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### RESEARCH ARTICLE

#### THE ANTI SARS-COV-2 VACCINES AND THE QUESTIONS THEY RAISE

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#### Abstract

Since it was first reported in late 2019, SARS-Cov-2 had a global impact on human physical and mental health, as well as on their social life and economic endeavor. In one year, the virus has infected over 115 million people, killed almost 2.6 million of them and left many others with long-term health sequelae. The SARS-Cov-2 pandemic has overwhelmed healthcare systems, interrupted routine care and prevented patients' follow-up. All these factors led to increased mortality from other chronic diseases. Further, the SARS-Cov-2 pandemic has caused an unprecedented disruption to education, economic trade, travel, social life, and has profoundly changed our way of living. In this review article we present the organization and the function of the immune system that protects us against diseases, the virology, the infection, the transmission and the pathogenesis of SARS-Cov-2, the disease Covid-19, the development of vaccines against SARS-Cov-2 and some of the questions raised by these vaccines, as well as suggested responses to them.

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#### Introduction:-

Since it was first reported in late 2019, SARS-Cov-2 had a global impact on human physical and mental health, as well as on their social life and economic endeavor. In one year, the virus has infected over 115 million people, killed almost 2.6 million of them and left many others with long-term health sequelae.

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Further, the SARS-Cov-2 pandemic has caused an unprecedented disruption to education, economic trade, travel, social life, and has profoundly changed our way of living.

In this review article we present the organization and the function of the immune system that protects us against diseases, the virology, the infection, the transmission and the pathogenesis of SARS-Cov-2, the disease Covid-19, the development of vaccines against SARS-Cov-2 and some of the questions raised by these vaccines, as well as suggested responses to them.

#### The Immune System

The decay of all systems through aging, erosion and diseases is ultimately due to causes from within these systems or originating in their environments. During their evolution living organisms have resisted this decay by developing defense mechanisms for protection (1 - 4).

Humans have developed an immune system made of highly specialized immune cells (B-cells, T-Cells, and APCs such as macrophages, dendritic cells etc.) which interact with the Major Histocompatibility Complex molecules (MHC-I and MHC-II) to produce a humoral and/or a cellular immune response. These are two interrelated responses of the immune system which use separate immune cells and have complementary but different roles.

#### **The Major Histocompatibility Complex (MHC):**

The MHC plays an essential role in the immune response. It is made of two classes of molecules (MHC-I and MHC-II)

**The MHC-I molecules** reside around the cell's protein factories such as the Rough endoplasmic reticulum (RER). Thus, their location within the cell gives them their specific function of monitoring the output and transport of proteins produced inside the cell. They specifically bind to antigens that have been synthesized in the intracellular environment like in the case of malignant neoplasms or intracellular infections. The cytotoxic CD8<sup>+</sup> T-Lymphocytes recognize and interact with antigens displayed in association with MHC-I molecules at the surface of the Antigen-Presenting-Cells (APCs), then proliferate and destroy diseased cells that display the same antigens. MHC-I molecules are expressed on all our cells. Thus, any cell which displays a foreign or abnormal antigen fragment that was not recognized as self during development and education of T-Cells, is destroyed by Cytotoxic CD8<sup>+</sup> T-Lymphocytes (1 - 7).

**The MHC-II molecules** reside around the vesicles which engulf and destroy extra-cellular antigens, such as the lysosomes and the endosomes. Thus, their location within the cell gives them their specific function of monitoring the content of these vesicles. They specifically bind to antigens that have been enzymatically processed in the lysosomes or in the endosomes. The Helper CD4<sup>+</sup> T-Lymphocytes recognize and interact with antigens displayed in association with MHC-II molecules at the surface of the APCs, then proliferate and stimulate Plasma B cells to produce neutralizing antibodies against free antigen in circulation and antigens displayed at the host-cell surface as well as circulating pathogens. MHC-II molecules are only expressed on our immune cells. Thus, the activity of the Helper CD4<sup>+</sup> T-Lymphocytes is focused on the cells which participate in the immune response, without impacting other cells in the organism (1 - 7).

#### **The Immune Cells:**

Our immune system uses immune cells (B-cells, T-Cells, and APCs such as macrophages, dendritic cells etc.) which express at their surfaces specific receptors (MHC-I, MHC-II, TCRs, CD4 and CD8) and mature through a multilayered education process to avoid attacking self-cells.

**B-cells or B-Lymphocytes** are so called because they originate and mature in the bone marrow. Plasma B-Cells can produce antibodies which specifically recognize and bind to foreign antigens in the circulation or those displayed at the surface of sick cells. These antibodies can be monoclonal (specifically bind to a single epitope on the antigen) or polyclonal (bind to multiple epitopes on the antigen).

**T-Cells or T-Lymphocytes** are so called because they mature in the thymus. They do not produce antibodies but are equipped with antibody-like molecules called T-Cell-Receptors (TCRs). Unlike antibodies, the TCRs only recognize antigen fragments produced in the intracellular environment and exported and displayed in association with MHC-I or MHC-II molecules at the surface of other cells. The TCRs are highly specific and precise in recognizing processed antigens.

Genes encoding antibodies and TCRs are assembled from short DNA fragments originating from different gene-families, in a process that can generate up to **10<sup>14</sup> combinations**. Therefore, no matter how rare an antigen is, there will always be an antibody or a TCR that can recognize it. Moreover, one should not be afraid to venture outside of our planet as our immune system has a good chance to meet antigenic challenges coming from new space frontiers which, with the progress in space technologies, will no longer be beyond our reach.

**T-Cells' education:** Both the humoral and the cellular responses of the immune system involve T-Lymphocytes but the nature and the outcome of the immune response is dependent on which subclass of T-Lymphocytes is activated as well as on the cytokines present in the cellular microenvironment where the maturation and the activation of the T-Lymphocytes are taking place (1 - 4).

Our T-Cells undergo a rigorous and stringent two-step process of education in the thymus to learn how to discriminate between self and non-self-peptides. This process was dubbed the positive/negative selection. It involves MHC-I, MHC-II, TCR, CD4 and CD8 molecules. The CD4 and CD8 molecules are co-receptors which are cross-linked with the TCRs by the MHC molecules during the maturation and the activation of T-Cell precursors into mature T-Cells.

The precursors of mature CD4<sup>+</sup> and CD8<sup>+</sup>T-Cells are thymocytes that express TCRs and both CD4 and CD8 receptors. These double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes undergo, in the thymus, a test of complementarity between their TCRs, and the MHC-I as well as MHC-II molecules that are presenting self-peptides at the surface of the APCs.

**Double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes** with TCRs that show no complementarity with the MHC-self peptides complexes are deemed useless and die by genetically programmed apoptosis. This is referred to as “death by neglect”.

**Double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes** with TCRs that show a strong complementarity with MHC-self peptides complexes would cause auto-immune diseases if freed in circulation. Therefore, they are negatively selected to die by antigen-induced apoptosis via TCR signaling. This is referred to as “clonal deletion or central tolerance”.

**Finally, double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes** with TCRs that only recognize MHC-I and MHC-II molecules which do not display self-peptides are saved from apoptosis by neglect and are positively selected to continue maturation.

