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What is Known and Don't Known about Current m-RNA Vaccines Anti-COVID-19 in the Experts' Opinions

Udristioiu A[1], Cojocaru M[1] and Farmazon I[3]*

[1]*Titu Maiorescu University, Bucharest, Romania*

[2]*Laboratory Medicine, Bucharest, Romania*

Abstract

The patients with convalescent SARS-CoV-2 have a neutralizing antibody response that can be detected until 11 months after infection and is predominantly directed against the S protein, through which the virus binds to membrane receptors. The results revealed that neutralizing antibody responses in case of SARS-S may bring protection against SARS-CoV-2 infection.

To ensure they have safety standards, COVID-19 vaccines were tested in large clinical trials. In these trials, a lot of people were recruited to assist to understand how the vaccines can offer public protection regardless races, ages, ethnicities or different medical conditions. World Health Organization (WHO) has chosen the best vaccine candidates, in order to develop fastest vaccine taking into account efficacy, safety standards, and provide them government support. Messenger RNA (mRNA) vaccines have the same efficacy and safety standards as any type of vaccines in the Europe and United States.

Conclusion: The advantages and risks for immunocompromised patients who received a vaccine against SARS-CoV-2 should be considered from case to case, with discussion of the incidence of infection in the community. Vaccines are not a perfect solution. Other measures should be still needed to be practice like social distancing, hand washing, wearing a mask until public health.

Keywords: ACE2 receptors; CRISPR-Cas12-13 technology; Messenger ARN; SARS-CoV-2 vaccine; Spike protein S

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Correspondence:
Aurelian Udristioiu, Department Molecular Biology, Titu Maiorescu University, Damboviciului, Street No: 2, Postal Code: 040051, District 4, Bucharest, Romania, Tel: 40723565637;
E-mail: aurelianu2007@yahoo.com

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Introduction

SARS-CoV-2 uses a protein called S or Spike to attach to the ACE2 receptors to the cells. ACE2 is an angiotensin converting enzyme found in cells in the lungs, heart, arteries, kidneys and intestines and to a lesser extent in other tissues. The ACE2 receptor catalyzes the hydrolysis of angiotensin II into angiotensin, decreasing blood pressure. Once the virus enters the cell, viral RNA is released, RNA polyproteins are translated, and replication and transcription of the viral RNA genome takes place by cleaving proteins and assembling the replicase-transcriptase complex at the ribosomes. Thus, the proteins are synthesized and "packaged" in the host cell, after which these viral particles are released, in turn infecting other cells.

The mRNA vaccine, instead of injecting the viral protein, injects a genetic material, messenger RNA that encodes this protein. The mRNA is basically the code that contains instructions and will be used as a template for creating proteins, and in this case, it contains instructions on how a Spike protein can be produced. So, instead of introducing the virus or Spike protein into the cells, he teaches the body how to produce such proteins on its own by giving it the template to produce them. The mRNA is composed of several elements, head, tail, two regions that indicate the beginning and end of the region containing that code, (http://www.eb.tuebingen.mpg.de/).

The ends of the mRNA, called the 5 'head and 3' poly (A) tails, are actually sequences of adenilic nucleotides and help protect the degradation code. The number of these sequences influences their lifespan before they are destroyed, because, over time, these ends are shortened, in the absence of specific polymerase [1].

The form of mRNA vaccines, liposome encapsulated mRNA also (saRNA) self-amplifying have undergone to preclinical evaluation. The immunogenicity and expression of SARS-CoV-2 spike trimers has been improved by prefusion-stabilizing mutations resulting in the mRNA-1273 vaccine, which formulated in LNPs induced both potent neutralizing antibody and CD8+ T cell responses

