Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review

COVID-19 vaccines: Frequently asked questions and updated answers

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ABSTRACT

At the end of December 2019, China notified the World Health Organization about a viral pneumonia epidemic soon to be named COVID-19, of which the infectious agent, SARS-CoV-2, was rapidly identified. Less than one year later, published phase 3 clinical trials underlined the effectiveness of vaccines utilizing hitherto unusual technology consisting in injection of the messenger RNA (m-RNA) of a viral protein. In the meantime, numerous clinical trials had failed to identify a maximally effective antiviral treatment, and mass vaccination came to be considered as the strategy most likely to put an end to the pandemic. The objective of this text is to address and hopefully answer the questions being put forward by healthcare professionals on the different anti-SARS-CoV-2 vaccines as regards their development, their modes of action, their effectiveness, their limits, and their utilization in different situations; we are proposing a report on both today's state of knowledge, and the 14 February 2021 recommendations of the French health authorities.

1. Introduction

Whereas the COVID-19 pandemic has occasioned over 100 million cases and more than 2.3 million deaths worldwide, the published results of pivotal trials of the first COVID-19 candidate vaccines have represented a source of genuine hope for the international community. Numerous countries have rapidly initiated a COVID-19 vaccination campaign; as of 12 February 2021, more than 150 million doses had been administered throughout the world (https://ourworldindata.org/covid-vaccinations). Numerous questions have been raised in France, not only by public health decision-makers, but also and especially by caregivers and practitioners in charge of informing the population, of defining and identifying prioritized individuals, and of setting up a nationwide vaccination campaign. Given the existing demand for simple and objective elucidation of the available data, the French Infectious Diseases Society (SPILF) was asked to draw up an informative summary document to be addressed to healthcare professionals.

2. Methodology

A working group proceeding under the supervision of the SPILF Vaccination-Prevention group identified the questions most frequently put forward by healthcare professionals. As regards each question, the literature was analyzed in view of providing a response based on the most recent data, while remaining within the limits of the knowledge amassed at the date of writing, and taking into full account the volume of continuing uncertainties. Several experts in vaccinology, infectious diseases and/or immunology were contacted and asked to reread and/or to participate in the drafting of responses. Given:

- the fact that questions are numerous;
- the plethoric and rapidly evolving nature of available data;

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- stakeholders' expressed need for immediately enlightening information, a methodology premised on systematic review of the literature was not applied.

The present document may consequently be viewed as expert opinion based on the elements at our disposal at a given point in time.

3. Generalities

3.1. What is the antigen targeted by Coronavirus disease 2019 (COVID-19) vaccines?

The majority of the vaccines being developed target the spike (S) protein of the virus, which is located at the surface of the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) envelope, enabling the latter to be bound to a cell receptor, the angiotensin-converting enzyme 2 (ACE-2, which is present in pneumocytes, enterocytes. . .) and enter into host cells; its contribution to infection is consequently central. Different studies have shown that were neutralizing antibodies to be triggered against the S protein, protection from infection would be afforded [1,2]; that is why spike protein represents the target of most of the vaccines developed in 2020.

3.2. What are the different types of COVID-19 vaccines?

Different vaccinal technologies, also known as platforms, are currently being applied; they can be divided into two categories [3,4].

3.2.1. Vaccines based on the whole virus

They may consist in a whole virus (in this case, SARS-CoV-2), inactivated by beta-propiolactone (example: the vaccines developed by Sinovac [Coronavac] and Sinopharm [Chinese-WIBP-Vero-Inactivated-Covid], by Valneva [VLA 2001], and by Bharat Biotech [Covaxin, BBV152]) or in a live but attenuated virus (example: the vaccine developed by Codegenix/serum institute of India [COVI-VAC]).

3.2.2. Vaccines based on a viral protein (here, the S protein) or on part of the protein

They comprise protein or virus-like particle vaccines (molecular S-protein aggregates), nucleic acid vaccines and viral vector vaccines.

Some of them are based on a non-modified protein in whole or in part, for example the viral vector vaccines developed by the University of Oxford-AstraZeneca [AZD1222, ChAdOx1-nCoV-19] and by the Gamaleya Research Institute [Gam-COVID-Vac, known as Sputnik V], the messenger RNA (m-RNA) vaccine developed by CureVac-GSK [CVnCoV] and the protein vaccines elaborated by COVAXX [UB-612], by Medicago [CoVLP], by Clover Biopharmaceuticals/GSK/Dynavax and by Sanofi Pasteur-GSK. While MSD drew up two replication-competent viral vector vaccines based on the measles virus and the vesicular stomatitis virus, their immunogenicity was deemed insufficient, as a result of which, their clinical development was suspended in late January.

The other types of vaccines are based on the modified protein in its prefusion form, for example the m-RNA vaccines developed by Moderna [Moderna COVID-19 Vaccine®, mRNA-1273] and by Pfizer-BioNTech [Comirnaty®, BNT162b2], the viral vector vaccine developed by Janssen Vaccines & Prevention (Johnson & Johnson) [Ad26.COV2.S] and the protein vaccine developed by Novavax [NVX-CoV2373].

A progress report on the preclinical and clinical development of the different candidate vaccines is updated weekly on the World Health Organization (WHO) website [5].

3.3. Do the vaccines contain adjuvants?

If live vaccines, RNA vaccines and viral vector vaccines do not contain adjuvants, this is due to the fact that by their very nature, they can satisfactorily stimulate the innate immune system. On the contrary, inactivated vaccines and protein vaccines necessitate adjuvants. Some of the COVID-19 vaccines now being developed contain aluminum or a number of other currently or soon-to-be commercialized adjuvants, orienting helper T lymphocytes toward TH1 polarization.

3.4. Why did one year suffice for COVID-19 vaccines to be developed and receive conditional market authorization?

3.4.1. Because the causative agent was rapidly characterized, and was found to be relatively stable

On 9 January 2020, the Chinese health authorities and the WHO announced the discovery of new coronavirus, which was promptly termed 2019-nCoV and presented as the agent responsible for the pneumonia cases of which the WHO had been apprised by China on 31 December 2019. As early as 10 January 2020, the complete viral sequence was rendered public. Even though it is indeed an RNA virus, SARS-CoV-2 is more stable than, for example, the influenza or the HIV virus. That is why the vaccines developed from viral sequences isolated in January 2020 were still valid in December 2020.

3.4.2. Because knowledge on coronavirus immunity was already present

Coronavirus immunity had been widely studied on the occasion of the alerts that occurred in 2002–2003 (emergence of SARS-CoV in China) and 2012 (emergence of MERS-CoV in Saudi Arabia). Animal models had been developed and phase 1 clinical trials of a DNA vaccine encoding the S protein of these two coronaviruses had highlighted the presence of neutralizing antibodies in vaccinated volunteers [6,7]. It was shown that the triggering of a response against S protein or the injection of neutralizing antibodies provided protection against the infection [1,2].

Using the published sequence of the SARS-CoV-2 genome, in a few days it was possible on the basis of DNA synthesis to produce m-RNA corresponding to S protein stabilized in a prefusion conformation by two proline residues at the site of the cleavage between subunits S1 and S2, in a manner particularly propitious to the induction of neutralizing antibodies. The abbreviated duration thereby obtained is in no way comparable to the time lapse needed for the protein proteins or the virus culture required in the context of classical vaccinal platforms.

By chance, rapidly conducted animal trials confirmed how simple it was to trigger an effective immune response against SARS-CoV-2.

3.4.3. Because previous, highly advanced research rendered possible the use of innovative vaccinal platforms

Well before the COVID-19 pandemic, nucleic acid and viral vector vaccine platforms had been widely utilized in studies with

animal models and in phase 1 and phase 2 clinical trials pertaining not only to the Zika virus, the rabies virus and the HIV virus, but also to the SARS-CoV and the MERS-CoV coronaviruses [2,8,9]. Years of work effectively contributed to elaboration of a SARS-CoV-2 vaccine. One key reason why the above-mentioned platforms were identified as being of the utmost interest for development of a vaccine against an emerging infectious disease is that they paved the way for expeditious elaboration of a vaccine candidate following identification of the causative infectious agent [10].

