HAUTE AUTORITÉ DE SANTÉ

REPORT

Immunological and virological aspects of SARS-CoV-2

Genetic variability, immune responses, vaccine platforms and animal models

25 November 2020

Description of the publication

Title	**Immunological and virological aspects of SARS-CoV-2** Genetic variability, immune responses, vaccine platforms and animal models
Work method	Literature review
Purpose(s)	Provide elements of knowledge to help understand and interpret the results of clinical trials on SARS-CoV-2 vaccine candidates
Targets concerned	Healthcare professionals, Journalists, General Public
Requester	*Direction générale de la santé* (Ministry of Health) (DGS)
Sponsor(s)	*Haute Autorité de santé* (French National Authority for Health) (HAS)
Project management	CTV (Technical Vaccination Committee) co-management: Jean-Daniel Lelièvre, Agnès Gautheret-Dejean SEESP-HAS (HAS Public Health and Economic Evaluation Department) co-management: Sophie Tchakamian, Project Manager, Anne Doussin (Assistant Head of Department), Michèle Morin-Surroca (Head of Department)
Literature search	Sylvie Lascols (Assistant Archivist), Marina Rennesson (Archivist)
Authors	Jean-Daniel Lelièvre, Agnès Gautheret-Dejean, Daniel Floret, Sophie Tchakamian
Declaration of interests	The members of the working group communicate their public declarations of interest to the HAS. They may be consulted on the website https://dpi.sante.gouv.fr. They were analysed according to the analysis grid of the HAS guidelines for the declaration of interests and management of conflicts of interest. The interests declared by the working group members were considered to be compatible with their participation in this work.
Validation	Version dated 25 November 2020
Updating	Nov 2020
Other formats	Summary available to download free of charge at www.has-sante.fr

This document and its bibliographic reference are available to download at www.has-sante.fr
Haute Autorité de santé – Communication and Information Department
5 avenue du Stade de France – 93218 Saint-Denis la Plaine Cedex. Tel.: +33 (0)1 55 93 70 00

Introduction

In accordance with its framework document and the referral of the Director General for Health on 13 July 2020, the HAS was tasked with drawing up intermediate recommendations concerning possible vaccination campaign rollout procedures, in anticipation of the arrival of a SARS-CoV-2 (Severe Acute Respiratory Syndrome-related CoronaVirus-2) vaccine.

In order to anticipate and support the scientific expertise of the HAS, a working group composed of members of the Technical Vaccination Committee was set up to produce a summary of the scientific knowledge (in particular, immunological responses, virological data, animal models and vaccine platforms) required to understand the results of clinical trials on SARS-CoV-2 vaccination.

This document has been divided into two parts:

- The key points that provide a summary of the most relevant data, serving as a framework to assess the results of clinical trials;
- Further details on the data presented in the first part.

This document is not intended as a presentation of the results of clinical trials on the SARS-CoV-2 vaccine candidates under development; these may be subject to a specific publication.

The information it contains is only valid on the date of publication and will be regularly updated.

In this document we have used the name: SARS-CoV-1 for SARS-CoV and SARS-CoV-2 for 2019-nCoV (initial name).

Methodology

This summary document on knowledge relating to the immune response to SARS-CoV-2 infection is based on a systematic review of the literature.

The search included publications in English and French, in the Embase, Lissa and Medline databases. Additional searches were performed in the WHO databases, in preprint databases and on the Science Direct website.

To supplement this review of the literature, systematic daily scientific monitoring of the BioRXiv and MEdRXiv preprint databases and Embase and Medline databases was also put in place. This work is ongoing.

In parallel, monitoring of the scientific press and the media was conducted.

Regular monitoring of clinical trials and their development was also carried out.

Regular updating of the “Key points” part of the document will be carried out by the working group based on published data and the literature monitoring system put in place by the HAS Documentation and Scientific Monitoring department.

The more technical literature search aspects can be consulted in appendix 1 of this document.

Since this document is an educational document, it was amended by the Covid-19 Task-Force working group and submitted to all the members of the Technical Vaccination Committee, as well as the guidance Board on 25 November 2020.

Key points

1.1. SARS-CoV-2: structure and variability

SARS-CoV-2 (Severe Acute Respiratory Syndrome-related CoronaVirus-2) belongs to the *Coronaviridae* family, genus *Betacoronavirus*, subgenus *Sarbecovirus*. Six other coronaviruses can also infect humans: SARS-CoV-1, MERS-CoV, HKU1, OC43, NL63 and 229E.

SARS-CoV-1 and MERS-CoV are phylogenetically close to SARS-CoV-2, replicate in the lung parenchyma and, like it, are responsible for an illness with potentially lethal lung damage. The other common coronaviruses only replicate in the upper airways and cause a common cold illness. It should be noted that the specific feature of SARS-CoV-2 is its propensity to multiply in the upper airways as well.