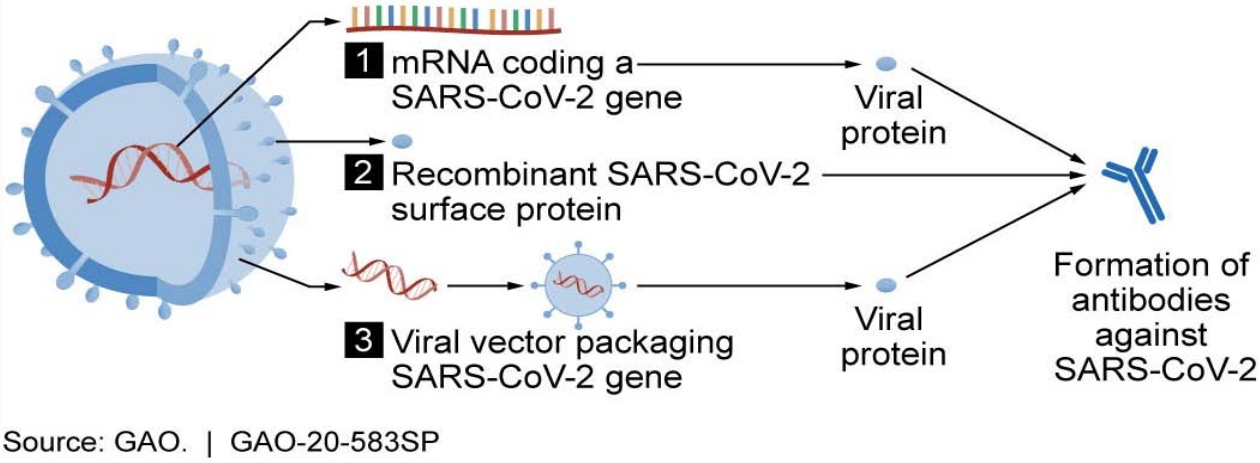

Figure 1: RNA vaccines in the form of mRNA, liposome encapsulated, have been subjected to preclinical evaluation mRNA coding a SARS-CoV-gene into the cells teach. The body how to produce such proteins on its own is given in the template to produce them.

and protected immunized mice against SARS-CoV-2 infection, (https://www.newscientist.com) (Figure 1).

In another research, mRNA has been adjusted in the 5′ and 3′ end un translated regions and in the open reading frame to guarantee the translation of ideal levels of protein (https://www.curevac.com/mrna-platform). LNP mRNA-based delivery has previously been demonstrated to provide protection against CHIKV challenges in immunized mice and has been applied for COVID-19 vaccine development by engineering several SARS-CoV-2 constructs (https://www.curevac.com/covid-19).

Main test

Messenger RNA (mRNA) based vaccines:

First results from the research (July 1st, 2020) revealed that immunization with BNT162b1, the modified RNA vaccine at doses of 30 and also 100 μg resulted in dose-related RBD-binding IgG concentrations and SARS-CoV-2 neutralizing titers. Also neutralizing titers were 1.8 to 2.8 fold higher than it was observed in a panel of COVID-19 [2].

The main vaccine candidate obtained high levels of neutralizing titers in lab animal models. Also another vaccine candidate has initiated a program with LNP mRNA-based delivery, which determined strong antibody responses in animal models, (https://www.daiichisankyo.com).

Vaccination should be the option for people irrespective of history of prior asymptomatic or symptomatic COVID-19 infection. Testing for the evaluation of previous infection is not recommended for vaccine decision-making only. What mRNA vaccine manufacturers said in 2020:

- -mRNA vaccines cannot give someone COVID-19; mRNA vaccines do not use the live virus that causes COVID-19.
- -mRNA vaccines don't interact or alter with DNA in any circumstances, mRNA don't enter in the nucleus of the cell where the DNA is preserved. After the instructions are finished, the cell disposes and removes the mRNA soon.
- -COVID-19 vaccines are only for non-pregnant adults, after other researches are ended, the COVID-19 vaccine perhaps will be available for children.
- Because a number of people required to have protection to achieve mass immunity is different by disease, the experts cannot predict the number of people who need to get the vaccine for achieving the mass immunity to COVID-19 [3].

The list of candidates for vaccine who published results from 1 to 3 phase clinical trials comprises three vaccines (Moderna mRNA-1273, Pfizer-BioNTech BNT162b2 and Oxford-AstraZeneca AZD1222) who have at least 85% to 90% efficacy in producing target levels of neutralizing antibody with excellent safety profiles, according to their respective pharmaceutical companies [4-8].