3.4.4. Because an unprecedented scientific and financial deployment has occurred

The COVID-19 pandemic occasioned a hitherto unequaled and exceptionally concerted response; the mobilization of states and research teams alike and the lightning-like creation of public-private partnerships facilitated and accelerated the development, the manufacture (in anticipation of industrial production) and the subsequent distribution of vaccines. The inter-institutional American "*Operation Warp Speed*" propelled the financing of several companies in their quest to develop vaccines based on novel technologies. Another telling example is that of "Covax", the vaccinal component of a worldwide collaborative effort driven by *Coalition for Epidemic Preparedness Innovations* (CEPI), *Global Alliance for Vaccine and Immunization* (Gavi) and the WHO, the common objective being to accelerate the development and production of diagnostic tools, treatments and COVID-19 vaccines and to ensure equitable access to all of the above.

3.4.5. Because clinical trials were conducted in record time

In the exceptional context of the pandemic, the different vaccine development stages designed to assess safety and effectiveness have swiftly dovetailed, at times overlapping, in keeping with the strictures of the regulatory health authorities [11]. Volunteers were rapidly recruited and the quantitative objectives regarding cases of COVID-19 were quickly attained. Without undue haste, the processes of data collection, analysis and verification were appreciably expedited. While the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) instituted procedures designed to fast forward the processes, there was no derogation from scientific rigor. For example, a "rolling review" consists in analyzing the data provided by laboratories every two weeks, thereby substantially shortening the overall duration of the process. As under other circumstances, only following conclusive demonstration of its quality, safety and effectiveness has a vaccine been approved by the competent independent authorities.

3.5. Why were the clinical trials of some vaccines suspended?

Whenever doubt arises on an eventual adverse effect of a given vaccine, trials are suspended. In different cases, an independent committee analyzes the relevant data and authorizes trial resumption in the absence of proof that the disease is in any way associated with the candidate vaccine [12]. So, it was that on 6 September 2020, trials of the adenovirus-based AZD1222 vaccine developed by AstraZeneca and the University of Oxford were suspended following the appearance of myelitis in one of the participants from the United Kingdom. Only after:

an independent committee of neurologists had concluded that the myelitis was idiopathic and
equally independent regulatory agencies had given their approval were the trials allowed to resume.

Another example: on 12 October 2020, due to the occurrence of an unexplained illness in one of its trial participants, the Janssen group suspended the development of its candidate vaccine AD26.COV2-S. Subsequent to evaluation of the relevant safety data by an independent supervisory committee, resumption of the trials was recommended.

3.6. What is the duration of monitoring necessary to establish the safety of a vaccine?

Up until recently, accumulated vaccinology experience showed that the side effects of vaccines occurred a few days (at most six weeks) after vaccination. And up until now, the responsibility of a vaccine in the onset of an autoimmune disease has yet to be demonstrated. One exception consists in the cases of narcolepsy reported after the administration of certain vaccines in the 2009 swine flu (H1N1) epidemic, particularly those using the ASO3 additive; while exceedingly rare, these cases were detected and identified by pharmacovigilance systems, the first signs having appeared a median of 3 months after the injection of vaccine [13]. In view of detecting the occurrence of this type of adverse event, the duration of monitoring in clinical trials exceeds six months [14].

The commercialization of COVID-19 vaccines raises the question of vaccination of the volunteers included in placebo groups before the end of a trial. Once a crucial moment has passed, it becomes impossible to compare the vaccine to a placebo so as to determine not only its effectiveness, but also any delayed side effects [15]. After all, there exists a theoretical risk that exceedingly rare adverse effects (frequency < 1/10,000) that remained unobserved during clinical trials may occur during a vaccination campaign. The French National Agency for Medicines and Health Products Safety (ANSM) has set up a twofold monitoring system:

- pharmacovigilance consisting in independent medical analysis of declarations as well as regular reports;
- pharmaco-epidemiology consisting in analysis of the data of the nationwide health data system (SNDS).

3.7. Can vaccines increase the risk of a severe form of COVID-19?

In certain cases, preexisting immunity of natural or vaccinal origin may favor severe forms of a given infection, either:

- because preexisting antibodies facilitate the infection of immune cells, particularly macrophages (the facilitating antibody phenomenon) or;
- because the vaccine-induced immune response proves conducive to a deleterious inflammatory reaction [16].

One example of the "facilitating antibody" phenomenon is to be found in dengue fever; previous infection by one of the four dengue serotypes increases the risk of severe dengue fever in the event of later infection by one of the other three serotypes [16]. The same risk has been reported with regard to vaccination against dengue fever [17], vaccinated individuals never having been infected by one of the four serotypes of the dengue virus are indeed at increased risk of a severe form of dengue fever; on the other hand, this risk has not been reported in vaccinated individuals with previous incidence of the former.

The phenomenon of deleterious macrophage polarization during inflammatory response has been observed with a viral vector vaccine based on a poxvirus, the Modified Vaccinia Ankara (MVA),

in a nonhuman primate model for SARS-CoV infection [18], and with other SARS-CoV vaccines in mouse models. In actuality, the deleterious antibodies were anti-spike antibodies oriented outside the receptor binding domain (RBD), a fact impelling some research teams to limit themselves to this domain in their development of a vaccine. As regards SARS-CoV-2, on the other hand, no *in vitro* or *in vivo* data in animal viral infection models or in clinical trials for vaccines has suggested the existence of this risk. That said, in the development of SARS-CoV-2 vaccines, special attention has been paid to this eventual risk. The number of participants included in studies having led to market authorization for the Pfizer-BioNTech, Moderna, AstraZeneca and Sputnik V vaccines tends to demonstrate that the above-mentioned adverse effect is not present during SARS-CoV-2 infection. As is the case with other hypothetically possible effects, it is monitored in real-life pharmacovigilance (phase 4).

3.8. Is there any danger in vaccinating someone who has previously had COVID-19 or been asymptomatically infected by SARS-CoV-2?

In phase 3 trials for the Pfizer-BioNTech, Moderna and AstraZeneca vaccines, previous COVID-19 incidence was an exclusion criterium. On the contrary, a positive PCR test result for SARS-CoV-2 in nasopharyngeal swab or positive serology on screening did not occasion exclusion. So it was that over a thousand volunteers who were shown to have had previous SARS-CoV-2 infection received at least one vaccine dose during the trials. Within the limits of this volunteer population, there was no signal of poor tolerance or occurrence of severe COVID-19. While the initial pharmacovigilance data have shown no serious incident, heightened reactogenicity has occasionally been reported [19]. The interest of (and indications for) COVID-19 vaccinations in persons recently exposed to or previously infected with SARS-CoV-2 will be considered later.

3.9. Once a certain threshold of vaccine coverage has been attained, will circulation of the disease be limited?

During the initial phases of the vaccination campaign, the objective will be to protect persons at risk of complications and, *a fortiori*, to avoid deaths and hospitalizations [20,21]. Prior to the emergence of "variants" of the virus, it appeared that herd immunity could be reached once 60% of the population would be immunized (according to the 1/R0-1 equation) [22]. The emergence of more transmissible variants (higher R0) is liable to increase the proportion of immunized persons necessary to effectively achieve "group effect", which would indirectly protect individuals who could not be vaccinated, and possibly bring the epidemic to a halt. The group effect would also bring into play the capacity of vaccines not only to prevent the disease, but also to circumvent asymptomatic infection; at this stage, the data pertaining to this outcome of vaccination have yet to be consolidated [23,24] (cf. the following question).

3.10. Do mutations of SARS-CoV-2 have an impact on vaccine effectiveness?

This was not the case with most of the mutations identified during the first year of the pandemic; since then, it has definitely become a justified fear.

Sequencing and continuous monitoring of circulating viral strains have been put into place on a worldwide scale, enabling researchers to follow the appearance of mutations in viral genomes and to detect the emergence of variants of concern whose mutations present epidemiological peculiarities.