SARS-CoV-2 is a helical capsid enveloped virus with a genome composed of positive-polarity, single-stranded RNA with around 30,000 nucleotides. The surface S (spike) protein binds to the ACE2 cell receptor, which is expressed in numerous tissues. It contains 2 subunits, S1 and S2, with S1 including the receptor binding domain (RBD) containing the receptor binding motif (RBM). Subunit S2 contains the fusion peptide. The closest human coronavirus is SARS-CoV-1, with which it shares the same genome organisation. As is the case for SARS-CoV-1 and MERS-CoV viruses, the main target of the neutralising antibody response is the spike protein.

SARS-CoV-2 is a virus that is liable to mutate. The nucleotide substitution per genome per year rate is estimated to be between $8x10^{-4}$ and $8.1x10^{-3}$. This rate is lower than that observed for HIV or the influenza virus, probably related to the existence of a corrective activity.

This rapidly led to the emergence of several clades[1] of this virus. The current classification includes 8: V, L, S, G (divided into GR, GH and GV) and O. In France, clade S, L, G, GR, GH and GV strains have been circulating to date. Clade GH strains predominate. In the S gene, 5 main mutations are observed in the strains circulating in France (L5S, D80Y, A222V, S477N and D614G).

Observed from February 2020, the D614G mutation was present in almost every country of the world and in France (>90% of strains). Since September 2020, A222V and S477N mutations have emerged in Europe, and, in particular, in France (10% to 30% of strains, respectively). Only the D614G mutation is thought to increase the transmissibility of those strains carrying it. It may increase the protein's capacity to twist, helping it to bind to the receptor.

Studying common coronavirus infections responsible for the common cold helps provide a better understanding of the biology of coronaviruses and their evolution in response to the immune system. This has only been studied with respect to the humoral (antibody-mediated) immune response.

In vaccinology terms, the question is whether SARS-CoV-2 is capable of rapidly undergoing genetic alterations resulting in it evading the immune response, thereby leading to a need to regularly change the antigens used in vaccines. The currently available data are as follow:

1. *In vitro*: the use of monoclonal neutralising antibodies as monotherapy leads to rapid selection of mutants resistant to neutralisation (effect not found following the use of several antibodies, which corresponds to the situation expected *in vivo* following infection or vaccination)
2. *In vivo*: viral strains presenting mutations in their RBD are present in very low levels among infected populations. SARS-CoV-2 with a D614G mutation, which is one of the most prevalent

[1] A clade (from the Ancient Greek: κλάδος / kládos, "branche"), also called a monophyletic or natural group, is a group of living or previously living organisms, including a specific organism and all its descendants.

strains of the virus at present, seems to be more sensitive to neutralisation. It should be noted that it has been shown that coronaviruses responsible for colds, such as HCoV-229E, acquire mutations in their RBD, thereby inducing resistance to neutralisation by antibodies. Hence, the existence of regular reinfection by these common coronaviruses may be explained more by the selection of mutants resistant to the adaptive immune response - particularly humoral - than by a loss of this response.

1.2. Animal models

Ideally, an animal model should 1) be susceptible to infection by the target pathogen, 2) enable reproduction of the pathophysiology of the infection in humans 3) have an immune system for which the mechanisms have been adequately studied and for which *in vitro* study systems are readily available 4) induce immune responses that closely resemble those obtained in humans 5) be easy to maintain.

Several animals can be experimentally infected with the SARS-CoV-2 virus. Mice, which are the animals most often used in preclinical experiments, are not susceptible to SARS-CoV-2 infection, but transgenic mice expressing the human ACE2 receptor are. Among rodents, hamsters appear to be the most relevant model to study SARS-CoV-2 infection, since this animal develops a respiratory illness similar to the one found in humans.

However, the most interesting and most studied model is the macaque non-human primate (NHP) model, its greater phylogenetic proximity with humans being an advantage, particularly for the study of immunopathology phenomena (see below). In addition, it has been possible to study the impact of age - a key factor in the severity of SARS-CoV-2 infection in humans - in this model. In the NHP, following a second viral challenge performed 35 days after the first, the following are observed:

1. Protection against SARS-CoV-2-induced lung disease;
2. The existence, nonetheless, of viral replication in the ENT system despite the virus being completely controlled in the lungs. This tends to suggest that natural adaptive immunity provides more protection against illness than infection.

The most relevant animal models for exploration of the post-vaccine immune response in SARS-CoV-2 infection thus appear to be 1) the mouse model for rapid exploration of the immune response and, in particular, the humoral immune response, 2) the NHP model, subsequently, to verify the clinical efficacy and the absence of any harmful effect of the immune response.