**Double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes** which have their receptors TCRs and CD8 cross-linked during their interaction with MHC-I molecules will develop into CD8<sup>+</sup> lineage. Cells of this lineage recognize and kill abnormal cells in the organism.

**Double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes** which have their receptors TCRs and CD4 cross-linked during their interaction with MHC-II molecules develop into helper CD4<sup>+</sup> T-Cells or suppressor CD4<sup>+</sup> Treg cells. Cells of these lineages enhance or suppress the activities of T-Cells and B-cells.

**The negative selection** is not a perfect process. Some of the double positive (CD4<sup>+</sup>-CD8<sup>+</sup>) thymocytes which recognize self-peptides still can escape death by neglect. But they still have to survive the process of positive selection, and the suppressor effect of circulating CD4<sup>+</sup> Treg Cells. If they succeed to escape this multilayered control system, they can cause autoimmune diseases.

**The Helper CD4<sup>+</sup> T-Cells' maturation pathways:** Naïve CD4<sup>+</sup> T-Cells develop in the lymphoid tissue into Helper CD4<sup>+</sup> T-Cells through one of the following maturation pathways: Th-1, Th-2, Th-17 or Treg (8).

**They mature through the pathway Th-1** in the presence of IL-12 and INF- $\gamma$ . Helper CD4<sup>+</sup> T-Cells of this pathway play a role in cellular immunity. The Th-1 immune response uses the Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) to stimulate the proliferation of phagocytizing cells (4). It activates cytotoxic CD8<sup>+</sup> T-lymphocytes and directs them to identify and destroy cells which become diseased by an intracellular pathological process such as in intracellular infections and in cancerous diseases.

**They mature through the pathway Th-2** in the presence of IL-4 and TNF- $\alpha$ . CD4<sup>+</sup> Helper T-Cells of this pathway play a role in humoral immunity. The Th-2 immune response inactivates the proliferation of phagocytizing cells (1 - 3). It involves Plasma B cells which produce antibodies that recognize and neutralize extracellular circulating antigens or pathogens, as well as cell-displayed abnormal antigens.

**They mature through the pathway Th-17** in the presence of IL-17 $\beta$ , and a low concentration of the Transforming Growth Factor ( $\beta$ TGF). Helper CD4<sup>+</sup> T-Cells of this pathway play a role in proinflammatory immune responses. The Th-17 pathway is upgraded in strong immune reactions.

**They mature through Foxp3 positive Treg** in the presence of a high concentration of  $\beta$ TGF. Helper CD4<sup>+</sup> T-Cells of this pathway play a role in anti-inflammatory immunosuppressive immune responses. The Treg pathway is upgraded

in poor immune responses which characterize many intracellular infections as well as autoimmune and cancerous diseases.

#### **The balance between the immune response pathways:**

The balance between the Th-1 and Th-2 immune responses determines whether the main immune response to a disease is cellular or humoral.

The balance between the Th-17 and Treg immune responses regulates the immune cells of Th-1 and Th-2 pathways and thus determines whether the immune response to a disease will succeed or fail (8).

However, an unbalanced upgrade of Th-2 and Th-17 pathways can overproduce cytokines that cause major inflammation, tissue destruction and necrosis. This overreaction of the immune system happens in many diseases such as in lepromatous leprosy where it causes serious damages to the host or can be fatal such as in the Cytokine Storm Syndrome and thrombotic syndrome observed in patients seriously affected by SARS-Cov-2(1 - 3, 9).

#### **Antigens, Immunogens, and Protective Immunity**

**An antigen** is a foreign substance which may or may not trigger an immune response.

**An immunogen** is an antigen which triggers an immune response.

**A protective immunity** is an immune response capable of clearing the immunogen from the body.

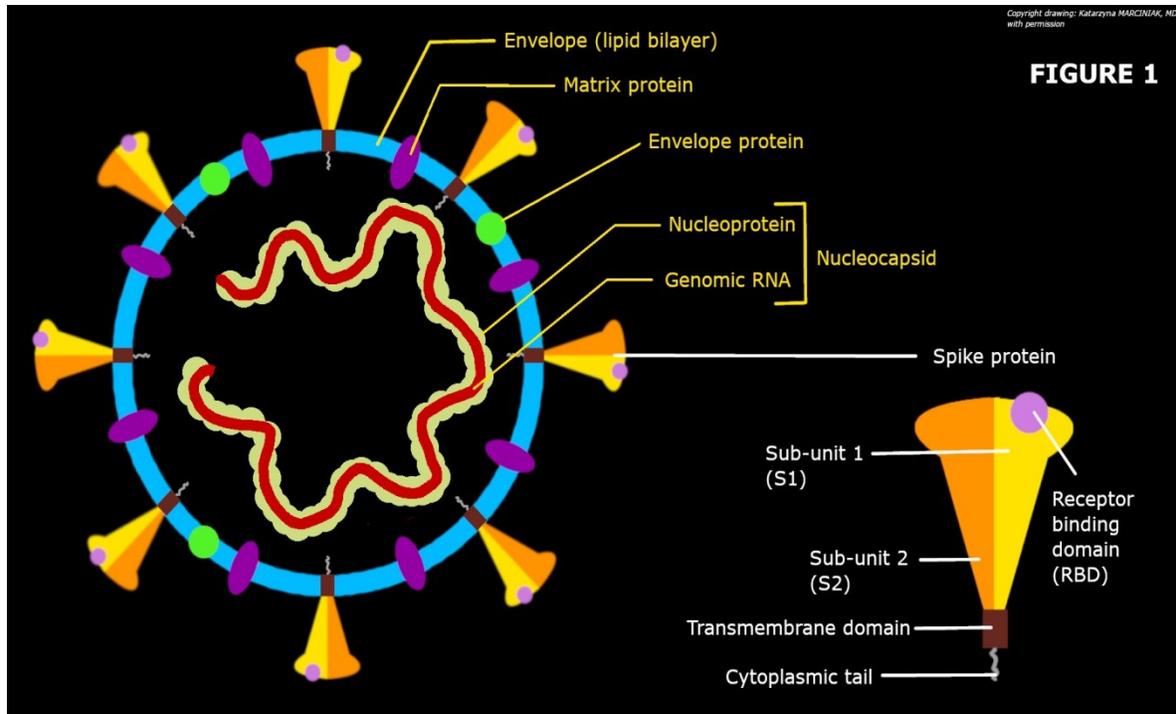
**The spike protein** (S-Protein) of SARS-CoV-2 is an immunogen which stimulates the immune system to produce neutralizing antibodies against SARS-CoV-2. For this reason, the S-protein is the focus of vaccine development against SARS-CoV-2 and is the target of rational drug design to produce therapies for Covid-19.

#### **The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2)**

**Coronaviruses** are so-called because of the appearance of a halo or crown when viewed under an electron microscope (Corona is the Latin word for crown or halo). These RNA viruses have a much higher mutation rate than DNA viruses because the RNA polymerase lacks the editing function present in the DNA polymerase. Thus, in addition to the high speed of virus production, RNA viruses can mutate within the host-cell at a much higher rate and further overwhelm the immune system through a mechanism that was dubbed “speed and shape change” by opposition to the mechanism of “camouflage and sabotage” associated with DNA viruses (20).