The variant detected in December 2020 in the United Kingdom (variant B.1.1.7, or 20I/501Y.V1) possesses a high number of mutations in comparison with the strain of reference, including a mutation modifying an amino acid in the receptor binding domain (RBD) of the S protein (mutation N501Y) [25]. The variant detected in the Republic of South Africa (variant B.1.351, or 20H/501Y.V2) and the variant detected in Japan during screening of persons returning from Manaus (Brazil) and subsequently identified as circulating in northern Brazil (variant P1, or 20J/501Y.V3) contain the same mutation (N501Y) and two other mutations in the RBD, (K417N/T and E484K) [26–28].

Pfizer-BioNTech et Moderna have announced that when compared to a virus of reference, serum from volunteers vaccinated with their respective vaccines possessed equivalent neutralizing activity with regard to a pseudovirus containing the mutations of variant 20I/501Y.V1 [29,30], while other studies have shown that different mutations, including E484, significantly reduce recognition of the S protein RBD by convalescent serum antibodies [31]. The Moderna laboratory has announced that in comparison with the reference strain, the serum neutralizing titers in volunteers vaccinated with their vaccine were six times lower with regard to variant B.1.351; that said, their titers remained superior to the levels considered as protective [30]. Wang et al. tested on different strains the neutralizing activity of:

- 20 convalescent plasmas;
- the serums of 22 persons having received two doses of the Pfizer-BioNTech or Moderna vaccines;
- monoclonal antibodies, including two from the Regeneron® laboratory [32]; they observed a diminution of the neutralizing activity of the antibodies with regard to the viruses presenting mutation E484K (diminution of six to eight times compared to the other variants).

As for mutation N501Y, which manifested a more pronounced affinity to the ACE2 receptor, it was not associated with reduced neutralizing activity of the natural antibodies. Lastly, the phase 2/3 studies by Novavax and Johnson & Johnson/Janssen, which were partially conducted in South Africa and Brazil (the variants were already present), showed a significant but relatively limited loss of clinical effectiveness of the vaccines against the variants. More specifically, as concerns its NVX-CoV2373 vaccine, in a phase 3 trial involving 15,000 participants in the United Kingdom in which the 20I/501Y.V1 variant represented more than 50% of the individualized strains, Novavax reported 89% effectiveness (95% CI: 75–95); in a phase 2b trial in South Africa (https://ir.novavax.com/node/15506/pdf), effectiveness came to 60% (95% CI: 20–80). Taken together, these data suggest that previous infections fails to afford complete protection from symptomatic reinfection due to the variant; one third of the persons included displayed positive SARS-CoV-2 serology. As for the Johnson & Johnson/Janssen laboratory, as regards the Ad.26.COV2.S vaccine they reported overall effectiveness of 66% (85% against the severe/critical forms); the proportion reached 66% in Latin America and 57% in South Africa (https://www.nih.gov/news-events/news-releases/janssen-investigational-covid-19-vaccine-interim-analysis-phase-3-clinical-data-released).

Given that the technology of mRNA vaccines is compatible with rapid development, new versions incorporating at least one sequence of variants could be elaborated in eight weeks and be awarded market authorization as a pharmaceutical variation; it bears mentioning that the composition of the lipid nanoparticles essential to intracellular vectorization of the mRNA would remain the same.

3.11. Will yearly vaccination be necessary, as is the case with the flu?

It seems unlikely that the different distancing and vaccination measures will totally eliminate the circulation of SARS-CoV-2; on the other hand, acquisition of long-term immunity in the highest possible proportion of the population is required, the realistic objective being to render the circulation as non-toxic as feasible. The duration of post-vaccinal (or post-infectious) protection and the emergence of immune-escape mutants are to be taken into account:

- the duration of post-vaccinal or post-infectious immunity is not presently known; while the available data lead us to believe that it may exceed six months, several factors come into play; according to the persistence time of neutralizing antibodies and the kinetics of the development of antibody titers during new exposure via memory B cells, booster doses may be necessary, at a cadence that remains to be defined;
- it also bears mentioning that of the virus acquires mutations through which it will no longer be recognized by the post-vaccinal or post-infectious immune response, and that if the epidemic has not been mastered, it may become necessary to organize vaccination on a regular basis, using a vaccine adapted to possible immune escape variants (cf. preceding question).

3.12. Can vaccination be used in post-exposure or during a documented SARS-CoV-2 infection?

Given the incubation period of COVID-19 (a median of 5 days) and the lapse of time between a 1st vaccinal dose and the protection afforded by RNA vaccines or the AstraZeneca vaccine (2 to 3 weeks) [23,24,33], vaccination is highly unlikely to represent an effective disease prevention strategy subsequent to documented exposure.

3.12.1. In the context of documented individual exposure

The French Health Authority (HAS) recommends that persons with documented SARS-CoV-2 exposure not be vaccinated before receiving screening results and/or prior to the end of a possible two-week isolation period [34]. As regards screened positive contact cases, with or without symptoms and with or without serology showing systemic immune response, the HAS recommends that vaccination preferably take place six months (not less than three months) after diagnosis of the infection, and that it consist in a single dose if immunocompetent (cf. question 6.11) [34]. If an infection occurs after the 1st dose of the vaccine and before the second dose has had the time to be administered, the 2nd dose will be given only three to six months after diagnosis of the infection [34].

3.12.2. In the context of collective exposure

For Ehpad or USLD residents, who evolve in environments (medicalized retirement homes) where SARS-CoV-2 can freely circulate for long periods of time, a vaccination campaign must not be delayed or postponed insofar as it could be preventive among the contact cases of contact cases (2nd generation of cases) [35]. Vaccination should be avoided only in the event of patent exposure (a roommate, for example), while awaiting the result of screening by PCR or antigen test. For example, when screening is carried out in an entire unit or establishment due to the fact that COVID-19 cases have been diagnosed or because the screening coincides with a period of planned vaccination, persons who are not contact cases and who present no signs of COVID-19 may be vaccinated. As regards screened and positive contact cases with or without symptoms and with or without serology showing systemic immune response, the HAS recommends that vaccination be postponed for three to six months (cf. question 6.11) [34]. If an infection is diagnosed after the 1st dose of a vaccine, the 2nd dose will be postponed for three to six months [34].

4. The functioning of nucleic acid vaccines (RNA, DNA)

4.1. What is a nucleic acid vaccine?

The main component of nucleic acid vaccines is desoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The DNA or RNA molecule provides coding for a viral protein that will be produced by our cells. The vaccinal DNA or RNA sequence is synthesized in a laboratory before being produced on an industrial scale. The protein of which it constitutes the genetic sequence is chosen because the immune response triggered against that protein affords protection from infection. For the SARS-CoV-2 vaccine, this is the spike (S) protein.

4.2. How do RNA vaccines function? [8,36]

In humans, genetic information is encoded by DNA, with its 46 chromosomes contained in the nucleus of each of our cells. DNA is transcribed into messenger RNA (mRNA), which leaves the nucleus and moves toward the cytoplasm, where it is translated into protein by the ribosome.

During intramuscular injection, vaccinal RNA enters into the muscle cells, namely the myocytes [8,37], and then the dendritic cells, which are also present in the draining lymph nodes [38]. The messenger RNAs are internalized in the cells via the endosomes before being released by fusion between the lipid nanoparticles and the lipids of the internal side of the endosome membrane [39]. On the one hand, the RNAs stimulate innate immunity, acting as an adjuvant [40]; on the other hand, they are translated in the cytoplasm into protein S. In the dendritic cells and the macrophages, protein S is presented to the T lymphocytes in one of two manners:

- by translation of the vaccinal mRNA into protein S (in parallel to stimulation of the innate immune system) if the cells have internalized the RNA;
- by phagocytosis of the cells expressing protein S.

The naive B lymphocytes capable of recognizing this protein are activated. These interactions contribute to the production of neutralizing antibodies by the B lymphocytes and to the generation of memory B lymphocytes, and also to the generation of a cytotoxic T lymphocyte response and of memory T lymphocytes. In the event of a subsequent encounter with the coronavirus, these memory cells will be capable of detecting and rapidly combatting the virus by means of a humoral response and destruction of the cells infected by SARS-CoV-2.