It is important to remember that there is a correlation between the dose used to infect the animal and the severity of the illness in animal models. However, the vaccine protection studies conducted with various candidates do not all use the same amount of virus to infect the animals. It is therefore necessary to carefully analyse the virological results to differentiate between the existence of true viral replication (presence of subgenomic RNA) and straightforward local persistence of the virus post-challenge (presence of genomic RNA only).

The results of studies conducted in animal models with the main SARS-CoV-2 vaccine candidates are reported in table 3.

1.3. Immunological analyses performed in SARS-CoV-2 vaccine clinical trials

Like all viruses, SARS-CoV-2 triggers a humoral (antibody-mediated) and cellular (cell-mediated) adaptive response.

1.3.1. Quantitative and qualitative humoral response

The quantitative anti-SARS-CoV-2 humoral response has been evaluated using different tests. However, at present, it is primarily based on the performance of ELISA tests. The viral antigens recommended in the context of serological tests to screen for infection are the S protein (spike protein), its RBD (Receptor Binding Domain), or the N protein (nucleocapsid protein). In the context of SARS-CoV-2 vaccines, the ELISA tests will usually only target the former two, since many vaccines (see below) only contain the spike protein as the antigen. The result is expressed as a percentage of positive subjects and as a geometric mean. There is no international standardisation of these tests, which are therefore difficult to compare with one another.

To analyse the qualitative anti-SARS-CoV-2 humoral response (neutralising antibody), microneutralisation tests are used. The sample of serum or antibody solution to be tested is diluted and mixed with a viral suspension. The mixture is then incubated to enable the antibody to react with the virus, then distributed on a culture of host cells susceptible to the virus. The serum concentration required to reduce the number of lysis plaques by 50% (PRNT50) compared to the virus alone provides a measurement of the amount of antibodies and their efficacy.

Some teams have optimised this test by replacing one gene - ORF7 (Open Reading Frame 7) - of SARS-CoV-2 with the gene encoding nanoluciferase (NLuc) or mNeonGreen, thereby enabling simpler and quicker detection of the infection following cultivation on cells (5h). This type of test requires the use of a BSL3 (Biosecurity Level 3) laboratory[2]. To avoid this problem, some teams use pseudoneutralisation tests. In this case, SARS-CoV-2 is replaced by a pseudovirus - i.e., another virus (vesicular stomatitis virus [VSV] for example, or lentivirus) - in which the gene for its envelope protein has been deleted on the genome and replaced by the gene encoding the SARS-CoV-2 spike protein. The rest of the procedure remains identical. The tests cannot be compared with one another because:

1. There is no international standardisation;
2. The viruses are not the same (virus or pseudo-virus);
3. The SARS-CoV-2 (or spike protein) strains may differ from one study to another;
4. The cell lines may also vary (Vero: monkey kidney cells, Huh7: human hepatocarcinoma cells, etc.). To overcome this problem, the authors generally compare the results obtained post-vaccination with those obtained following natural infection, but it is still necessary for the samples chosen to be comparable in terms of sampling schedule and severity of the illness presented by the patients.

This humoral immune response is generally evaluated 4 weeks after vaccination.

[2] Contained secure laboratory handling infectious agents that can be contagious through the air and that can have serious or even fatal consequences.

1.3.2. 1.3.2 Cellular immune response

The anti-SARS-CoV-2 T cell response also appears to be important to control the infection.

The most rapid - but hence the most rudimentary or basic - test to evaluate the T cell response is the IFN-γ ELISPOT assay. The principle of this test is to measure the specific cellular responses to an antigen by quantifying the number of T cells producing IFN-γ. T lymphocytes are collected and then stimulated with overlapping peptides corresponding to the protein of interest (spike protein in the case of the current SARS-CoV-2 vaccines). Then, after 24 hours, the production of IFN-γ by these cells is analysed using an ELISA method with an anti-IFN-γ antibody. Sorting of CD4+ and CD8+ T cells can be performed before conducting the test in order to analyse which lymphocytes produce IFN-γ, but this makes the test more complex and is not routinely performed. In order to better analyse and separate the CD4+ and CD8+ T cell responses, the use of flow cytometry is preferred.