**SARS-CoV-2 is an enveloped positive sense RNA coronavirus.** It is made of an RNA genome wrapped with a **nucleoprotein** (N-protein), **accessory proteins**, a **bilayer phospholipid envelope**, an **envelope protein** (E-protein), a **membrane protein** (M-protein), and a **spike protein** (S-protein) which protrudes from the surface of the virus body and gives it its specific appearance. The proteins N, M, E and S are structural proteins. The structure of SARS-Cov-2 is shown in **Figure-1** here-below.

**SARS Cov-2 has a genome** of 29,881 base pairs (GenBank no. MN908947) which is organized into a positive single-stranded RNA. The RNA genome of SARS-Cov-2 encodes in the cytoplasm of the infected host-cells sixteen non-structural accessory proteins which form a Replication-Translation Complex (RTC). The RTC uses the positive sense viral genome as a template to produce a negative sense RNA. The latter is then used as a template to produce positive sense viral RNA molecules to be packaged into newly produced viral particles. The RTC also mediates the synthesis of the N-protein which forms, in association with the viral genomic RNA, the Nucleocapsid complex. The N-protein also plays a role in RNA synthesis.



The SARS-Cov-2 RNA genome further encodes in the Rough Endoplasmic Reticulum (RER) for the structural proteins E, M and S. After modification in the RER-Golgi complex these structural proteins form in association with phospholipids the virus envelope (22). The E and M-proteins are also needed for viral assembly. The S-protein is used to attach to and to enter into the host-cell.

**The N-protein** contains N and C termini domains (NTDs and CTDs) which bind RNA molecules. It has highly conserved serine-arginine domains (SR/RS) across species and is enriched in basic amino-acid residues. These structural attributes allow the N-protein to wrap and coil the acidic positive sense RNA molecule into a nucleocapsid complex. This process is highly efficient and specific for positive sense RNA molecules, which have the correct sequence and size to form mature viral particles. The N-protein also interacts with the M-protein to facilitate the process of viral genomic packaging in the newly produced virions (23, 24).

**The S-protein** is a transmembrane glycoprotein which mediates the attachment, the fusion and the entrance of the virus into the cell via the Angiotensin Converting Enzyme II (ACE-2) receptor. It is made of 1273 amino acids, which assemble into a homotrimer on the surface of the virus giving it the specific decoration of Coronaviruses. The 1273 amino acid residues are organized from the N-terminal to C-terminal direction, in several domains including the **peptide signal (SP)**, the **subunit (S1)** which contains 685 amino acids, the **subunit (S2)** which contains 588 amino acids, the **Furin** cleavage site located at the S1 / S2 junction, the **transmembrane domain (TM)** and the **cytoplasmic tail (CT)**, among others.

The S1 subunit contains a Receptor Binding Domain (RBD) which uses its Receptor Binding Motif (RBM) to recognize and bind to the ACE-2 receptor. The S2 subunit facilitates the fusion between the virus envelope and the cell membrane of the host-cell (16). The surface location of the S-protein made it a main target for the host immune response. The humoral immune response produces antibodies which recognize antigenic determinants on the S-protein and prevent the infection of host-cells by SARS-Cov-2, hence protection against the disease Covid-19. A study which followed 1241 seropositive patients for 127 days and 11052 seronegative patients for 188 days found zero cases of symptomatic infection and 3 cases of asymptomatic infection in the seropositive group but 89 cases of symptomatic infection as well as 79 cases of asymptomatic infection in the seronegative group (10). Another study showed that immune memory cells were detectable for up to 8 months after infection with SARS-Cov-2 (11). The conclusion is that antibodies protect against SARS-Cov-2 infection. Thus, the S-protein is the focus of vaccine

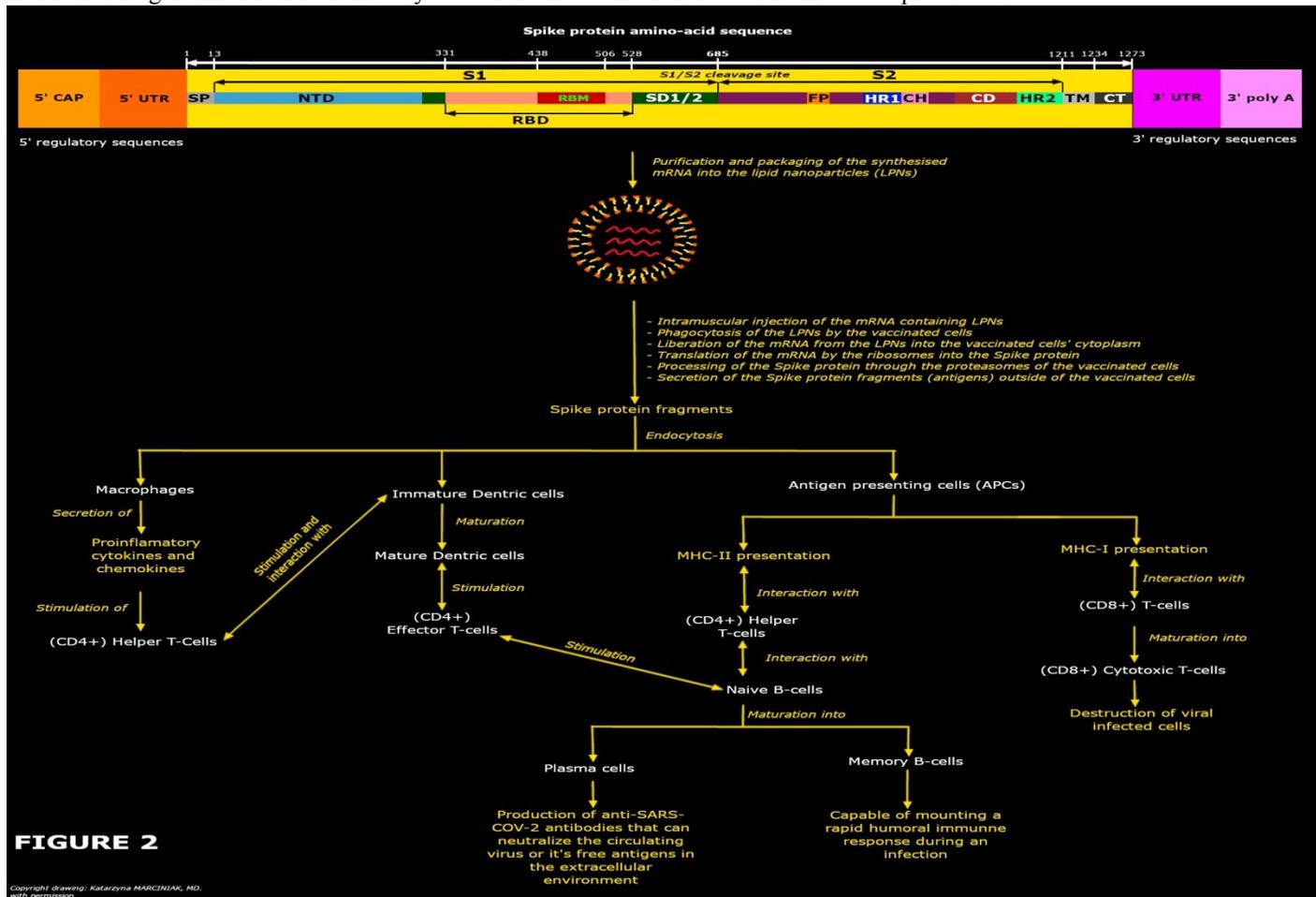
development against SARS-Cov-2, and a target for rational drug design to produce therapies against Covid-19. The organization of the amino-acid sequence of the S-protein is shown in **Figure-2** here-below.