4.3. Can RNA vaccines modify our genes?

The high molecular weight of the RNA molecules prevents them from being freely disseminated through nuclear pores. During protein synthesis, information circulates in the DNA→RNA→protein direction by means of the nuclear export signals (NES) present on the messenger RNA molecules, the objective of these sequences being to cross the nuclear pore complex. In order to enter the nucleus, the vaccinal RNA molecules need to possess a nuclear localization sequence (NLS) [41]. Since the expression of the antigen of vaccinal interest is to be found in the cytoplasm, the mRNAs utilized in vaccines do not possess this NLS sequence.

4.4. Can viral RNA be transcribed by endogenous retroviruses?

With their reverse transcriptase enzyme, retroviruses are RNA viruses capable of "reverse transcription" (DNA from an RNA template). For example, HIV can reverse transcribe (RNA to DNA) and then be integrated in the genome by means of another viral enzyme, retroviral integrase.

Our genome contains remnants of ancient viruses that infected our long-gone ancestors, hundreds of thousands of years ago; they are known as endogenous retroviruses. Their sequences are often considered as fossils; they can generate neither reverse transcriptase nor an integrase [42]. It appears highly unlikely that vaccinal RNA could generate DNA sequences that would subsequently be incorporated into the genome of the host cell.

4.5. And DNA vaccines: can they modify our genes?

In DNA vaccines, nucleic acid is present in a plasmid form, which cannot be incorporated into chromosomal DNA. Moreover, the vaccine does not contain the enzyme (integrase) that would enable its integration. While other DNA delivery systems have been used with this objective in mind, they employ different technologies, and have little or nothing to do with vaccinal DNA.

4.6. Why are "nanoparticles" used to carry vaccinal RNAs?

As for vaccinal RNA, it cannot be injected in a "naked" form, which before being able to enter a cell would be immediately and considerably degraded. In order to avoid the phenomenon of degradation and so as to favor cellular internalization, mRNA molecules are associated with a mixture of four different lipids. One of the lipids is positively charged, the objective being to complex negatively charged mRNA molecules [43]. The three other lipids are first cholesterol, then a lipid possessing 18-carbon chains (comparable to cell membrane lipids), and lastly a lipid conjugated to a polyethylene glycol (PEG) chain. The lipid particles/mRNA have a diameter approximating 100 nm and are colloidally stable due to the effects of steric repulsion between the PEG chains. Once they have been injected, the nanoparticles fuse with the cell membranes and release RNA, which can then be translated into antigen proteins, from which the adaptive immune response originates.

4.7. Can the polyethylene glycol (PEG) contained in the lipid nanoparticles of RNA vaccines be toxic?

PEGs constitute a class of compounds of which the molecular weight ranges from 200 to more than 10,000 Da. After oral or intravenous exposure, the PEGs are excreted, mainly in changed form, in feces and urine [44]. PEGs with high molecular weight are often used in the medical industry due to their hydrosoluble and liposoluble properties. In daily life, they combat constipation (macrogol) by oral route. When conjugated to the active ingredients of drugs administered by parenteral route, the PEGs increases the half-life of these medications, which are known as "pegylated" interferons, in the treatment of hepatitis, of certain cancers and multiple sclerosis as well as hematopoietic growth or coagulation factors, anticancer chemotherapies, pegylated naloxone, pegylated certolizumab, etc.:

- the theoretical risk of release of ethylene glycol following PEG administration has not been shown to exist; in studies involving repeated doses and elevated levels of exposure, the toxicity typical of ethylene glycol has not been found;
- while allergic reactions to PEG are exceedingly rare, they do occur. Given their experience regarding other medicinal products, registration agencies consider PEGs and lipid nanoparticles to be satisfactorily tolerated. In mRNA vaccines, the PEGS with molecular masses of 2000 Da have been shown to form long threads at the exterior of the nanoparticles, which as a result do not aggregate [45]. The anaphylactic reactions observed in mRNA vaccines are quite probably associated with preexisting anti-PEG antibodies [46].

4.8. How can we place into perspective the technology applied in nucleic acid vaccines?

Up until quite recently, no duly registered RNA vaccine existed, even though researchers have been working on the subject for over 20 years [47]. The technology has continuously improved, thereby ensuring increased safety. RNA vaccines have been tested in humans against the Zika virus, the flu, rabies and the cytomegalovirus (CMV), with approximately 600 participants included in the relevant trials. As for SARS-CoV-2 RNA vaccines, they have been assessed in phase 3 trials on several tens of thousands of persons, been granted market authorization, and been administered to several million individuals since the commencement of vaccination campaigns in December 2020 (https://ourworldindata.org/covid-vaccinations).

This is likewise the case with DNA vaccines, which have shown promising results in animals, and for which clinical trials were already underway prior to the development of SARS-CoV-2 DNA vaccines. What is more, four DNA vaccines have received the regulatory approvals necessary to their commercial utilization, the objective being to protect several animal species from viral diseases and to treat oral malignant melanomas in dogs [48].

4.9. What are the advantages of nucleic acid vaccines?

They can be readily and rapidly produced. They are synthesized in a laboratory, and it is not necessary to manipulate the virus. Moreover, once the vaccine has been injected, the protein of interest is naturally produced by our cells; it takes on the aspect and conformation that it usually has in the virus and induces a specific and complete immune response (antibodies and lymphocyte T). This represents an appreciable advantage over inactivated or subunit vaccines, for which the immune response is incomplete (antibodies alone).

4.10. What are the efficacy and immunogenicity data on the Pfizer-BioNTech and Moderna vaccines derived from animal models of SARS-CoV-2 infection and phase 1 and 2 clinical trials?

In an animal model (rhesus macaques) of SARS-CoV-2 infection, injection of two doses of 30 or 100 μg of RNA from Pfizer-BioNTech vaccine provided protection from subsequent experimental infec-

tion [49]. In the phase 1 and 2 clinical trials, the neutralizing antibody titers obtained after two vaccine doses were comparable or superior to those found in convalescent plasma. While they were generally higher on 18-to-55-year-olds than in 65-to-85-year-olds, the responses were comparable to the levels observed in convalescing patients in the two age groups. A dose of 30 μg of mRNA was chosen for the phase 2 and 3 trials [50].

With the Moderna vaccine, injection of two doses of 10 or 100 μg of RNA rendered SARS-CoV-2 indetectable at the pulmonary level in Rhesus macaques that were vaccinated before being infected by nasal and tracheal route [51]. That said, only the 100 μg dose enabled sterilization of all the nasal and pulmonary samples. In the phase 1 trials, satisfactory levels of neutralizing antibodies similar to those found in convalescents were observed after two doses [52]. Antibody levels were higher following the more elevated dose (100 μg), and a significant increase was observed following the second dose; a similar response has been observed in more elderly persons [53]. Lastly, persistence of the antibodies at a significant level was demonstrated for up to 119 days after the 1st dose among the participants in these early phases, and it was higher than the level observed among convalescents [54].

5. The functioning of viral vector vaccines

5.1. How do viral vector-based vaccines function?

For thirty years, the relevant technology has been widely explored in two fields: anticancer and anti-infective vaccination. In this type of vaccine, a non-pathogenic virus triggers an immune response against the disease of interest [9]. The virus utilized is called a “viral vector”; in other words, the viruses are attenuated or naturally non-pathogenic in humans; they cannot be replicated. Their genome has been modified through insertion of a DNA or RNA sequence of the protein of interest, in this case the S protein of SARS-CoV-2. Once the viral vector has been injected, it infects the host cells, and delivers to them its DNA or RNA; the cell machinery thenceforth expresses the vaccinal protein, which is taken up by the antigen-presenting cells permitting activation of the T and B lymphocytes. What is more, the viral nature of the vector allows for development of an activation signal addressed to the immune system, and consequently to the establishment of an immune response of interest. The vaccines can be divided into two categories.

5.1.1. Non-replicating viral vectors

Human and nonhuman adenoviruses are widely utilized in non-replicating viral vector vaccines. As common viruses, they account for mild symptoms (cold, flu. . .). The candidate vaccines developed by the University of Oxford-AstraZeneca and by Johnson & Johnson/Janssen are based on adenoviruses (chimpanzee adenovirus on the AstraZeneca vaccine [ChAdOx1, AZD1222], human adenovirus in the Janssen vaccine [Ad26.COV2.s]), as are the Gamaleya candidate vaccine [rAD26-S, and then rAd5-S] and the CanSinoBIO vaccine [Ad5] developed in China (human adenovirus). As these adenoviral vectors are replication-defective, it may reasonably be concluded that once the virus has infected a cell, no other virus can be produced.