Analysis of cytokine production by flow cytometry (intracellular staining [ICS]) is a more expensive method requiring access to a flow cytometer. However, it makes it possible to identify which cell produces a given cytokine. The preparatory phase is the same as for the previous test and the lymphocytes are incubated with overlapping peptides for the antigens of interest. After 24 hours, the lymphocytes can be analysed and the CD4+ T cells with Th1 polarisation (production of IFN-γ and/or IL-2 but not IL-4, IL-5 and/or IL13) can be differentiated from those with Th2 polarisation (production of IL-4, IL-5 and/or IL-13 and CD40L). For CD8+ T cells, another, smaller peptide pool will be used and the production of IL-2, TNFα or IFN-γ will be examined, or the expression of CD103a, perforin or granzyme (the latter proteins being associated with their capacity to destroy infected cells). As is the case with antibodies, these tests are not standardised on an international level and therefore the results are not quantitatively comparable from one study to another; at most, the relative proportions of the Th1/Th2 responses for the CD4+ T cells can be compared.

This T cell response is generally evaluated 2 weeks after vaccination.

1.4. Immunity and vaccines against coronaviruses other than SARS-CoV-1 and MERS-CoV

The existence of an immunopathological component seems to be one of the important characteristics of coronavirus infections and, in particular, the resulting COVID-19 illness, responsible for severe forms, in both humans and animals; this suggests a rationale for the use of immunomodulating treatments, either antiviral (type 1 interferons) or anti-inflammatory (corticosteroids) in moderate to severe forms.

This is an important point, since this innate immune response has effects on the adaptive response and hence it is difficult to make strict parallels between the adaptive response observed following a natural infection and that expected after vaccination.

Study of the immune response following coronavirus infection in animals reveals:

1. The need for an antibody and T cell response to ensure protection,
2. The rapid decrease in immune response, in particular humoral,
3. The sometimes harmful role of this immune response with, as a model, infectious peritonitis virus in cats, characterised by an ADE (antibody dependant enhancement) phenomenon, leading to propagation of the infection *via* infection of the macrophages.

As indicated previously, the human coronaviruses responsible for common cold illnesses (HKU1, OC43, NL63 and 229E) are viruses that multiply in the upper airways (like SARS-CoV-2), but not in the lung parenchyma (unlike SARS-CoV-1, SARS-CoV-2 and MERS-CoV). The humoral component

of the adaptive response against these viruses has been studied, but no data are available concerning the lymphocyte response. Infection with these viruses triggers a systemic (IgG) and mucosal (IgA) humoral response, with the presence of neutralising antibodies. In contrast with the systemic response, the mucosal response is associated with control of multiplication of the virus in the upper airways. Despite a high seroprevalence rate in the adult population, these viruses are responsible for regular and cyclical epidemics. Clinical data, in particularly following viral challenge tests in volunteers, appear to indicate that this phenomenon is more related to the emergence of viral mutants less susceptible to the neutralising response than to the loss of this response.

It should be noted that vaccines exist against several animal coronaviruses (these are listed in table 4), but that there is currently no vaccine against human coronaviruses causing the common cold.

1.5. Immunity and vaccines against SARS-CoV-1 and MERS-CoV

SARS-CoV-1 and MERS-CoV were originally epidemics responsible for a high mortality but very fortunately were rapidly controlled. Following these epidemics, major efforts have been made to characterise the immune response against these viruses, in parallel with the development of the first vaccine candidates.

1.5.1. Humoral immune response

As regards the humoral response against these viruses, the presence of neutralising antibody activity directed against the spike protein is noted, the protective function of which has been demonstrated in animals (passive antibody transfer and vaccination).

The concomitant development of antibodies and pulmonary signs in patients, as well as the positive correlation between antibody levels and infection severity, have prompted the hypothesis that antibodies may be involved in the pathophysiological mechanisms that cause lung damage. This ADE phenomenon, only suspected in humans initially, has been found in several vaccine studies conducted in mice and was demonstrated in a SARS-CoV-1 vaccine model in macaque monkeys. Vaccination with an MVA (Modified Vaccine Ankara) vaccine (see below) expressing the spike protein induced a neutralising antibody response in this model, but exacerbated the lung damage following viral challenge. This harmful antibody activity was linked to activation of macrophages *via* their receptor to the Fc fragment of immunoglobulins. This macrophage activation effect was also observed *in vitro* with the sera of subjects having presented severe forms of the disease. In other *in vitro* studies, a possible role of the quantity of neutralising antibodies in this harmful effect is observed, with this dose-dependent effect not having been observed *in vivo* in the model cited above. The specificity of the antibodies appears to be important in the development of this ADE effect. Since the antibodies with a harmful effect are directed against certain epitopes located outside the RBD, this led some authors to select only this part of the spike protein as the antigen structure for a vaccine.

Finally, although the persistence of neutralising antibodies following SARS-CoV-1 infection was long considered to be relatively short (2 to 3 years) - like the B memory response - very recent data have challenged this idea by demonstrating the presence of neutralising antibodies more than 10 years after infection.