Main SARS-CoV-2 vaccine candidates use new mechanisms or the conventional ones to acquire an immune response. Conventional methods used attenuated inactivated virus and recombinant viral protein vaccines to develop immunity. New process includes replication-deficient, adenovirus vector-based vaccines that contains the SARS-CoV-2 spike protein and mRNA-based vaccines that encode for a SARS-CoV-2 spike protein [9,10] (Figure 2).

After the vaccine, if the person contacts the SARS-CoV-2 virus, it is recognized by the immune system, who is ready to attack it. For protection against COVID-19, the immune cells and antibodies will work together, preventing the virus to enter into the body's cell or kill the virus destroying infected cells [11], (Scheme 1).

Mostly clinical trials of vaccines are focused on the spike coronavirus protein and its variants as the main antigen of COVID-19 infection. Platforms who had been evolved in 2020 involved nucleic acid technologies (nucleoside-modified messenger RNA and DNA), non-replicating viral vectors, live attenuated viruses, and inactivated viruses, peptides, recombinant proteins [12,13].

Excepting one of the vaccine candidates, other were tested require two separate inoculations separated by three 3 to 4 weeks. New challenges for approving the SARS-CoV-2 vaccine are manufacturing to sale, reconstitution and administration, particularly for the lipid nano-particle mRNA-based vaccines, which require low temperatures for adequate vaccine preservation, distribution, storage conditions. Most importantly, a willing public is essential for receiving the vaccine.

Messenger RNA vaccines against SARS-CoV-2

Eric J. Topol
Scripps Research, La Jolla, CA, USA
Correspondence: etopol@scripps.edu
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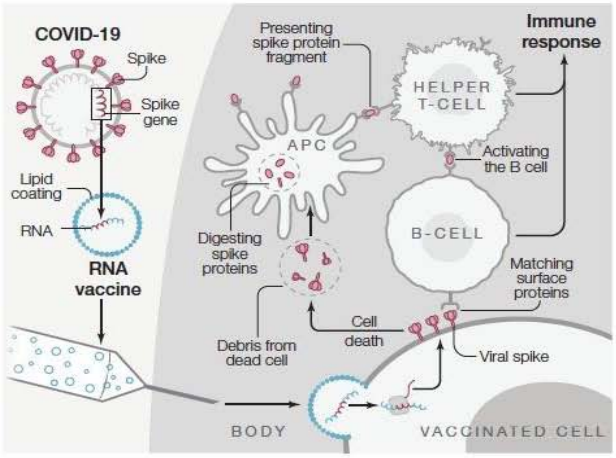


The first two vaccines proven to be effective for inhibiting COVID-19 illness were both mRNA, achieving 95% efficacy (and safety) among 74,000 participants (half receiving placebo) after intramuscular delivery of two shots, 3–4 weeks apart.

NAME
Pfizer-BioNTech and Moderna SARS-CoV-2 mRNA vaccines

APPROVED FOR
Emergency authorization, ages 16 and older, vaccination against SARS-CoV-2 infection

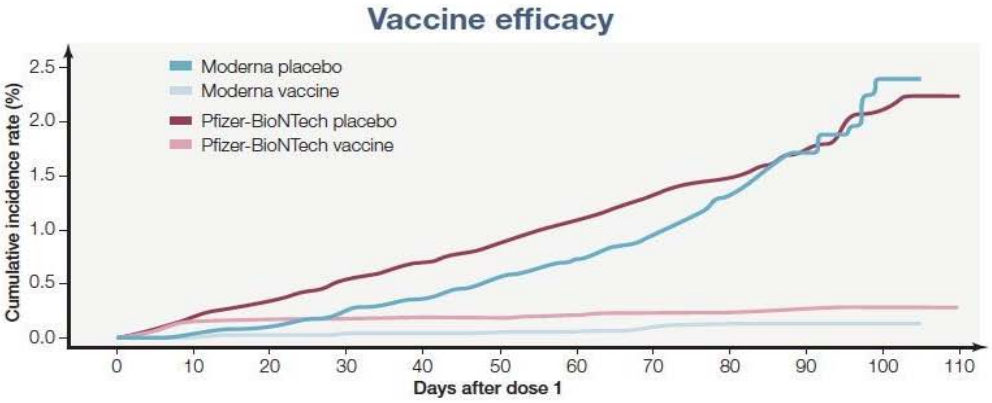
TYPE
mRNA in lipid nanoparticles

MOLECULAR TARGETS
The viral spike (S) glycoprotein

CELLULAR TARGETS
The vaccine induces B cell production of antibodies to the virus's spike protein. T cells are also elicited, particularly CD4+ and CD8+ against the SARS-CoV-2 spike protein.