But not all antibodies produced after SARS-Cov-2 infection are necessarily protective. Certain non-neutralizing antibodies can complicate the immune response by facilitating the entry of the virus into target cells through a mechanism that was dubbed "Antibody-dependent Enhancement" (12). This could explain why the use of plasma from convalescing subjects as a treatment has yielded mixed results (61, 62).

**The mechanism of attachment and entry** of SARS-Cov2 into the host-cell requires a rearrangement of both the S-protein and the ACE-2 receptor. SARS-Cov-2 uses its S-protein to attach to the ACE-2 receptor present on the host cell cytoplasmic membrane. Then it either fuses its membrane with the host-cell cytoplasmic membrane, or it is endocytosed via the endosomal pathway and once inside the host-cell it fuses its envelope with the endosome membrane. The Entry of SARS-Cov-2 into the host-cell via the endosomal route is mediated by acidic pH and endosomal cathepsins. The Entry of SARS-Cov2 by fusion with the host cell cytoplasmic membrane is mediated by proteases such as Transmembrane Serine Protease Type 2 (TMPRSS2) or Human Airway Trypsin (HAT), and Furin which is a membrane endopeptidase that shuttles between the Trans-Golgi Complex and the surface of the host-cell. On the one hand TMPRSS2 cleaves the ACE-2 receptor, and on the other hand it primes the precursor of the S-protein to assist the Furin to act on the cleavage site located at the S1 / S2 junction and separate the S1 and S2 subunits.

In both cases, SARS-Cov-2 succeeds to release its positive sense RNA genome and its essential enzymes into the cytoplasm of the host-cell.

Inside the host cell cytoplasm, the viral genome undergoes nucleocapsid uncoating to dissociate the N-proteins from the RNA. The genomic RNA is then ready to be transcribed and translated. The first to be produced is a



SARS-Cov-2 replicase polyprotein which is cleaved by the viral proteases into 16 non-structural accessory proteins that will form the RTC. Then a negative sense RNA template is synthesized. The stage is then set for SARS-Cov-2 to hijack the cellular machineries and reproduce itself as described above. Newly produced essential SARS-Cov-2 enzymes, S, M and E proteins, and the nucleocapsids are packaged into virions which bud into the lumen of the RER-Golgi apparatus to form mature virions which are released from the host-cell to start a new cycle of infection and propagation (22).

#### **SARS Cov-2 has a multiple cell tropism.**

Since the ACE-2 receptor is present on cells of multiple organs, SARS-Cov-2 can infect and multiply in all these organs. This multiple cell tropism of SARS-Cov-2 and its RNA genome give it the advantage of evolving more rapidly, of crossing the species barrier and propagating in other hosts, of infecting several vital organs of the body and of expressing itself in multiple clinical forms.

#### **Covid-19, the disease caused by SARS-Cov-2**

As with many other pathogens, the clinical course of infection with SARS-Cov-2 will be dependent on the inoculum and the virulence of the viral variant circulating in the population. Generally, the following phases are distinguished:

##### **The incubation phase:**

After contracting SARS-Cov-2, the incubation phase will last about 5 days. During this phase the virus continues to multiply in the body at high speed, but the infected person has not yet developed symptoms. The amount of virus accumulated in the body reaches a maximum between the 7<sup>th</sup> and 10<sup>th</sup> days after infection and then begins to drop over the following days under the pressure of the immune system.

##### **The clinical phase:**

The symptoms begin around the 6<sup>th</sup> day after infection and will continue to develop even though the amount of the virus in the body begins to drop under the pressure from the immune system. This phase can take several forms.

**In the weak or silent forms**, the patient does not develop symptoms and has no idea that he had contracted SARS-Cov-2. He will only discover this fact when his physician asks for anti SARS-Cov-2 antibodies in a routine laboratory blood-test examination that takes place weeks after exposure.

**In the active form** the patient begins to develop symptoms from the 6<sup>th</sup> day after infection. The intensity of these symptoms varies from one person to another. Some patients will develop a moderate form of the disease which peaks between day 10 and 14 and then returns to normal on 15<sup>th</sup> day after infection. Other patients will have their symptoms worsen with hyper-inflammation (Cytokine Storm Syndrome) requiring hospitalization and even admission in the intensive care unit (ICU). Some of the critical cases will sadly end in fatality. In fact, the human body's immune response to infection with SARS-Cov-2 is a double-edged sword. By trying to protect us, our immune system will also participate in the severe complications of the disease (9).

Patients at high risk of morbidity and mortality include the elderly and those suffering from chronic diseases [pulmonary, hepatic, renal (insufficiency, dialysis, CKD), arterial hypertension, cardiovascular (thrombotic, myocarditis, arrhythmia, heart failure, coronary syndromes), neurological (Dementia), endocrine (diabetic mellitus type 1 and 2), non-hematological malignant neoplasm, hematological neoplasm, immunodeficiency, transplants, Down Syndrome etc.].

#### **Vaccination**

**The first vaccination** in human would have been a passive maternal immunity in which the first women who got pregnant was able to immunize her fetus via transplacental transport of protecting antibodies during her pregnancy.

**Active vaccination** relies on the two responses of the immune system. Humoral immunity which uses antibodies to neutralize the virus circulating in the extracellular medium, and cellular immunity which uses cytotoxic T-Lymphocytes to destroy infected cells that are resistant to antibodies.

**Many technology platforms** were developed to design and prepare vaccines for a variety of diseases. Several of these technologies are today being implemented to develop vaccines against SARS-COV-2. The main types are listed in **Table-1** shown here-below.

<b>Table-1</b>					
<b>Institution</b>	<b>Vaccine Type</b>	<b>Antigen targeted</b>	<b>Doses</b>	<b>Storage</b>	<b>Efficacy</b>
Pfizer-BioNTech	mRNA	Spike Protein	2	-70°C	95%
Moderna	mRNA	Spike Protein	2	-20°C	95%
Novavax	Subunit	S & M proteins	2	+4°C	90%
Sinopharm	Inactivated Whole Virus	Whole virus	2	+4°C	79%
Johnson and Johnson	Non-Replicating Vector	Spike Protein	1	+4°C	72%
Astra Zenica	Replicating vector	Spike Protein	2	+4°C	62% - 90%
Gamaleya National center	Replicating Vector	Spike Protein	2	+4°C	92%

1) Vaccines based on **whole viruses**, inactivated or attenuated by various mechanisms

2) Vaccines based on **pathogen-protein-subunits** or based on **virus-like-particles (VLPs)**

3) Vaccines based on **replicating** or **non-replicating** viral vectors. This technology dates back to the 1980s. It uses non-pathogenic DNA viruses to infect the host-cells. The viral vector is genetically engineered to transport, deliver and express inside the host-cells, genes encoding immunogens that elicit a protective immunity in the host. However, live vaccines are risky in immuno-compromised individuals.