1.5.2. Cellular immune response

Studies concerning the T cell response against these viruses indicate that it plays an important role in recovery and protection against infection. Several authors stress the importance of analysing the Th1/Th2 ratio in the CD4+ T cell response, with a predominantly Th1 response being associated with a better prognosis. Some authors have nonetheless suggested that it is the Th17 response and not

the Th2 response that is pathological. This clearly has an evident impact on the choice of potential vaccine adjuvants. For example, it has been demonstrated that the use of aluminium in animal models, known to polarise the CD4+ T response towards the Th2 pathway, was not associated with a less good response, supporting the hypothesis of a potentially detrimental role of the Th17 pathway. Finally, the persistence of the T cell response appears to be long (>10 years).

The results of several phase 1 trials on vaccines against these viruses have been reported with various vaccine candidates, particularly for MERS-CoV. These clinical trials reveal a good overall safety of the vaccines, a high seroconversion rate at the end of the vaccine schedules but with an effect that wanes over time and a low induction of neutralising antibodies (25 to 40% and less than 10% after 1 year), with the T cell response (evaluated by IFN-γ ELISPOT) appearing to be greater and to be maintained for longer. There are no phase 2/3 studies making it possible to guarantee the efficacy of these strategies and hence the transposability of the immunological results in clinical terms. These results are summarised in Table 6.

1.6. Immunity against SARS-CoV-2

Currently available data indicate that infection with SARS-CoV-2 is accompanied by a primarily IgA and IgG-type antibody response, with the IgM response appearing to be less significant. The seroconversion rate in symptomatic subjects is high, peaking on D14. In subjects with fewer symptoms, the antibody peak appears to be staggered, as is the case with SARS-CoV-1 infection. While the antibody response is directed against numerous proteins, the neutralising response appears to be mainly directed against the spike protein. A good correlation is found between the detection of antibodies by ELISA and a neutralising activity of these antibodies in all the studies in which this correlation was investigated. The specificity of the antibodies appears to be high and no cross-reactivity was found with common coronavirus infections, whereas this does exist in the context of past infection with SARS-CoV-1. Factors positively correlated with the seroconversion percentage and the antibody level are disease severity, age and male gender. No ADE-type effect has been demonstrated to date in SARS-CoV-2 infection. Finally, it should be noted that no induction of specific antibodies can be observed in patients with few or no symptoms, in whom a mucosal IgA response and/or a T cell response has nonetheless been demonstrated.

A CD4+ and CD8+ T cell response is found in subjects infected with SARS-CoV-2. It is more common than the antibody response, also being found in asymptomatic patients. Recent data indicate that this T cell response is broad and directed against all the virus proteins; the CD4+ T cell response is broader and more marked in severe forms, with the reverse being observed in moderate forms, in which the CD8+ T cell response is dominant. The protective CD4+ T cell response is of Th1 type, while a Th17 response is visibly harmful and associated with pulmonary symptoms of the infection. In contrast with what is observed with antibodies, a marked cross-reactivity is found between the specific CD4+ T cell response and, to a lesser extent the CD8+ T cell response against SARS-CoV-2 and against common coronaviruses. The impact of this cross-reactivity is not known, but if it were to prove to be effective and provide a certain degree if protection against the infection or against the severity of the disease, it would have a significant impact on the risk of resurgence of new epidemic outbreaks. This could also lead to consideration of the diversity of antigens to be included in a vaccine, which should not be limited to only those present in the spike protein.

With over 53 million cases of COVID-19 currently having been reported worldwide, the number of reported cases of reinfection remains anecdotal. These clearly documented reinfections, numbering around ten, because they involve different viruses in the two infectious episodes, were observed in relatively young, non-immunocompromised subjects. The absence of immunological studies coupled

with virological studies unfortunately means that it is impossible to know the reasons for these reinfections at present: absence of initial adaptive response, loss of this response or selection of viral variants resistant to this response. However, potential asymptomatic reinfections with strains similar or otherwise to those of the first infection have not been demonstrated, since they have not been studied.

1.7. Vaccine platforms against SARS-CoV-2

At the end of October 2020, according to the WHO list, almost 200 vaccine candidates for SARS-CoV-2 were under development, using eight different technological platforms: live attenuated vaccines and inactivated vaccines, protein subunit vaccines, viral genetic material-based vaccines (RNA and DNA), replicating or non-replicating viral vector-based vaccines, and virus-like particle (VLP) vaccines, with the DNA and RNA platforms never previously having led to vaccines being marketed for human use.

In the case of SARS-CoV-1, it has been shown that antibodies directed against the spike protein can neutralise the virus and prevent infection (see chapter). Hence, the immense majority of SARS-CoV-2 vaccines under development contain at least part of the spike protein, which may be limited to the S1 domain or to the RBD (see below).