EFFECTS ON TARGETS
Antibodies bind to target sites on the SARS-CoV-2 surface glycoprotein and either neutralize it or inactivte virions for destruction and clearance by the immune system.

DEVELOPED BY
BioNTech/Pfizer and Moderna/NIH VRC



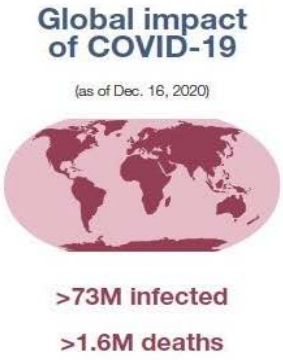
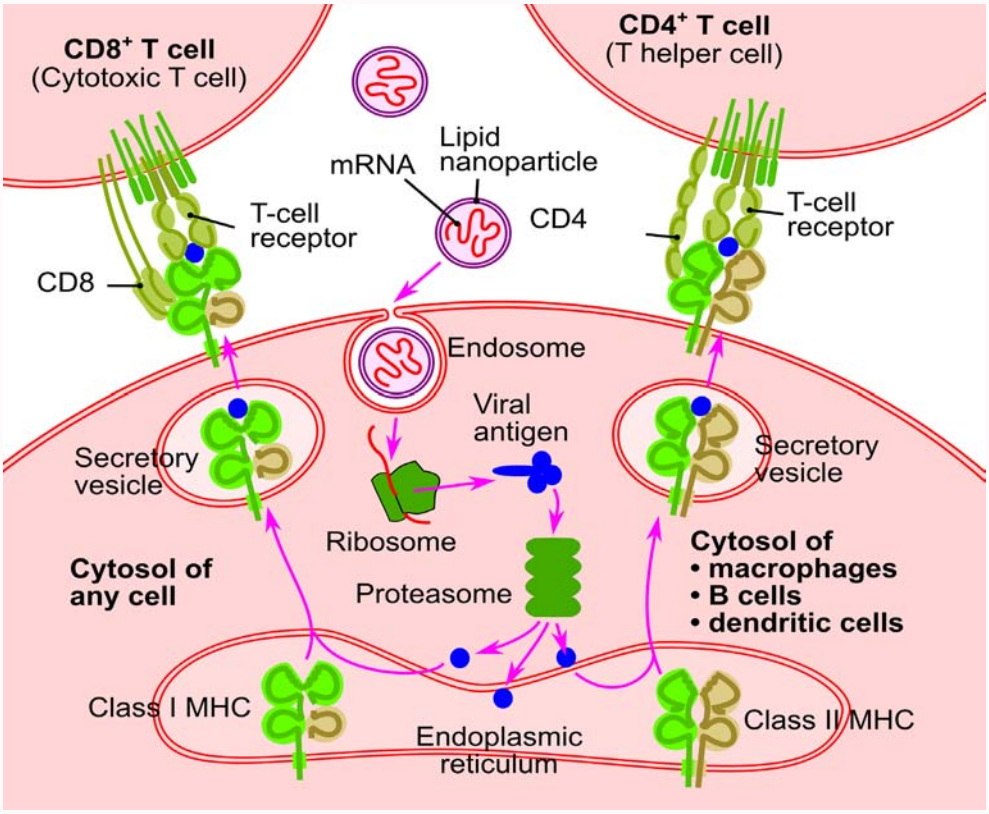


Figure 2: Messenger RNA vaccine against SARS-CoV-2. Leading SARS-CoV-2 vaccine candidates use either conventional or novel mechanisms of action to elicit an immune response in patients. Conventional methods included attenuated inactivated (killed) virus and recombinant viral protein vaccines to develop immunity.

The CRISPR-Cas12-13 technology is one of the solutions for gene editing, being a versatile, simple program, which simultaneously analyzes RNA and DNA chain sequencing based on a genetic panel. CRISPR-based nucleic acid detection methods can function at a constant temperature (37°C), different from RT-PCR-based methods, who needs an expensive thermal cycler.

