immune response specific for this protein (31).

Another major barrier to vaccine development is the higher mutation rate of RNA viruses compared to DNA viruses, resulting in higher genetic diversity. Moreover, RBD of protein S is the most variable region in the coronavirus genome. Therefore, ADE and higher genetic diversity should be considered as essential factors for the current development of vaccines, future modifications and antibody-based drugs against SARS-CoV-2 (30).

❖ ***Vaccine that induces cytotoxic T cells (CTL), without antibodies correctly***

The immunological goal of an ideal vaccine for SARS-COV-2 is to elicit CoV-specific cytotoxic T responses, to kill infected cells in the absence of antibody generation. CoV-infected patients with mild or asymptomatic symptoms develop less antibody responses, CoV-specific CTL responses are mainly controlling their disease.

The more severe the disease, the greater the antibody response is detected in patients in the convulsive phase (32). Vaccine-induced CTL can protect against CoV transmission if CTL kills CoV-infected cells before the virus begins to multiply.

❖ ***Cells immunopathology***

During early testing of experimental vaccines for SARS-CoV, some experimental animals developed after immunization lung or liver histopathology events, characterized by significant infiltration of lymphocytes, monocytes, and eosinophils (33).

In-depth literature analysis suggests that TH17 responses (T-helper 17 cells) may direct these cellular responses after immunization with inactivated viruses and attenuated vaccines, virus vectors, and other vaccine elements. In part, this demonstrates the link between the development of TH17 cells and IL-6 (interleukins 6), an increased cytokine in patients with COVID-19, who experience cytokine storm reactions. Aluminum, an adjuvant that promotes TH2-type immunity, reduces immunopathology and highlights the importance of selecting vaccine with appropriate adjuvant delivery platforms (31).

❖ ***Difficulties in developing vaccines during a pandemic.***

The costs of developing one or more vaccines, including the clinical development and distribution process are approximately of US $ 2 billion. These cost estimations presume a successful development process, reaching final marketing authorization approval or license under emergency use guidelines. An important amount of funds are needed during all stages of COVID-19 vaccine development, preclinical and clinical phases.

There is a major disruption to the candidates' journey from Phase II to III, which partially reflects the very high costs of Phase III trials (34). Approximately development of phase III trials study is responsible for about 70% of the total development costs.

❖ ***Glycolysis of viral proteins***

Glycosylation is one of the most critical forms of modification after protein translation and it is an important way to regulate the localization of proteins and their role. However, high protein glycosylation levels can work as a sort of "invisible camouflage", which may help the virus escape successfully from recognition by the human immune system and increase his survival rates. Therefore, the higher the rate of glycosylation, the greater the probability that the virus will escape the immune response and lower the vaccine development's success rate (35).

However, the use of mRNA-based vaccine technology, with a focus target only to the S protein, and not the whole virus, can lead to the production of S protein antibodies from the human immune system, without viral glycosylation effect.

CONCLUSION

✓ ***The role of pharmaceuticals and clinical trials.***

Pharmaceutical companies are contributing to the fight against COVID-19 on numerous fronts. COVID-19 has driven the pharmaceutical sector and industry to be the center of global attention, playing a key role, through their scientific and economic resources and by devoting itself to the development of diagnostics tools, effective treatment and safe vaccines.

As a science-driven industry that aims to address the world's greatest healthcare challenges, the biopharmaceutical industry is positioned in the best way possible to respond quickly to the COVID-19 pandemic.

More than ever before, the onset of COVID-19 pandemic pushed towards effective coordinated international cooperation, between private and public sectors and institutions, producing incredible results on the research, development and production of diagnostics, drugs and vaccines.

The pharmaceutical industry is fully committed to bringing its unique expertise to:

- ✓ Discovery of new indications for existing drugs in the treatment of COVID-19
- ✓ Accelerate research and development of safe and effective vaccines and new specific drugs.

- ✓ Develop diagnostic tests and provide them to the market continuously.
- ✓ Provide the necessary tools, medicines and vaccines on an ongoing basis.
- ✓ Support to the global health system.
- ✓ ***Vaccines, finally near us…***

Numerous platforms and products have been under development during months of efforts and studies.

Among those with the greatest potential are genetic vaccines (DNA and RNA-based platforms, with already approved representants), followed by those with recombinant subunits.

RNA and DNA vaccines can be produced rapidly because they do not require culture or fermentation, but rather use synthetic processes. To date, there are ongoing approval processes worldwide for RNA vaccines (Pfizer/BioNTech and Moderna, approved from FDA, EMA and other local RA), Regulators worldwide are working intenstively, to evaluate data submitted and approve global use of as many as possible vaccine representants.

The approval of COVID-19 vaccines is just one step (a huge one) but it is fundamental to have virtuous global administration and distribution, in order to reduce to the minimum delays and monitor closely any possible efficacy or safety issues. Further studies and real-world data will tell us, if COVID-19 vaccines will be remembered as a "history of unprecedented medical and scientific success," changing the course of the pandemic and helping the return to the "old boring and not so bad normality..."

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Conflict of interest

None declared.

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