Although more recent platforms have been widely used in SARS-CoV-2 vaccine development strategies, several teams have used traditional platforms: live attenuated vaccines (LAV), inactivated vaccines (IV) and protein vaccines (PV). These are vaccines for which the manufacturing methods do not differ greatly from those used for vaccines against other pathogens. Since they require BLS3 laboratories for their production, LAV are not at an advanced stage of clinical development. At present there are five IV-type vaccine candidates for SARS-CoV-2 in the clinical development phase. An aluminium-adjuvanted IV is currently being tested in a phase 3 trial. The different PVs vary depending on:

1. the type of cells on which they are produced (production using a eukaryotic system such as insect cells, yeast, or even on plants, or production using a prokaryotic system, such as *E. Coli*), with proteins produced on eukaryotic systems being the closest to those produced *in vivo*;
2. the structure of the protein used (spike protein or RBD of this protein only in order to minimise the risks of ADE) (see above). Virus-like particle (VLP) vaccines are also in development. The structure of these VLPs increases the immunogenicity of PVs. It should be noted that PVs require an adjuvant (see below).

The technology for these traditional vaccines is well known and their large-scale production in accordance with good manufacturing practice is relatively simple for PVs.

In the context of emerging and/or re-emerging diseases - as has been the case with the Ebola or Zika viruses, for example - it seemed preferable to have platforms that could be easily used in order to be able to rapidly develop vaccines capable of triggering humoral and cellular responses and not requiring high doses. Therefore, the vaccines are no longer based on the use of whole viruses or some of their proteins but on gene fragments encoding the proteins of interest. A differentiation can be made between two types: viral vectors and nucleic acid vaccines (DNA and RNA).

These vaccines are based on the use of nucleic acid sequences corresponding to the antigen structures liable to trigger a protective immune response. They have the advantage of:

1. being specific, like PVs, since they only use the part of viruses of immunogenic interest;
2. enabling all the post-translational protein modifications normally observed *in vivo*, as with protein vaccines produced on eukaryotic systems;
3. being potential universal platforms with a standardised production method;

4. theoretically requiring a smaller amount of vaccine, since the proteins are produced *in vivo* in sufficient quantities *via* activation of the cell production system.

For viral vectors, the construction mechanism is relatively simple. It involves using a virus with little pathogenicity (in particular an adenovirus) or that has been rendered non-pathogenic (MVA, VSV, measles virus) and incorporating into its genome the sequence encoding the protein of interest (spike protein in the case of SARS-CoV-2). Since these vaccines are viruses, they do not require adjuvants. With these vaccines, the response against the vector itself may pose a problem and result in a reduced response against the antigen of interest, this having been clearly demonstrated with adenoviral vectors but not with the other viral vectors. The viral vectors at the most advanced development stage use adenoviruses (Ad). These viruses – for which there are more than 90 serotypes - are genetically stable, can infect the dendritic cells, are relatively easy to modify and have a high level of thermal stability. These viruses are modified with a view to their use as vaccine platforms and are thus made non-replicating. Three adenoviruses are currently being used:

1. Ad5, which has the advantage of having been widely tested as a viral vector (Ebola virus, HIV vaccine, in particular); however, it is a common serotype in humans and hence the anti-SARS-CoV-2 immunological responses need to be interpreted on the basis of anti-Ad5 antibody levels;
2. Ad26: this platform has been used for an Ebola virus vaccine and is also being used in phase 2b/3 trials for an HIV vaccine and an RSV vaccine;
3. A chimpanzee adenovirus, ChAdOx1, for which far fewer clinical data are available in humans; apart from phase 1/2 results for a SARS-CoV-2 vaccine, only the findings of a phase 1 trial conducted with a MERS-CoV vaccine are available.

These three adenoviral platforms are currently being used for SARS-CoV-2 vaccines in phase 3 trials.

The first DNA vaccines were developed thirty years ago. DNA vaccines offer the advantage of being relatively simple to produce and of inducing a humoral and cellular response. They induce humoral and cellular responses but these are of low intensity, requiring the repetition of vaccine injections for optimum efficacy. They can be produced at low cost and are very stable, which are not insignificant advantages. While there are no human vaccines of this type with a marketing authorisation, veterinary vaccines using this technology are available.

RNA vaccines are without doubt the least advanced in terms of length of development but they have the advantage of an optimum safety profile (due to their translation in the cytosol of cells, they do not need to penetrate the cell's nucleus and the risk of their genetic material being incorporated into the genome of the host is eliminated) and of being, by their very nature, particularly potent inducers of danger signals within the host cell. There are two types of RNA vaccine in development: 1) small, non-amplifying mRNA molecules encoding the antigen of interest, and 2) larger self-amplifying mRNA molecules that encode a viral replicon of an alphavirus in addition to the antigen of interest. While these vaccines are still relatively little-studied in humans at present, promising results have been published in the context of the development of a SARS-CoV-2 vaccine and two developed candidates are currently in phase 3 trials.