The method is more advantageous, having the ability to analyze up to thousands of samples at once, the ability to examine each sample for changes in multiple RNA sequences. The targeted nucleases, Cas12a and Cas13a, gave researchers the ability to control virtually any genomic sequence, allowing easy creation of isogenic cell lines for the study of human disease and promoting exciting new possibilities



Scheme 1: The vaccinated person comes into contact with SARS-CoV-2; the immune system recognizes the virus and be prepared to attack it. The antibodies and immune cells can work together to kill the virus, prevent its entry into the body's cells and destroy cells that are infected, thus helping to protect against COVID-19. (Layers miRNA Lipid nanoparticle; Endosome Ribosome; Viral antigen; Proteasome; e-Endoplasmic reticulum; Class I MHC, Class II; MHC Cytosol of any cell Cytosol of •macrophages•; B cells•; dendritic cells; Secretory vesicle CD8 CD4 T-cell receptor; CD8+ T cell (Cytotoxic T cell) CD4+ T cell (T helper cell)

for human gene therapy.

The immunocompromised patient populations who have hematological diseases may have attenuated or don't have a response to SARS-CoV-2 vaccines:

- Primary or secondary immunodeficiencies which concern adaptive immunity.
- B cell directed therapies [e.g., blocking monoclonal antibodies against CD20 or CD22, bispecific agents like blinatumomab, CD19 or CD22-directed CAR-T cell therapies, BTK inhibitors].
- Splenectomy or functional asplenia [e.g., sickle cell disease].
- T cell directed therapies [e.g., calcineurin inhibitors, anti-thymocyte globulin, alemtuzumab.
- Splenectomy or functional asplenia [e.g., sickle cell disease].
- Many chemotherapy regimens.
- Hematopoietic Cell Transplantation (HCT), especially within the first 3 to 6 months after autologous HCT and often longer after allogeneic HCT.
- Underlying aberrant immunity [e.g., Graft-*vs.*-Host Disease (GVHD), graft rejection, absent or incomplete immune reconstitution, neutropenia ANC<500/μL, lymphopenia ALC<200/μL].
- High-dose corticosteroids (μ20 mg per dose or >2 mg/kg/day daily prednisone or equivalent) [14].

Following considerations for previous testing before administration of SARS-CoV-2 vaccine at immunocompromised patients include: peripheral blood B and T cell immunophenotype, CBC with differential, quantitative immunoglobulins (IgG, IgM, IgA), Staphylococcal, Pneumococcal titers to reveal if a patient has the potential to have a protective immune response. The effect of these parameters targeting the responses to SARS-CoV-2 vaccines is unknown for a long time.

In the case of RNA vaccine candidates, the side effects that were reported until now have been similar to those which were found in the seasonal influenza vaccination, but could be more regular given the 2 inoculations schedule for most SARS-CoV-2 mRNA vaccines, fever (<50%), injection site soreness (<60%), cephalalgia (<42%), arthralgia (<24%) and fatigue (<28%).

It will be recommended at least 2 up to 4 weeks before to the planning of immunosuppressive therapy, splenectomy or transplant if the plan targeting the vaccine against SARS-CoV-2 will be made. Given the circumstances the patient has received or is receiving an immunosuppressive therapy, it will be recommended to get the vaccine after six months holding the therapy in order to increase his immunity [15,16]. Most researchers recommend vaccinations only if it is safe to apply, despite that the general population will have a protection rate higher as their [17]. There is needed more information even if measuring titers can be useful to estimate the response. For now, higher doses or too many inoculations with an approved vaccine against SARS-CoV-2 isn't recommended [18].