5.1.2. Replicating viral vectors

The vaccines developed by MSD are examples of replicating viral-vectored vaccines. One of them brings into play the vesicular stomatitis virus, from which infections in humans are generally asymptomatic or responsible for mild illness (flu. . .). Researchers have replaced part of its RNA sequence by RNA coding for the protein S of SARS-CoV-2. After infection of a host cell, the cell manufactures and expresses protein S; given that the vaccinal virus is replication-component, it goes on to infect other cells, which infect still other cells, and so on. It bears mentioning that these replicative vectors cannot be used in immunodepressed patients. On 25 January 2021, it was announced that due to non-optimal immunogenicity in phases 1 and 2 trials, MSD had decided to suspend clinical development of the two vaccines, which are based on the vesicular stomatitis and the measles vaccine virus [55]. Other replicating viral vectors are currently being developed [5].

5.2. How can we place viral vector vaccines into perspective?

Their development dates back to the 1980s. The dengue vaccine (Dengvaxia®) utilizes the vaccinal strain of the yellow fever virus expressing genes with the structure of each of the four dengue viruses. More recently, MSD applied this technology and used the vesicular stomatitis virus to develop a vaccine designed to combat the Ebola virus (Ervebo®); it was approved by the FDA and the EMA, and has led to the vaccination of tens of thousands of persons. Other vaccines of this type are currently being developed, including vaccines against chikungunya, Zika, Nile fever, RSV, HIV. . . The Johnson & Johnson/Janssen COVID-19 candidate vaccine draws upon the AdVac® (human adenovirus 26) technology platform, which has also been used to develop and manufacture their combined Ebola vaccine (Zabdeno® and Mvabea®), approved by the European commission, and to construct its Zika, RSV and HIV candidate vaccines (https://www.janssen.com/infectious-diseases-and-vaccines/vaccine-technology). In addition, the AdVac® technology platform has contributed to the vaccination of several tens of thousands of people in the framework of the Janssen experimental vaccine programs.

5.3. What are the efficacy and immunogenicity data of the AstraZeneca vaccine derived from animal models of SARS-CoV-2 infection and from phases 1 and 2 clinical trials?

Similarly to RNA vaccines, viral vector vaccines possess interesting immunogenic properties. Specifically and independently of the proteins they express and the genomic information they transport, their viral particles are recognized by the innate immune system, thereby facilitating the constitution of a cytotoxic (CD4 and CD8) cellular and humoral immune response. In a macaque model of SARS-CoV-2 infection, vaccination by one or more doses of the ChAdOx1 vaccine provided clinical protection and significantly reduced viral load in the bronchoalveolar lavage (BAL). On the other hand, nasal viral load did not differ between vaccinated and control animals [56]. In a phases 1 and 2 trial involving a thousand volunteers, the immune response provided by one or two doses was humoral (increase of anti-protein S antibody titers 28 days after the first dose, and presence of neutralizing antibodies) and cellular (specific LT response at D14) [57]. The second vaccine dose (D56) occasioned an appreciable increases of the anti-protein S neutralizing antibody titers and of Fc-mediated antibody activity, including phagocytosis by the neutrophiles and macrophages, as well as activation of the complements and the NK cells [58].

6. What have we learned about RNA vaccines and viral vector-based COVID-19 vaccines through phase 3 clinical and real-life data?

6.1. What are the RNA vaccines in phase 3 of clinical development?

In Europe, two RNA vaccines have been given approval:

- the Pfizer-BioNTech vaccine [BNT162b2] for COVID-19 prevention in persons over 16 years of age was approved on 21 December 2020 by the European Medicines Agency (EMA) and registered under the name of Comirnaty®;
- the Moderna vaccine [mRNA-1273] for COVID-19 prevention in persons over 18 years of age was approved on 6 January 2021 by the EMA and registered under the name of COVID-19 Vaccine Moderna®.

Another RNA vaccine, the CVnCoV Vaccine of CureVac, is currently under evaluation by the EMA [5]. Three DNA vaccines have also reached phases 2/3 of clinical development [5].

6.2. What are the viral vector-based vaccines in phase 3 of clinical development?

The AstraZeneca-University of Oxford [ChAdOx1-S-AZD1222-Covishield] vaccine for COVID-19 prevention in persons over 18 years of age was approved on 29 January 2021 by the EMA and registered under the name of COVID-19 vaccine AstraZeneca®; three other relevant vaccines are those of:

- CanSino Biological Inc./Beijing Institute of Biotechnology [Recombinant novel coronavirus vaccine];
- the Gamaleya Research Institute [Gam-COVID-Vac];
- Johnson & Johnson/Janssen [Ad26.COV2.S] [5].

6.3. What is the degree of efficacy of the Pfizer-BioNTech and Moderna RNA vaccines?

Evidence of the efficacy of these vaccines was found in two exceedingly large phase 3 double-blind, randomized, placebo-controlled clinical trials [23,33]. These trials, involving approximately 43,000 participants (Pfizer) and 30,000 participants (Moderna), were respectively 95% (95% CI: 90–98) and 94% (95% CI: 89–97) effective with regard to occurrence of symptomatic COVID-19 seven or 14 days after the second dose of the vaccine. Concretely speaking, for the Pfizer-BioNTech vaccine, out of 170 cases of symptomatic COVID-19, eight occurred in the group of vaccinated volunteers versus 162 in the placebo group; for the Moderna vaccine, out of 196 cases of symptomatic COVID-19, 11 occurred in the group of vaccinated volunteers versus 185 in the placebo group. Relative efficacy did not vary according to age category or gender or in persons with underlying medical problems; other results will help to more sophisticatedly determine whether efficacy varies among and/or between different groups of persons.

As for efficacy with regard to severe forms, in the Moderna clinical trial there were 30 cases of severe COVID-19, including one death, in the placebo group, *versus* none in the vaccinated group; in the Pfizer-BioNTech clinical trial, there were nine severe cases in the placebo group and one in the vaccinated group.

The duration of the protection afforded by the vaccine is not yet known. The study period in for the clinical trials was inferior to two months; the antibody level observed 119 days after the 1st dose of the Moderna vaccine (in persons having received the 2nd dose 28 days after the 1st) suggest that the protection lasts as least as long. The main obstacle to protection persistence is encountered when variants emerge, in which case the problem would be not the duration of immune response, but rather the progressively more pronounced inadequacy of the protection afforded.

Real-life vaccinal effectiveness will be assessed in the framework of European studies designed to include a large number of subjects.

6.4. What is the degree of efficacy of the AstraZeneca viral vector-based vaccine?

The published clinical efficacy data for the AstraZeneca vaccine are derived from two pooled phase 3 clinical trials involving approximately 24,000 volunteers, of whom half received the ChAdOx1 nCoV-19 vaccine, while the other half were given the meningococcal ACYW vaccine or a placebo [24]. The majority of the participants (88%) were 18 to 55 years old; only 4% were over 70, and few of them presented with comorbidities. Assessed in terms of prevention of symptomatic COVID-19 occurring at least 14 days after the second vaccine dose, overall vaccinal effectiveness (as reported in interim analysis of around 12,000 participants) was estimated at 70% (95% CI: 55–81). Out of the 131 confirmed cases of COVID-19, 30 were found in the vaccinated group, and the remaining 101 in the control group. In the sub-group of volunteers in the United Kingdom having mistakenly received only a half-dose of the vaccine as a first injection, the reason being a modification of the modalities of quantification of the viral particles, vaccinal efficacy was estimated at 90% (95% CI: 67–97), independently of age of vaccinated persons. While a hypothesis according to which immunization against the viral vector (in this case nonhuman, chimpanzee adenovirus) following the first injection would render the second dose less effective has been put forward, preliminary trials did not show a correlation between the titers of neutralizing anti-ChAdOx1 antibodies and an increase in the titers of anti-SARS-CoV-2 antibodies between the 1st and the 2nd vaccinal dose [56]. That said, and in order to circumvent the possibly deleterious effect of anti-vector immunity, the Gamaleya Research Institute (Russia) utilized two different adenoviruses for the first (rAd26) and the second (rAd5) Gam-COVID-Vac vaccination (Sputnik V) [59].