Although these new platforms are interesting, experience of their use in humans nonetheless remains limited.

1.8. The adjuvants used with SARS-CoV-2 vaccine candidates

Unlike live attenuated vaccines, RNA vaccines and those based on the use of a viral vector, the other types of vaccines may require the addition of an adjuvant for optimum efficacy.

The adjuvants having been used during the development of vaccines against other coronaviruses pathogenic in humans are: aluminium, MF59, Montanide ISA51/CpG, Matrix-M™, Q21, AS01/AS03n, Delta inulin (Advax™)+CpG, Toll-like Receptor (TLR) ligands, rOv-ASP-1 and Protollin. Among the 42 SARS-CoV-2 vaccine candidates in clinical development, at least 11 of them contain adjuvants: aluminium, Matrix-M™, Advax™, MF59, AS04 and CpG 1018.

Adjuvants can have several functions, in particular the capacity to increase the immunogenicity of vaccines, and to modulate and target the immune response. The preferential induction of a Th1-type CD4+ T cell response, as well as the induction of a CD8+ T cell response appear to be useful to protect against or control coronavirus infections, and particularly SARS-CoV-2. Of the adjuvants listed above, it can be noted that aluminium and MF59 preferentially trigger a Th2 response, AS04 and CpG 1018 preferentially induce a Th1 response and Matrix-M™ and Advax™ induce balanced Th1/Th2 responses. It is important to note that aluminium has yielded some interesting results with SARS-CoV-1 vaccines despite its Th2 polarisation considered as being unfavourable; in addition, this adjuvant offers the advantage of a long history of use with a well-known safety profile, which is not the case for the others.

1.9. Mucosal immunity and vaccination

Mucosal immunity plays a fundamental role in infection control. However, the role of this immunity is predominantly a tolerogenic one against antigens in food in the digestive system. Antiviral humoral responses in the lungs are dominated by IgG, which are diffused from the blood, whereas locally produced secretory IgA fulfil this role in the upper airways. The main SARS-CoV-2 vaccine candidates are administered via the intramuscular route and cannot effectively induce the production of secretory IgA. Consequently, their effect will, in principle, more concern blocking of viral multiplication in the lungs (illness severity) than in the airways. This has been demonstrated in animal models.

The main problems posed by the development of a vaccine administered by the mucosal route are related to immunotolerance phenomena. The mucosal surfaces are continuously exposed to antigens, leading to the development of an antigen-tolerant micro-environment. Despite that, several vaccine candidates aimed at inducing a mucosal response have been developed against animal coronaviruses, MERS-CoV and SARS-CoV-1. Studies conducted with these vaccines in animals revealed the induction of a mucosal response and protection against infection where this was analysed, demonstrating the relevance of these strategies. While several “mucosal” SARS-CoV-2 vaccine candidates have demonstrated their efficacy in animal models, only two vaccines using a non-replicating viral vector (Ad5) are currently in phase 1 of development in humans.

1.10. Published phase 1 and/or 2 trial results

At present, phase 1 and/or 2 trial results have been published for 11 vaccine candidates. The main results are summarised in Table 1.

These are often interim results, with a short follow-up period, limiting their interpretation; phase 3 results will have to be conducted, including, in particular, subjects at risk of severe forms of Covid-19, in order to confirm the safety and immunogenicity data and enable the vaccine efficacy to be assessed.

As of 12/11/2020 according to the WHO list, 48 vaccine candidates were in the clinical development phase, including 11 candidates in phase 3, corresponding to 24 trials in progress.

For the vaccine candidates in phase 3 of development, four are non-replicating viral vectors (AstraZeneca and Janssen vaccine, Russian Gamaleya vaccine and Chinese Cansino vaccine), four

are inactivated vaccines (three Chinese vaccines and one Indian vaccine), two are RNA vaccines (Moderna and BioNTech/Pfizer vaccines) and the last one is a protein vaccine (Novavax vaccine).

At present, all the phase 3 trials being conducted concern vaccines that are administered by the intramuscular route and according to a two-dose schedule with variable intervals (0-14 days, 0-21 days or 0-28 days), except for the ChadOx1 vaccine candidate from AstraZeneca,which is administered as a single dose in three of the five trials currently under way, and the Ad5 nCov vaccine candidate from Cansino, which is administered as a single dose in the two trials being conducted.