4) **DNA-based vaccines**. This technology dates back to the 1990s. These vaccines require the technique of electroporation to facilitate the entry of DNA into host-cells and increase its immunogenicity.

5) Vaccines based on **mRNA** which is non-replicating and non-self-amplified. The mRNA molecules are encapsulated in lipid nanoparticles called amphipathic micelles or LPNs for stability and to facilitate their passage through the cell membrane. This vaccine is not stable at room temperature, hence the need to store it in a frozen form at a very low temperature. Once inside the host recipient cell, the micelles release their mRNA content into the cytoplasm of the cell where it is translated into proteins that are processed and exported to the surface of the APCs to elicit a response from the immune system (**Fig-2**). The translated mRNA is degraded and does not enter the nucleus of the recipient cells or integrate into their genetic material.

**In a summary**, mRNA constitutes a minimal genetic vector which is non-infectious, non-immunogenic by itself, non-integrative, rapidly synthesized in the laboratory, packaged and effectively delivered, stable and efficiently translated by the ribosomes in the cytoplasm of the recipient cell, and is degraded after translation.

**Anti SARS-Cov-2 vaccines**: One year after the outbreak there are hundreds of vaccine projects in development, a few of which are already approved and used in vaccination campaigns, and several more are in clinical trials (**Tables-1**).

In this article we only discuss the mRNA vaccines encoding the S-protein of SARS-Cov-2. The mRNA is produced in vitro from a DNA template containing the open reading frame of the S-protein flanked at its 5-prime end by a Cap1 structure and an untranslated region (UTR) to confer mRNA stability, to stimulate mRNA biogenesis (splicing and transport) and to increase mRNA translation efficiency, and at its 3-prime end by an UTR and a polyA tail. The purified mRNA is encapsulated in lipid nanoparticles (LPNs) to enhance its uptake by the vaccinated cells (**17-19, 21, Fig-2**)

## Questions & Answers

### Why Should we develop vaccines against SARS-Cov-2?

Vaccines in general are the crown jewel of medicine. In contrast with therapeutic drugs, their use is less frequent, their protection from disease is for much longer and their cost is much lower. Vaccines are very important in supporting the economy by reducing the severity of diseases in the population and thus keeping it healthy and thriving (**30**). During the SARS-Covid2 pandemic an increase in all-cause mortality was reported (**31**). We cannot

wait for mass-population immunity (herd immunity) to be achieved by natural infections as this has been shown not to be an option in certain countries (32).

### **Can the foreign DNA or mRNA delivered into the cells of the vaccinated host integrate into and modify their own genetic material?**

A vaccine should be based on facts and scientific knowledge that change and evolve over time. To overcome the epidemic, we must act quickly but on solid principles. Still many uncertainties and many questions remain unanswered. The decision must be made according to the particularities of each person. As a reminder, human cells, with the exception of mature red blood cells, carnified cells of the skin, hair and nails, are nucleated eukaryotic cells. They have their genetic material in form of DNA organized and structured into chromosomes. The chromosomes are located in a separate compartment, the nucleus, which is surrounded by an active nuclear membrane. The communication of the nuclear membrane with the surrounding cytoplasm is highly regulated by the cell.

The human genomic DNA is made of regions called EXONS which encode for (or are expressed into) proteins, and regions called INTRONS which are non-encoding (or not expressed, or silent, or interfering). The introns make up the majority of human DNA. The exons are organized into genes and regulatory sequences. The latter govern the transcription of the encoding sequences within the gene [Open Reading Frame (ORF)] into mRNA, in the nucleus. The mRNA then leaves the nucleus to be translated into proteins by the ribosomes in the cytoplasm.

The process of protein synthesis in eukaryotic cells begins in the nucleus by transcribing the genes into primary mRNA molecules which undergo splicing (excision of introns and connection of exons) to produce mature mRNA molecules. The latter exit the nucleus to be translated into proteins by the ribosomes in the cytoplasm.

The insertion of a DNA fragment into another DNA molecule may not have any consequences or may cause profound changes. Depending on the position of the insertion, it can activate a new ORF, or inactivate an existing one. For these reasons the safety of directly injected or vectorized DNA vaccines raises legitimate questions about their safety. In principle, vaccines using genetic material derived from an infectious agent should not pose a risk to the vaccinated host given the care taken in the development of such vaccines. Also, we must not lose sight of the fact that even classical inactivated or live attenuated vaccines contain the entire infectious agent with its lipids, carbohydrates, proteins and of course its genetic material (DNA and RNA). Moreover, we have many years of experience with mRNA vaccines in malignant neoplasms and certain infectious diseases. In addition, mRNA is delivered into the cytoplasm of the recipient cell where it is translated by the ribosomes then is degraded after a short life. Therefore, it should not come in contact with the nucleus of the recipient cell where the chromosomes are located. But the use of replicating viral vectors which carry DNA molecules that obey to the same enzymatic molecular transactions as the human DNA raises questions about the possible interaction and integration of the viral DNA into the genetic material of human cells, and the consequences of such event (63 – 66).

### **Are our cells capable of degrading and recycling synthetic mRNA?**

The incorporation of ribonucleotides into RNA synthesis occurs only on the 3-prime carbon of the ribose molecule in nature. However, chemically it is also possible to use 2-prime carbon. Therefore, since the mRNA vaccine is made of synthetic mRNA molecules obtained by transcription from a DNA template:

- 1) Does the mRNA vaccine only include mRNA molecules that have been elongated on the 3-prime carbon?
- 2) What was done to avoid elongation on the 2-prime carbon or to get rid of such mRNA molecules if the vaccine produced contains a mixture of C-2 prime and C-3 prime mRNA molecules?
- 3) The mRNA is degraded after translation. Are human cells capable of degrading mRNA synthesized by elongation on 2-prime carbon that does not occur in nature in living systems?
- 4) Was the transcription process used to produce the mRNA vaccine proven not to produce partially synthesized mRNA fragments?
- 5) Does elongation on the C-2 prime produce partially synthesized or instable full mRNA molecules?
- 6) Does the mRNA vaccine only include a homogeneous population of mRNA molecules which were fully transcribed from the DNA template?
- 7) Does the mRNA vaccine include partially synthesized mRNA molecules and if yes what was done to remove them?
- 8) Are the partially synthesized mRNA molecules packaged in the LPNs with the full mRNA molecules?
- 9) Are the partially synthesized mRNA molecules stable inside the LPNs, and inside the vaccinated host?
- 10) Are the partially synthesized mRNA molecules translated into shorter polypeptides in the vaccinated host?