No hospitalization due to COVID-19 occurred in the vaccinated group, versus ten in the control group.

Given the ages of the populations included in the phase 3 trials of the AstraZeneca vaccine, the French Health Authority (HAS) has first recommended that it be used only in persons under 65, starting with health sector professionals and medical-social professionals (whatever their ages) and in 50-to-64-year-olds presenting with comorbidities. Individuals aged 65 years or more, on the other hand, should be vaccinated by means of an RNA vaccine [60].

6.5. Are RNA vaccines and the AstraZeneca vaccine effective from the first dose onward?

In the studies demonstrating the effectiveness of mRNA vaccines, two doses administered at an interval of three or four weeks were scheduled. Supply chain pressures, the appearance of more transmissible variants, and the orientations of recommendations from other countries underscore the key importance of the “time interval” issue. In the pivot study by Pfizer-BioNTech, the vaccinal efficacy of the complete course of vaccination was 95% (95% CI: 90–98) for symptomatic COVID-19 occurrence at least seven days after the 2nd dose and the vaccinal effectiveness of the 1st dose was 87% (95% CI: 69–95) for symptomatic COVID-19 occurrence at least 10 days after the first dose [33]. As concerns the results of the Moderna vaccine trial, they were comparable after one and following two doses [23]. Primary analysis of the phase 3 AstraZeneca trials, currently available in preprint, showed vaccinal effectiveness

of 76% (95% CI: 59–86), without any case of COVID-19 that was severe or necessitated hospitalization starting from the 22nd day after the 1st dose [61]. The main argument against an increased interval between the two vaccinal doses (and, *a fortiori*, against utilization of a single dose) is that after a 2nd dose, humoral immune response is 10 to 100 times greater [53,54], suggesting the probability of more prolonged protection, of a more pronounced effect on transmission and, quite possibly, of more marked action against the variants. Conversely, the evidence in favor of an early vaccinal campaign advocating (at least initially) a single dose, are:

- the high effectiveness, not significantly inferior to that of two doses, of one dose;
- the evident interest, in light of the epidemic emergency, of the possibility of vaccinating twice as many persons during the first stages of the campaign.

Two elements seem to corroborate the second set of arguments: "real life" effectiveness in Israel, after a single dose of the Pfizer vaccine [62] and the efficacy of the Johnson & Johnson/Janssen vaccine (https://www.nih.gov/news-events/news-releases/janssen-investigational-covid-19-vaccine-interim-analysis-phase-3-clinical-data-released) and of the Gamaleya vaccine after a single dose [63] (that said, the vaccines were not those of the mRNA variety).

For the time being, France and the majority of European countries have decided to maintain a two-dose vaccination schedule.

6.6. Are the COVID-19 vaccines approved in Europe effective with regard to asymptomatic infections?

The phase 3 clinical trials of the RNA vaccines approved in Europe were drawn up in order to demonstrate efficacy against the COVID-19 disease (symptomatic SARS-CoV-2 infection), and only in the event of symptoms did the relevant protocols stipulate a search for infection [23,33]. While the initial results tend to show excellent efficacy against symptomatic episodes of SARS-CoV-2 infection and the severe forms of COVID-19, as of now there are no consolidated results concerning protection from asymptomatic infection or its transmissibility. In the Moderna vaccine trial, nasopharyngeal PCR was carried out prior to administration of the second dose; 39 patients in the placebo group had positive PCR SARS-CoV-2 results with no clinical signs *versus* 15 in the vaccination group. While not altogether conclusive, these results are interesting, highlighting a possible effect of the vaccine with regard to asymptomatic forms.

In the clinical trial of the AstraZeneca vaccine about which an intermediate analysis has been published, some of the participants underwent systematic weekly testing, the objective being to detect asymptomatic infections [24]. A half-dose/full dose regimen afforded 58% protection from asymptomatic infections (95% CI: 1–83%); on the other hand, a full dose/full dose regimen failed to provide significant protection. According to primary analysis of phase 3 trials (available in preprint format), vaccination lowered the PCR positive rate by 67% (95% CI: 49–78) after the 1st dose and by 50% (95% CI: 38–59) after the two doses [61].

Before being able to assess (and to understand) the possible effect of certain vaccines on transmission, it will be necessary to collect complementary clinical information and mucous immunity data. Even though symptomatic infected persons are obviously highly implicated in transmission of the virus, results concerning vaccine effectiveness against asymptomatic infection and transmission will probably be less spectacular than their effect on the symptomatic forms.

6.7. What are the most frequently encountered adverse effects of RNA vaccines?

An overwhelming majority of the adverse events observed during clinical trials of RNA vaccines appeared the day after vaccination and generally lasted for fewer than 3 days [23,33]. In many cases, the events represented signs of reactogenicity: injection site reaction, asthenia, headache, myalgia, chills or fever. Systemic effects were the most frequent after the 2nd dose and in persons under 65 years of age; more often than not, they were minimum to moderate. While paracetamol intake remains possible, for the time being it is not recommended as a systematic preventive measure.

The data on the frequency of reactogenicity symptoms in the two trials came from a "solicited" sub-group of which the members were requested during the seven days following each injection to report and quantify any adverse events. For example, in the clinical trial of the Moderna vaccine, during the seven days following the 2nd dose, 23% of the persons in the placebo group reported headaches (*versus* 59% in the vaccination group), and 23% of the persons in the placebo group (*versus* 65% in the vaccination group) complained of fatigue [23]. More frequent occurrence of axillary adenopathy in the group of vaccinated volunteers (0.3 and 1.1% in the Pfizer-BioNTech and Moderna trials, respectively) than in those having received the placebo was likewise noteworthy. In only one case was the adenopathy classified as a "severe adverse effect".

6.8. Are COVID-19 vaccines responsible for facial paralysis?

Several cases of facial paralysis (Bell's palsy) have been reported in the clinical trials of Pfizer-BioNTech and Moderna [23,33]. In the Pfizer-BioNTech trial, there were four cases of facial paralysis, two of which were ascribed to the vaccine, as opposed to none in the placebo group. Facial paralysis appeared in one case on day 37 after dose 1 (the participant did not receive dose 2), and on days 3, 9 and 48 after dose 2 in the three other cases. In the Moderna trial, there were three cases in the vaccinated group, as opposed to one case in the placebo group. In the AstraZeneca trials, three cases of facial paralysis occurred in the two groups [24]. In point of fact, frequency of occurrence of facial paralysis among the vaccinated volunteers was close to what is to be expected in the general population (15 to 40 cases among 100,000 persons a year) [64]. Since commercialization of the vaccines began, these different events have been closely monitored. The French pharmacovigilance data on the Pfizer-BioNTech vaccine corresponding to the period from 27 December 2020 to 29 January 2021 (and to approximately 1.5 million vaccinations) reveal six cases of facial paralysis, including four cases of typical peripheral facial paralysis having occurred 1, 5, 10 and 15 days after vaccination [65].

6.9. What have been the severe adverse effects reported with RNA and AstraZeneca vaccines?

In the phase 3 trials of the Pfizer-BioNTech and the Moderna vaccines, the frequency of severe adverse events was not higher in the vaccinated group than in the placebo group (0.6% and 1% respectively in the vaccinated group *versus* 0.5% and 1% respectively in the placebo group). The reported frequencies were likewise comparable in the AstraZeneca trials (0.7% in the group having received

the AZD1222 vaccine and 0.8% in the placebo or meningococcal ACYW vaccine group). Most of the events (appendicitis, cholecystitis, myocardial infarction, cerebrovascular accidents. . .) were considered by the investigators as being unassociated with the vaccine.