Cellular stress and autophagy process: Evidence shows that

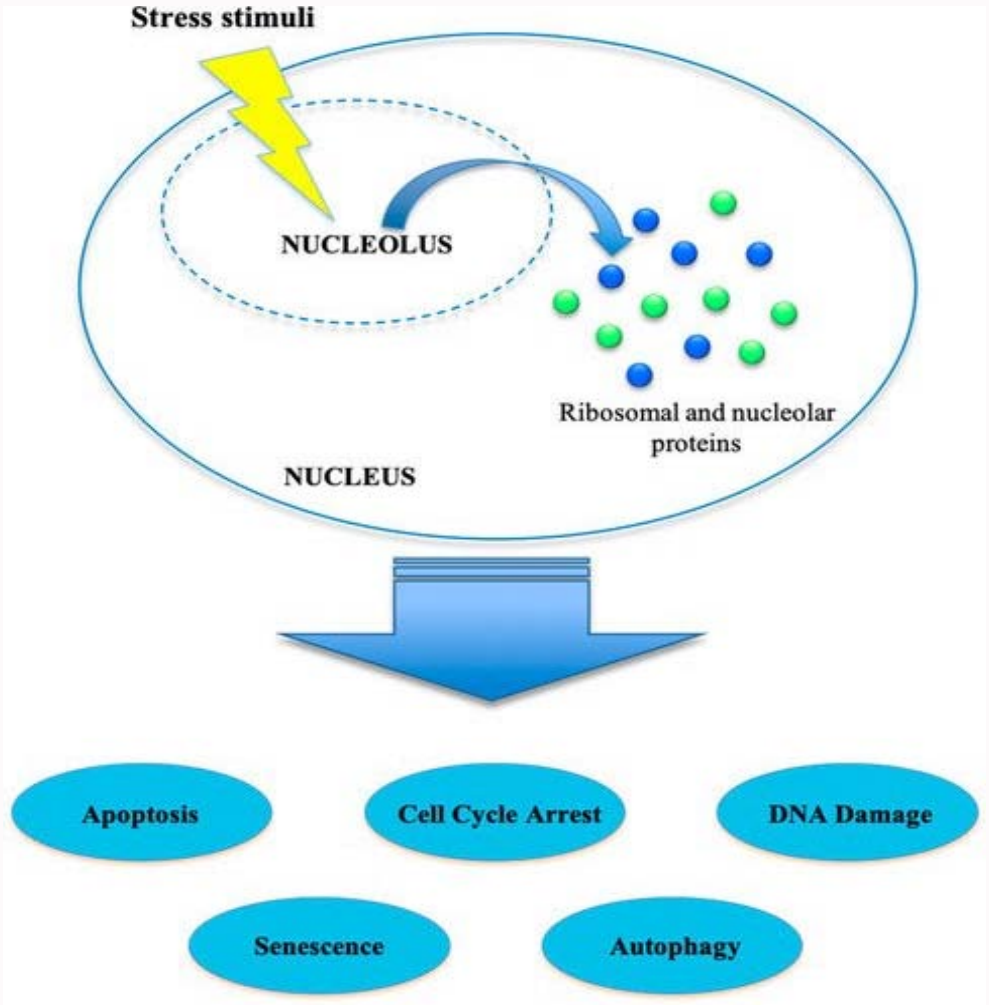


Figure 3: Several stress stimuli can activate a cellular-stress-response pathway known as nucleolar stress. This condition is mediated by different ribosomal proteins and/or nucleolar proteins that are released from the nucleolus to the nucleoplasm, leading, through the activation of specific pathways, to apoptosis, cell-cycle arrest, DNA damage, senescence and/or autophagy.

autophagy induction is connected to the alteration of the expression of certain ribosomal proteins. Disruption of the ribosomal P complex activates stress-mediated autophagy. Diverse researchers indicate the implication of the nucleolus-resident RNA Polymerase I (Pol I), the main protagonist in ribosomal RNA precursor transcription, in the nucleolus-mediated autophagy [19]. It is acknowledged that the specific inhibition of Pol I cause nucleolar disruption and therefore to the translocation of several nucleolar proteins from the nucleolus to the nucleoplasm. The final phase in the autophagic process is the fusion of autophagosomes with lysosomes in order to degrade the autophagosome content [20].

A sub-nuclear compartment, known as the nucleolus, is identified as main actor in the cellular-stress response. The activation pathway of nuclear stress determinate activation of apoptosis, in cell-cycle blocking, senescence and DNA damage. Functional and genomic studies have assigned to nucleolus novel non-canonical roles, other than well-known several proteomic, nucleolar canonical function as site of the ribosome biogenesis, such as genome stability, cell cycle control, cellular senescence and biogenesis of Ribonucleoprotein Particles (RNPs) [21] (Figure 3).