11) What happen to the polypeptides produced from partially synthesized mRNA molecules that are stable and translated?

We haven't yet seen published studies on these subjects. But given the complexity and efficiency of our biochemistry it is possible that our cells can handle it. If not, would this cause a long-term problem of cellular and genomic toxicity, especially if the vaccine has to be taken repeatedly? We have raised these questions before, and we are waiting for anyone with a sufficient and convincing answer (25 - 28).

We suggest the following preliminary basic research experiments to answer these questions. The first type of experiments would consist of determining whether the product of an in vitro synthesis of mRNA from a DNA template without cells, contains mRNA molecules whose elongation was made on the C-2 prime carbon. If so, what proportion does this type of molecules represent in the mixture? The second type of experiments would consist of blocking the C-3 prime carbon and synthesizing an mRNA by elongation only on the C-2 prime carbon. The third type of experiments would consist of injecting this C-2 primemRNA into cell cultures and measuring its toxic effect. Radioactive labeling or tagging of C-2 prime or C-3 prime may be one way to monitor mRNA synthesis in vitro (25 - 28).

The developers of mRNA vaccines and therapeutics may have already performed these studies since they should have shown biocompatibility studies to get approval from regulatory bodies. Or maybe academic and other independent groups have conducted research in this field. If so, please share your findings with us.

#### **Can a genetic vaccine induce or exacerbate autoimmune diseases?**

Yes, it is possible, but such complications would not only be seen with genetic vaccines. In theory, they can happen with any vaccine. For a vaccine to elicit or exacerbate an autoimmune response, it must confuse our immune system and cause it to fail in distinguishing between its own normal cells, and foreign antigens or self-abnormal cells. Autoimmune diseases develop when autoreactive T-Cells can make it through all the multilayer maturation processes and further escape the suppression by the immune system (29). However, many studies have shown the presence of autoantibodies in the sera of patients who were infected with SARS-Cov-2 (70 - 84). Some of these antibodies and the diseases they are associated with are listed in **Table-2** shown here-below.

#### **Can the vaccine cause autoimmune diseases similar to those caused by the natural infection?**

Vaccines directed against SARS-Cov-2 proteins such as the S-protein cause the production of antibodies which block the peptide domain used by the S-protein to anchor itself to the cytoplasmic membrane of the host-cells, at the ACE-2 receptor. In addition, these vaccines activate subclasses of T-Lymphocytes which confer a cellular immune response. There is a need to investigate whether the antibodies produced, or the T-cell subclasses activated by these vaccines, do or do not cross react with the peptide domains of angiotensin-2 and therefore block it also? If such a possibility exists, even at a minimal level, the consequences for our health would be very serious given the important role of angiotensin-2 in our physiology. The same question can be extended to all other vaccines using other SARS-Cov-2 proteins such as the M-protein, and to all known human proteins.

We propose the following preliminary experiments in basic research to try to answer these questions. From the SARS-Cov-2 genome, we can deduce the amino acid sequences of all of its proteins such as the S-protein whose mRNA is used in vaccines already available on the market. The amino acid sequences of many human proteins can also be obtained from the Human Genome Project database. Therefore, it is easy to determine the degree of homology between SARS-Cov-2 proteins and known human proteins. It is also known that the immune response generated by the natural SARS-Cov-2 infection, or elicited by the vaccine, is both humoral and cellular.

<b>Autoimmune antibodies</b>	<b>Autoimmune diseases</b>
Anti Nuclear Antibodies (ANA)	Systemic Lupus Erythematosus, Sjogren Syndrome, Scleroderma, Rheumatoid arthritis
Anti- $\beta$ 2 Glycoprotein I (Anti- $\beta$ 2 GPI)	Anti-Phospholipid Syndrome (thrombosis, thrombocytopenia, obstetric complications)
Anti-Angiotensin Converting Enzyme 2 (Anti-ACE-2)	Constructive vasculopathies
Anti-Annexin V	Systemic Lupus Erythematosus, Rheumatoid arthritis, Anti-Phospholipid Syndrome, Thrombosis
Anti-Cardiolipin	Systemic Lupus Erythematosus, Rheumatoid arthritis, Anti-Phospholipid Syndrome, Vasculitis
Anti-Contactin Associated Protein 2 (Anti-CASPR 2)	Encephalitis, Epilepsy, Ataxia, Neuropathic pain, Morvan's Syndrome, Isaac's Syndrome, Psychoses
Anti-Cyclic Citrullinated Peptides (Anti-CCP)	Rheumatoid arthritis
Anti-Heparin PF4	Thrombocytopenia, Venous Thrombosis
Anti-Muscle Specific Kinase (Anti-MuSK)	Myasthenia Gravis
Anti-Phosphatidylserine	Anti-Phospholipid syndrome, Venous Thrombosis, ST-elevation Myocardial Infarction
Anti-Prothrombin	Anti-Phospholipid syndrome (thrombosis, thrombocytopenia, obstetric complications)
Anti-Ro52	Myositis, Scleroderma, Liver diseases, interstitial lung disease, Connective Tissue Diseases
Anti-Ro60	Systemic Lupus Erythematosus, Sjogren Syndrome, Rheumatoid arthritis
Anti-Type I Interferons (Anti-IFNs)	Spread of Microbial Infections
Cytoplasmic Anti-Neutrophil Cytoplasmic Antibodies (C-ANCA)	Immunodeficiency, Vasculitis, Glomerulonephritis, Cholangitis, Inflammatory Bowel Disease
Lupus Anti-Coagulant (LAC)	Anti-Phospholipid Syndrome (thrombosis, thrombocytopenia, obstetric complications)
Perinuclear Anti-Neutrophil Cytoplasmic Antibodies (P-ANCA)	Glomerulonephritis, Polyarteritis Nodosa, Churg-Strauss Syndrome, polyangitis
Rheumatoid Factor	Rheumatoid Arthritis, Sjogren Syndrome, Viral infections
	Auto Immune Haemolytic Anemia
	Guillain Barré Syndrome
	Immune Thrombocytopenic Purpura
	Kawasaki Disease
	Miller Fisher Syndrome

Rapid experiments would consist of determining whether the sera of patients who have contracted the natural infection or who have been vaccinated react with human proteins which have homologies with the proteins of SARS-Cov-2. More detailed and laborious experiments would first consist of isolating and cataloging the antibodies produced as well as the subclasses of activated T-Lymphocytes, from naturally infected patients as well as from vaccinated patients. Second, the antigenic domains recognized respectively by these antibodies and these activated T-Lymphocyte subclasses are mapped on the SARS-Cov-2 proteins. We could then investigate whether these recognized antigenic domains also existed on human proteins and if they were also recognized. Cross-reaction against human proteins would suggest the possibility of autoimmune reactions (25 – 28).

As we explained above RNA viruses mutate frequently. This process can generate mutants that are more infectious, more virulent, more resistant to treatments, more evading to vaccines, more deceiving and confusing to the immune system, and possibly more lethal. Several investigators have reported the detection of autoantibodies in naturally infected patients (49 – 60, 70 - 84). Would the vaccines cause autoimmune disorders by eliciting autoantibodies and/or cross-reactive T-Cell subclasses? Only time will tell.