Conversely, some exceedingly rare severe events have been considered by investigators as being associated with vaccination. In the Pfizer-BioNTech trial (19,000 vaccinated participants), they consisted in shoulder injury (by mistaken intra-articular vaccine injection?), ventricular arrhythmia lasting eight days, and axillar adenomegaly. In the Moderna trial (15,000 vaccinated participants), they consisted in one case of severe vomiting, two cases of transient facial swelling and one case of rheumatoid polyarthritis. No severe anaphylactic reaction to vaccination was reported in these trials; that said, persons with severe allergy history were not included. In the phase 3 AstraZeneca trials (12,000 participants received the AZD1222 vaccine), one case of transverse myelitis was viewed as possibly associated with the vaccine.

Since the outset of commercialization and vaccination campaigns in the United Kingdom, several cases of "severe and immediate allergic reactions" have been reported; some of the persons concerned were previously known as being susceptible to allergy. In the United States on 18 January 2021, among the side effects declared to the Vaccine Adverse Event Reporting System (VAERS), the CDC identified 47 and 19 cases of anaphylaxis with approximately 10 million and 7.5 million administered doses of the Pfizer-BioNTech and Moderna vaccines respectively, that is to say occurrence frequency of 4.7 and 2.5 out of a million vaccinated persons [66] (to be compared with the frequency of severe allergy accidents, one out of a million with the different commercialized vaccines [67], and of penicillin-related anaphylaxis, which is estimated at 1–5 per 10,000 treatments [68]). More than 90% of the reported events involved women, and approximately one third of the affected persons presented with anaphylaxis history. Median time elapsed between vaccine injection and symptom occurrence was 10 minutes [66]. Initial French pharmacovigilance data show four severe anaphylaxis cases for approximately 950,000 doses [65].

To conclude, the cumulative data are reassuring; the benefit/risk ratio in populations for which vaccination has been recommended is distinctly favorable. It bears mentioning that the mortality rate in cases of SARS-CoV-2 infection is an estimated at 0.5–1.4%, and that it exceeds 10% in persons over 70 years of age.

6.10. Can persons with allergy history be vaccinated with an RNA vaccine?

Vaccination is contraindicated for persons with hypersensitivity to a vaccine component, particularly PEG, or with a history of allergy polysorbate (risk of cross-allergy or cross-reactivity with the PEG contained in the vaccine). PEG or macrogol, a polyether compound, is used in different parenteral medicines (cf. question 4.7, non-exhaustive list). It can also be found in the coating of numerous treatments (antibiotics and NSAIDs in particular); in an exceptional situation, PEG is itself the allergen.

Given the reassuring data collected since worldwide launching of the immunization campaign, the *Fédération française d'allergologie* has recommended that allergy or anaphylaxis history not constitute a systematic contraindication to vaccination [69]. The policy to be adopted should take into account a description of the allergy, the substance currently or potentially at issue, and the conclusions of a possible overall allergy assessment: proven anaphylaxis due to PEG or polysorbate: no vaccination, history of anaphylactic reaction to a treatment containing PEG or polysorbate, and absent assessment of imputability to allergy: postponed vaccination while awaiting the allergy assessment, except in cases where vaccination is clearly necessary, history of immediate reaction without sign of severity to a medicine or other substance of which the coating contains polysorbate (for example, isolated urticaria or face swelling without laryngeal edema after Ciflox® intake): vaccination plus prolonged observation (30 minutes), history of delayed allergic reaction (occurring more than two hours later) to PEG, polysorbate, or another substance: vaccination and "standard" observation (15 minutes), history of anaphylactic reaction to a known medicine with identification of the allergen (different from PEG or polysorbate) after allergy assessment: vaccination plus prolonged observation (30 minutes).

In actual practice, administration of an mRNA vaccine must be carried out in a structure capable of managing a severe immediate hypersensitivity reaction. All vaccinated persons must remain under observation for 15 minutes (or more, according to previous history). In the event of a severe or immediate allergic reaction after the 1st dose of a vaccine, the 2nd dose is contraindicated [69].

6.11. Should persons with a history of SARS-CoV-2 infection be vaccinated – and if so, when?

Early in the history of the pandemic, it became evident that short-term reinfection was quite infrequent. It is for that reason that the French Health Authority (HAS) initially recommended that persons having had documented SARS-CoV-2 infection not be given priority for vaccination (or, more precisely, not until at least 90 days after the infection) [20].

More recently, other elements have provided evidence on the subject:

- on the one hand, T and B cell responses persist several months after SARS-CoV-2 infection, including (albeit at less elevated levels) in persons having had an asymptomatic form of the disease, and increased antibody titers and cellular response in primate models after a second exposure suggest the establishment of immune memory [70–73];
- on the other hand, monitoring of cohorts of persons having had a PCR-documented infection or positive serology has shown, in comparison with control groups, that the infection rate is reduced by 83 to 100%, while the rare cases of reinfection are in the overwhelming majority asymptomatic and generally occur at six to seven months [74–77].

Lastly, several preprints have shown that in the target population, a single vaccine dose triggers a substantial rise in IgG and IgA titers and in those of neutralizing antibodies [19,78,79]. In one of these studies, vaccinal injection procured neutralizing titers against divergent strains (variant 20H/501Y.V2 and SARS-CoV-1) [79]. A real-life study on the immunogenicity of the 1st dose of the Pfizer-BioNTech vaccine involving 514 health professionals revealed antibody titers ten times higher than those found in persons with an infection history [80].

It is in this context that on 12 February 2021, the HAS recommended that injection for non-immunosuppressed persons with a history of symptomatic or asymptomatic SARS-CoV-2 infection not be administered during the first six months, and that it consist in only one injection, to be considered as a booster shot [34].

6.12. Should pregnant women receive an RNA COVID-19 vaccine or the AstraZeneca vaccine?

As the mRNA and AstraZeneca vaccines are devoid of infectivity, there is no reason to fear maternal, fetal or embryonic infection associated with a vaccine injected during pregnancy. Moreover, the first teratogenicity studies in animals show no effect on embryonic and fetal development or on reproduction (non-finalized studies for the AstraZeneca vaccine). In clinical trials of the RNA vaccines of Pfizer-BioNTech and Moderna, while pregnant women were excluded, 23 and 13 pregnancies respectively nonetheless took place, in the group of vaccinated participants as well as the group receiving the placebo. No untoward event occurred among the pregnant women. Given the currently available date, the *Centre de référence sur les agents tératogènes* (CRAT) in France and the American College of Obstetricians and Gynecologists (ACOG) consider that vaccination with mRNA vaccines is possible during pregnancy, *a fortiori* if there exist risk factors exposing the pregnant woman to the eventuality of a severe form of the disease. The person concerned must imperatively be informed of the expected benefits and risks (of reactogenicity) [81,82]. On principle and as far as practicable, it is preferable to vaccinate after ten weeks of amenorrhea, and with a RNA vaccine. If the pregnant woman has poorly tolerated the first dose, whatever the vaccine, it is advisable, in coordination with her physician or midwife, to put off the second dose until after the end of the pregnancy. If the first dose was administered at a time when the pregnancy remained unknown, there is nothing to worry about, either for the mother or for the future child, whatever the vaccine. If the first dose has been satisfactorily tolerated, the vaccination calendar can be observed normally. Lastly, there is no time limit to be respected between a COVID-19 vaccination and the outset of a pregnancy [81].

6.13. Can breast-feeding women receive an RNA Covid-19 vaccine or the AstraZeneca vaccine?

There currently exist no safety data on COVID-19 vaccines for breast-feeding women (they have been excluded from clinical trials) or pertaining to their effects on breast-fed infants or milk production. That much said, the vaccines approved in Europe and in the United States are devoid of infectivity and show no expected passage into the bloodstream, and the CRAT and the CDC consider that vaccination by means of an mRNA vaccine can be envisioned in a breast-feeding woman [81,83]. There is no reason to think that matters will be different with regard to the AstraZeneca vaccine; in Great Britain, breast-feeding women belonging to a targeted group are allowed to have it injected [84].

6.14. Can persons with autoimmune disease receive an RNA COVID-19 vaccine or a viral vector vaccine?

The vaccines stimulate the innate immune system by interacting with the toll-like receptors, inducing an “alarm signal” and the expression of certain cytokines. Pro-inflammatory cytokines and interferons are conducive to the recruitment and activation of immune cells, and also to the acquisition of immune memory. A hypothesis has been put forward according to which immune response could be deleterious in individuals seemingly predisposed to inflammatory reactions and, possibly, to autoimmune disease; as of now, this risk remains theoretical, and the above-mentioned phenomena have never been observed with regard to any vaccine.