Currently, phase 3 trials only include adult subjects. One trial with ChadOx1 conducted by Oxford University/AstraZeneca schedules the inclusion of children aged between 5 and 12 years and the BNT162 trial conducted by BioNTech/Pfizer schedules the inclusion of subjects from the age of 16 years.

Concerning subjects over the age of 55, the majority of trials do not indicate an upper age limit for enrolments. Specifically, five trials indicate that they recruit subjects over the age of 60 years and, conversely, four trials do not include subjects aged over 55 or 60 years.

Table 1: Vaccine candidates for which phase 1 and/or 2 trial results have been published to date

Vaccine candidate	Sponsor	N injections (Ni) N Volunteers (Nv)	Safety(*)	Immunogenicity	Stability	Reference
Ad5 nCoV	Cansino Biologics	Ni = 1 (3 doses ≠) Nv = 108	Fever, local pain SAEs: fever+	ELISA D28: 100% (HD)	?	Zhu *et al.*, 2020 (1) Ramasamy *et al.*, 2020 (2)
AZD1222 (ChadOx1)	Oxford University Astra-Zeneca	Ni = 1 (n=2 for 10) Nv = 543	Fatigue, local pain SAEs: fever, headaches	ELISA peak D28 Nab D28: 100% (n=35) ELISPOT peak D28	?	Folegatti *et al.*, 2020 (3)
CoronaVac	Sinovac	Ni =2 (D0, D21) Nv = 243	Fever, local pain No SAEs	ELISA D35 100% Nab D35: 97%	?	Xia *et al.*, 2020 (4)
mRNA-1273	Moderna	Ni = 2 (D0, D28) (3 doses ≠) Nv = 45	Fatigue, fever, local pain SAEs: chills, fatigue	ELISA D57 = Convalescent Nab D57 = Convalescent T cell Th1 profile	-20°C	Jackson *et al.*, 2020 (5) Anderson *et al.*, 2020 (6)
BNT162 mRNA (RBD only)	Pfizer, BioNTech	Ni = 2 (D0, D21) (3 doses ≠) Nv = 36	Fatigue, local pain, SAEs: fever	ELISA D35 = Convalescent Nab D35 = Convalescent	-80°C Then 2-8°C if repackaged	Mulligan *et al.*, 2020 (7)
NVX-CoV2373 (protein+ adj)	Novavax	Ni =2 (D0, D21) Nv = 108 (83 vaccine+adj, 25 vaccine)	Benin SAEs: headaches, asthenia	ELISA D35 V+Ad>V> Convalescent Nab D35 V+Ad>V> Convalescent T cell Th1 profile	-80°C for years 1 month at 2-8°C	Keech *et al.*, 2020 (8)

Ad26	Janssen	Ni =2 (D0, D56) (2 doses ≠) Nv = 1045	Local pain, fever, headaches, fatigue, myalgia SAE: malaise, fever, dizziness	ELISA D28: 99% Nab D: 92% (18-55 years) to 100% (≥65 years) T cell Th1 profile, Th1/Th2 ratio = 1 to 68.5	Distribution at 2-8°C	Sadoff *et al.*, 2020 (9)
Virus Sputnik V (rAd26-S+rAd5-2)	Gamaleya	Ni = 1 Nv = 36	Fever, Headaches No SAEs	ELISA D21: 100% Nab D42: 100% (for rAd26-S+rAd5-2 only); = convalescent serum T cell Th1 profile?	?	Logunov *et al.*, 2020 (10)
Inactivated V.	Wuhan	Ni = 3 (D0, D28, D56) Nv = 96	Pain, fever No SAEs	ELISA peak D42 / ELISA D42: 100% Nab peak D70 / Nab: 97.6% (medium dose, phase2) T cell Th profile: absence of observed cell response	?	Xia *et al.*, 2020 (4)
BBIBP-CorV Inactivated V.	Beijing Institute of Biological Products, Sinopharm	Ni = 1 or 2 (D0, D14 or D0, 28) Nv = 192 (phase1) Nv = 448 (phase2)	Fever, fatigue No SAEs	ELISA D28: 100% (18-59 years) / 92% (>=60 years) for the dose of 4µg Nab peak D42 (phase1): Nab D42: 100%	?	Xia *et al.*, 2020 (11)
VLP vaccine	Medicago	Ni = 2 (D0, D21) Nv = 180	Pain, headaches, fatigue SAE: fatigue (AS03+)	ELISA D42: 99.1% Nab peak D42 Nab D42: 91.3% (PRNT)	?	Ward *et al.*, 2020 (12)

				T cell Th1/Th2 profile		

(*) SAE: serious adverse event from grade 3, without taking into account causality

Find all our publications at
www.has-sante.fr

HAS
HAUTE AUTORITÉ DE SANTÉ

Advancing quality in the fields of
health and social care services