The developers of mRNA vaccines and therapeutics, and the regulatory bodies may have already asked themselves the same questions and probably have some confidential answers. The bottom line is it comes down to trusting the scrutinizing process of the multilayered system on the benefit/risk ratio of getting vaccinated.

### **Will vaccines have long-term consequences on our health?**

mRNA used in the vaccine is synthesized in vitro rather than being biologically produced. Therefore, it is much quicker to produce than conventional vaccines. mRNA vaccine technology is not new. It was in the development for decades. Since the mRNA degrades easily at ambient temperature, the progress was very slow. In 2005 researchers discovered that packaging the mRNA into small lipid particles called LPNs or micelles increases its stability. Thus, the use of mRNA in vaccine development became an achievable goal. Micelles can fuse with the cell membrane of the host-cell and the endosome membrane to release their mRNA content in the cytoplasm of the host-cells where the mRNA is translated by the cell machinery to produce the protein encoded by the mRNA (**Fig-2**). The questions we asked at this level have been detailed above and elsewhere. (25 - 28)

### **What do the proponents say about the safety of the mRNA vaccines?**

The security is the first concern in the mind of many including health professionals (34). Therefore, the issue of safety was analyzed, investigated and scrutinized. The arguments presented in favor of the safety of SARS-Cov-2 mRNA vaccines include past experience with this type of vaccines (39 – 41), the careful testing of the vaccine following all required steps in clinical trials (17), enrollment of large numbers of individuals in the clinical trials, setting short time intervals for the manufacturers to report their data to regulating bodies, and continuous monitoring for side effects in vaccinated individuals (36). Further, the relatively short time spent to develop these vaccines was due to the type of technologies used and in no way was due to skipping safety steps or lack of vaccino-vigilance or pharmaco-vigilance. (35). Moreover, the vaccine is synthesized in vitro without cell culture and therefore is free from cellular contaminants and does not cause more allergies (42). Finally, animal studies have shown that mRNA is quickly degraded (37) and remains localized in the area where it is injected (38).

### **What do the critics say about SARS-Cov-2 Vaccines?**

Many groups are against vaccination in general. They claim that vaccines cause chronic disorders such as autism, diabetes, asthma, allergies and multiple autoimmune diseases. They criticize the way clinical trials are designed, performed, and analyzed. They believe that vaccine products are tested on small non representative groups of volunteers, that the safety data is collected over a short period of time which does not allow for the detection of slow developing side effects such as chronic autoimmune and degenerative diseases. They insist that a proper clinical trial should include no less than 500,000 patients and a follow up for side effects of no less than 7 years.

A recent article published by a well-known critic of vaccination suggests that the mRNA molecules used in recently approved anti SARS-Cov-2 vaccines contain sequences which can be recognized by intrinsic proteins such as TAR-DNA-binding-Protein-43 (TDP-43) and Fused in sarcoma protein (FUS). This interaction would then convert TDP-43 and FUS to assume their prion-disease causing state. Further, it was theorized that if the anti SARS-Cov-2 mRNA vaccine induces TDP-43 and FUS to aggregate in their prion-based conformations, this will lead to neurodegenerative illnesses such as Alzheimer and Amyotrophic Lateral Sclerosis (ALS) diseases. Furthermore, it was theorized that the binding of SARS-Cov-2 S-protein to the ACE-2 receptor would cause the Zinc-containing Angiotensin Converting Enzyme 2 to unload its Zinc molecules which in turn will cause the TDP-43 to assume its pathologic prion state. Moreover, the author touches on the controversy surrounding the origin of SARS-Cov-2 and suggested that if SARS-Cov-2 was a man-made biological weapon, then vaccinating with the S-protein would put the humanity in danger in the case of a second attack using a pathogen guided by the S-protein used in vaccination. Finally, the author warns that whatever the natural infection may cause would be of no comparison to any serious illness that may be caused by a vaccine administered to a much larger number of people, globally (48).

### **Are the anti SARS-Cov-2 vaccines going to protect against all the viral mutants?**

It's possible because SARS-Cov-2 genetic variability is much lower than in other viruses for which we already have effective vaccines such as Influenza virus, Measle, Hepatitis B virus, Mumps, Ebola etc. (33). Moreover, the vaccine will lower the transmission of mutants in the population. However, there are also published reports which show that Pfizer/BioNTech vaccine BNT162b2 elicits two-third less neutralizing antibodies against a laboratory mutant mimicking the South African B.1.351 SARS-Cov-2 variant (47). More studies are needed to understand what has caused the downgrading of the production of neutralizing antibodies. Is it the vaccine? or is it the viral mutant?

After starting vaccination campaigns in many countries, the debate concerning the efficacy of these vaccines against SARS-Cov-2 South African variant B.1.351, Brazilian variant P.1, British variants B.1.1.7 (67) and other future variants has intensified (47). One solution to the emergence of SARS-Cov-2 variants which are resistant to current homogeneous mRNA vaccines which encode for the wild type of the S-protein would be the development of new

heterogeneous vaccines made of a mixture of mRNA molecules which encode for the wild and the different mutated types of the S-protein.

Such a task would be quickly accomplished by the pharmaceutical industry because the technology involved is easily adaptable and rapidly implemented. The relative part of each type of mRNA molecules in the vaccine mixture would be optimized to elicit a protective immunity against SARS-Cov-2 wild type and its mutants (68).

**Will the vaccine solve all the problems?**

No. The vaccine already protects against severe forms of SARS-Cov-2 infections and this is already a big step forward. But the vaccine will not solve everything for everyone right away. First, the vaccines were only tried on adults and not children. Second, it is not known whether the vaccines will prevent the transmission of infection, and whether they will confer herd immunity. We do not know the impact of different pathologies and treatments on the immune response to vaccines and we do not know their effectiveness in older people.

The solution may be in the development of therapeutic antibodies that both neutralize the circulating virus and stimulate immune cells to phagocytize the virus and produce a protective immunity (14)

**Do we have to continue safety precautions after vaccination?**

Yes. The vaccine protects against the severe forms of the infection but does not stop re-infection or transmission of the virus.

**Do all vaccines in development produce the same effect?**

No. An experimental study has shown that mice vaccinated with mRNA produced a more robust immune response in terms of long-lived B-memory immune cells and neutralizing antibodies than mice vaccinated with the S-protein plus adjuvant. It also appears that vaccines based on mRNA or genetically modified viruses induce protective humoral and cellular immune responses against severe forms of SARS-Cov-2 infection (95% and 90% of efficacy), unlike vaccines based on proteins and adjuvants (13).

**How does the mRNA vaccine look like?**

The vaccine does not come as a single dose in a pre-filled syringe like other vaccines you use to take. The mRNA-based SARS-Cov-2 vaccines are supplied in multidose vials. A dose of 0.5 ml contains, according to the producers, from 30 to 100 µg of single-stranded mRNA which encodes for the S-protein of SARS-Cov-2. The mRNA was synthesized in vitro from a DNA matrix, without cells, and encapsulated into lipid nanoparticles (LPNs), then added to a suspension of phospholipids, cholesterol, polyethylene glycol, trometamol, acetic acid, sodium acetate, sucrose and water.