In view of attenuating this phenomenon, and of ensuring that it does not entail destruction of the RNA before it has had the time to be translated into protein, the RNA in the Pfizer-BioNTech vaccines is composed of nucleotides slightly different from natural nucleotides (pseudo-uridines) [85]. CureVac has chosen not to modify the nucleotides.

In the phase 2/3 clinical trial of Pfizer-BioNTech, a history of dysimmune disease was not an exclusion criterion, provided that it was not unstable. With median follow-up of 2 months after the 2nd vaccinal dose, no onset of dysimmune disease was signaled [33]. In the Moderna trial, one case of rheumatoid polyarthritis occurred in the group having received the vaccine [23].

The *Société française de rhumatologie* recommends not waiting for the disease to be controlled before proposing vaccination, if the person is eligible [86].

No case of Guillain–Barré syndrome (GBS) following vaccination has been signaled among the participants in the clinical trials of the Pfizer-BioNTech, Moderna, or AstraZeneca vaccines, nor, as of 22 January 2021, had French pharmacovigilance observed any case of GBS possibly associated with a vaccine [65].

6.15. Can immunodeficient persons (undergoing immunosuppressant or related treatment) receive an RNA COVID-19 vaccine or the AstraZeneca vaccine?

These conditions do not constitute a contraindication to a nucleic acid or a non-replicative viral vector vaccine (such as the AstraZeneca vaccine), which are devoid of infectivity. In some situations, it may be reasonable to postpone vaccination at the onset of autoimmune disease. On the other hand, most immunodepressions and immunosuppressive treatments are liable to have a negative impact on vaccine effectiveness with regard to humoral and cellular immune responses. Descriptions of chronic COVID-19 in patients suffering from humoral immunodeficiency or receiving an anti-CD20 monoclonal antibody suggest that humoral response is probably necessary as a means of controlling viral infection. It may be feared that patients develop insufficient post-vaccinal acquired immunity. Clinical trials and cohort studies are underway or upcoming, the objective being to more precisely determine the immunogenicity and tolerance of the vaccines currently available in France among persons at risk of lessened immune response, some of whom are quite elderly. Several learned societies (the *Société française de rhumatologie* and the *Institut national du cancer*) have issued recommendations concerning the indication and modalities for vaccination of the patients concerned [86,87].

6.16. Can children be vaccinated?

With the exception of the 16-to-18-year-old adolescents tested by Pfizer-BioNTech, the Pfizer-BioNTech, Moderna and AstraZeneca vaccines have not been tested in a pediatric population, for which vaccination is consequently not presently indicated. However, a small number of children presenting with risk factors for severe and at times deadly infections (congenital interferon deficiency, transplant recipients...) may benefit over the coming weeks or months from derogations. More broadly, vaccination of all children in the framework of a strategy aimed at achieving herd immunity the young population is not being envisioned in the short-term.

7. Administration of the vaccines approved in Europe

7.1. What should be the time interval between two injections of the Pfizer-BioNTech and Moderna vaccines?

The Pfizer-BioNTech vaccinal schedule comprises two doses (30 μg, 0.3 mL each), administered by intramuscular route with

an interval of 21 days. As for the Moderna vaccine, the two doses (100 μg, 0.5 mL) are administered by intramuscular route with an interval of 28 days. In the clinical trials, some participants have not perfectly respected the schedule; as a result, only limited data on early or late administration of the second dose are currently available.

As regards early administration of the 2nd dose, according to the CDC it can occur up until three or four days before (the recommended) D21 or D28 [35]. If by mistake it were to occur earlier, it would be necessary to have it repeated.

As regards the possibility of late administration of the 2nd dose of the Pfizer-BioNTech vaccine, on 7 January the French ANSM issued the authorization and on 22 January the HAS put forward the recommendation to postpone the 2nd vaccination up until D42, the objective being to enlarge single dose vaccination coverage among target populations in a constraining context [88,89]. Their argumentation was essentially based on the fact that some participants included in clinical trials received their 2nd dose of a vaccine more than 21 days (and up to 42 days) after the first, and that vaccinal effectiveness was visible starting on D12, that is to say well before administration of the 2nd dose. As for the WHO, on 5 January it pronounced itself in favor of an interval ranging from 21 to 28 days between the two doses of Pfizer-BioNTech, and declared that under exceptional circumstances, the interval could be prolonged, but without exceeding 42 days [90]. In the absence of certified data on the duration of the protection afforded by the 1st dose of a vaccine, and given the risk that in the face of emerging variants, a single dose not enable development of a sufficient immune response, on 26 January the French health minister announced that the 2nd dose would have to be administered between D21 and D28 [91].

In actual practice, if the recommended interval between the two doses were to be exceeded, the second dose would have to be administered as expeditiously as possible, without their being any need for more than two doses.

Errors of administration must be reported in pharmacovigilance.

7.2. What should be the time interval between the Pfizer-BioNTech or Moderna vaccines and another vaccine?

Due to the highly theoretical risk of interference between the immune responses provoked by the two vaccines, a minimal interval of 14 days between an RNA-based vaccine and another vaccine is recommended. However, in the event that this time interval were not to be observed, it would not be necessary to modify the schedule and/or administer a supplementary dose of one of the two vaccines.

7.3. What should be the time interval between passive COVID-19 immunotherapy (anti-SARS-CoV-2 antibodies or convalescent plasma) and vaccination?

At present, there do not exist any vaccination data concerning infected persons having received monoclonal antibodies or convalescent plasma in the context of COVID-19 treatment. Given the estimated half-life of these treatments and a number of observations suggesting that reinfection during the six months following the initial infection is infrequent, it is recommended to postpone vaccination for at least 90 days.

7.4. What should be the time interval between treatment by polyvalent immunoglobulins and vaccination?

For persons receiving polyvalent immunoglobulins (by intravenous or subcutaneous route), RNA vaccine administration is possible, without it being necessary to observe a given time interval before or after infusion. As of now, the amounts of anti-SARS-CoV-2 antibodies contained in these treatments are negligible and do not seem liable to antagonize the development of a protective antibody response.

7.5. Should a surgical procedure or COVID-19 vaccination be postponed when they are scheduled for practically the same time?

Any necessary operation can be performed, whatever the time interval with regard to COVID-19 vaccination. In actual practice, it is permissible to avoid programming vaccination in immediate proximity (a few days) to the operation, insofar as it might complicate interpretation of certain symptoms, such as postoperative or post-vaccinal fever.

7.6. What should be the time interval between a SARS-CoV-2 infection and COVID-19 vaccination?

This point is developed in paragraphs 3.12 and 6.11.

7.7. Can RNA vaccines and the AstraZeneca vaccine be administered subcutaneously?

No. The vaccine must be injected intramuscularly; that is the route of administration having been used in clinical trials.

In subjects presenting with primary hemostasis disorders or coagulation, and in persons taking anticoagulants, low-volume vaccination can be carried out in the deltoid muscle with a thin needle (ideally 25G, and a length of 25 mm for a normal-sized injection site), followed by pronounced and prolonged, facilitated compression (2 minutes). Persons presenting with highly severe hemostasis disorders or coagulation can be vaccinated on a case-by-case basis, if the possible benefits clearly outweigh the drawbacks associated with administration [92]. Use of the subcutaneous route exposes the patient to the risks of less satisfactory immunogenicity and heightened local reactogenicity [93].

7.8. Must the two vaccinal doses be administered in the same arm?

In preclinical trials of nucleic acid vaccines, the first and second (booster) injections are carried out in the same muscle. The protocols for the phase 3 trials of the RNA and the AstraZeneca COVID-19 vaccines propose preferential injection of each vaccinal dose in the non-dominant arm. In the absence of precise data on the modalities of circulation of lymphocytes and antigen-presenting cells after injection of the mRNA vaccine, it is preferable to use the same arm for the 2nd dose.

Ethical statement

Sans objet.

Contribution of authors

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Disclosure of interest

The authors declare that they have no competing interest.

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