**Are there any immediate side effects?**

Yes, but at low frequency. Those vaccinated mostly reported fatigue, headache, nausea, muscle and joint pain, pain and / or swelling and / or itching at the injection site and fever or chills. These side effects were more severe in young patients than in the elderly, and after the second injection than the first one (15). Other side effects have also been reported such as: syncope, shortness of breath, change in heart rate, swelling of the lips or face, discomfort in the throat, rash, hives, stomach pain and vomiting. Rare effects reported include facial paralysis, hypersensitivity to the vaccine, and severe allergic reactions (anaphylaxis).

**Should patients who have a special condition receive the mRNA vaccine?**

The Center for Disease Control and Prevention (CDC) has issued on **February 10, 2021** interim considerations for the use of mRNA vaccines currently authorized in the US (69). These considerations are summarized in (Table-3) shown here-below. The reader is advised to consult the CDC website for updates, and the local authorities in his country for specific instructions on the use of mRNA vaccines.

<b>Table-3</b>	
<b>Type of Situations you may have in your practice</b>	<b>What should you recommend to your patient about vaccination with mRNA vaccines?</b>
Known severe allergic reaction e.g. anaphylaxis	<b>No</b>
Previous allergic reaction to an mRNA vaccine	<b>No</b>
Hypersensitivity to Polyethylen glycol (PEG)	<b>No</b>
Age group less than 16 years old	<b>No</b>
Age group more than 16 but less than 18 years old	Pfizer/BioNTech
Age group 18 years old or more	Pfizer/BioNTech or Moderna
Coadministration with other vaccines	<b>No</b> . Minimum interval of 14 days. Except if the benefit of the second vaccine outweighs the risk
Need for a booster beyond the two doses	Has not been established
Testing for SARS-Cov-2 infection before vaccination	<b>No</b> need for testing. But patient should not have active symptoms
Patient with prior SARS-Cov-2 infection	<b>Yes</b> , except patient should not have active symptoms
Patient with active SARS-Cov-2 infection	<b>No</b> . Wait until symptoms are completely resolved and patient has met the isolation requirements
Patient who contracts SARS-Cov-2 after the first dose	<b>Yes</b> , but only after symptoms are completely resolved
Patient who received Monoclonal antibodies or convalescent plasma	<b>Yes</b> , but after 90 days, except if the treatment received was not specific to Covid-19 treatment
Patient who received Monoclonal antibody or plasma after first dose	<b>Yes</b> , but wait for 90 days before giving the second dose, except if the treatment was not for Covid-19
Can I take the vaccine as prophylaxis after exposure	<b>No</b> , because SARS-Cov-2 has a short preincubation period of 4-5 days, thus, it would not be effective
Congregate setting & vaccinated after exposure	<b>Yes</b> , if the resident does not have symptoms. It is a different rule than for non-congregate setting
The immunocompromised patient	<b>Yes</b> , if there is no contraindication to vaccination. But, lack of data about safety and benefit
I was vaccinated while taking immunosuppressive treatment	<b>No</b> need for revaccination after treatment is stopped and immunocompetence is regained
Patient with autoimmune conditions	<b>Yes</b> , But should be counseled about the lack of data about safety and efficacy
Patient with Guillain Barré Syndrome	<b>Yes</b> , if no contradiction to vaccination
Patient with Bell's palsy	<b>Yes</b> , if no contraindication to vaccination
Patient who received injectable dermal filler	<b>Yes</b> , if no contradiction to vaccination
Do I have to take a pregnancy test before taking the vaccine?	<b>No</b> recommendation
I took the vaccine should I stop trying to get pregnant?	<b>No</b> need to avoid pregnancy
I am pregnant should I take the vaccine?	Decision left to the patient after being counseled about the lack of safety data during pregnancy
The Lactating patient	Decision left to the patient after being counseled about the lack of safety data during breast feeding

### **Is a second dose of the vaccine necessary to have a stronger immunity?**

Maybenot. Recent scrutinizing of the data submitted to the FDA by Pfizer/BioNTech has shown that their vaccine BNT162b2 was actually highly effective with one dose. Given the worldwide shortage in vaccine, investigators have proposed the delay of the second dose until those in need have received the first dose (35,43 - 45). In its reply Pfizer stated the following: "we would like to emphasize that alternative dosing regimens of BNT162b2 have not been evaluated. The decision to implement alternative dosing regimens resides with health authorities; however, we at Pfizer believe that it is critical for health authorities to conduct surveillance on implemented alternative dosing schedules to ensure that vaccines provide the maximum possible protection" (46).

### **Should the vaccine be mandatory?**

No, because the vaccine is described as a tool that protects against severe forms of the disease and not as a tool that prevents the transfer of the virus from one person to another. The patient must therefore retain the freedom to decide

for his health and his body. On the other hand, if the vaccine prevents contagion and we cannot otherwise control the spread of the virus, mandatory vaccination becomes an option.

**Should a vaccination card be issued to the vaccinated?**

Yes. Proof of being vaccinated against certain diseases is already an obligation to travel to certain countries. But none of these situations quite compare to that of SARS-Cov-2 which affects the whole planet. There is therefore a conflict between the right to freedom of choice and the obligations that may be imposed depending on our type of work, our travel habits and our social activities profile etc. These fears are likely to motivate many of us to take the vaccine, even though being vaccinated does not mean being immune from getting the infection. For those of us who cannot get the vaccines due to severe health conditions, maybe isolation and quarantine are the solution.

**Are all these questions about SARS-Cov2 vaccines justified and why do we have to scrutinize the vaccines when the virus is doing worse during the natural infection?**

In one year, SARS-Cov-2 has infected almost 115million and killed almost 2.6million, globally. Although these numbers are devastating, they respectively represent 1.44% and 0.0325% of the world population estimated at 8 billion. Therefore, a vaccine that is supposed to be administered to 70 to 80% of the world population in order to bring SARS-Cov-2 under control must be highly scrutinized because any serious error could cause harm that by far surpasses what SARS-Cov-2 can do through natural infection.

We are an enthusiastic supporter of vaccination which is the crown jewel of modern medicine. We have spent several years of our life conducting research in this field (1 - 3). However, even if the questions are annoying, they should be answered in a thoughtful manner. Questions about the safety of the SARS-Cov2 vaccination can be resolved scientifically without indulging in unwarranted speculation. This can be answered by conducting basic research in immunology, cellular and molecular biology, microbiology and biochemistry. Our role as scientists and practitioner of the healing art is to openly debate the issues in a free academic environment. The pharmaceutical industry and regulatory agencies may then be interested in funding this type of research. It is not acceptable that drugs designed for use in humans become like other technological products which are sold to consumers before correcting their design flaws and then delegating the management of those problems to a customer service.

The pharmaceutical industry is a major player in health care. We have no doubt that its scientists conduct their research and development with diligence and ensure that their products are safe and effective.

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