A wide range of stress stimuli may impair the nucleolar structure and/or function, leading to a complex cellular response, namely nucleolar stress, able to activate p53-dependent or -independent signaling pathways. Under physiological condition, cytosolic p53 protein exerts a negative regulation on autophagy through a transcription-independent mechanism. This inhibitory effect involves the AMPK–mTOR signaling pathway *via* inhibition of AMPK and, consequently, activation of mTOR. Nuclear p53 acts at different levels: It induces the expression of many autophagy-related genes, such as ATGs, ULK1 and Parkin; it inhibits the mTOR pathway *via* activation of AMPK or by increasing PTEN expression; and it induces Beclin 1 through BAX and Bcl-2 regulation [22].

The quality-control systems of the Endoplasmic Reticulum (ER) selectively establish tracking of the well-folded proteins and aim the misfolded ones for proteolysis. Different conditions can alter the ER homeostasis, such as hypoxia, nutrient deprivation, drug-induced toxicity, acidosis, and irradiation, resulting in the accumulation of the misfolded and unfolded proteins within the lumen of the ER and, therefore, contributing to ER stress development [23].

The inhibition of rRNA transcription and activation of pro-death autophagy were as a result of the anticancer properties of this nanoplatform [24]. A better understanding of the stress pathways as nucleolar and endoplasmic reticulum stress and autophagy could open novel avenues for investigating specific and effective

pharmacologic targets for new drug development and therapeutic approaches in processes of death autophagy [25,26].

MicroRNAs (miRNAs) are evolutionarily conserved non-coding RNAs that involve 19 to 25 nucleotides and imply by cleavage from 70 to 100 nucleotide hairpin precursors. They hybridize to complementary messenger RNA (mRNA) targets and the most frequent function by inhibiting translation of specific mRNAs. miRNAs have offered a new dimension to our understanding of complex gene regulatory networks. miR-NAs can operate as oncogenes or as tumor suppressor genes in leukemia's, although the data for as for the last are more limited. Also, it is known that some type of microRNA(miRNA), suppressed, like the microRNA, miR-15a/16-1 cluster from 13q14-Minimal Region Chromosome, (MDR), causes development of indolent B cell-autonomous, clonal lympho-proliferative disorders observed in humans hematological diseases [27].

Many studies have indicated that aberrant expressions of microRNAs have a role in the initiation and development cancer. p53 has been reported to be down regulated past 50% in different cancer diseases and moreover, it was predicted to be the target gene of miR-214 using bioinformatics software programs. Furthermore, luciferase reporter vectors were constructed and it was confirmed that p53 is a target of miR-214. Following the transfection of miR-214 into in cancer diseases, especially breast cancer cells, were found that the over expression of miR-214 markedly enhanced cell invasion through the down regulation of p53 expression. Instead, the over expression of p53 repealed the effects of miR-214. To conclude, this research proves that miR-214 functions as an oncogene, at least partly by promoting cell invasion through the down regulation of p53 [28].

Moreover, their localization, together with the levels of miRNAs and target mRNAs, and the affinity of the miRNA-mRNA interaction, are important for gene regulation. It is not known the relation which can be between a microRNA (miRNA and messenger RNA (mRNA) in the cytoplasmic cellular stress but is expected a negative correlation between expression levels of these putative target mRNAs and the corresponding miRNAs [29].

Malignant transformation is defined by disorder of diverse cellular processes that have been the subject of detailed structural studies, biochemical and genetic, but only currently has evidence emerged that many of these processes appear in the context of biomolecular condensates of RNA molecules with related functions.

RNA molecules play regulatory roles in diverse biomolecular condensates, including the nucleolus, transcriptional condensates, co-transcriptional splicing condensates, nuclear speckles, paraspeckles, and stress granules. Localization of nuclear condensates can be mediated by proteins that bind to specific DNA or RNA sequences (left), and cytoplasmic condensates can form at sites on the plasma membrane and regulation of condensates can occur at many levels, for example, Post-translational Modifications (PTMs) of molecules or the presence of RNA may change the properties that influence formation insights into dysregulated metabolic processes in cancer [30].

The RNA is degraded by a sequence specific process known as RNA interference, (RNAi), in eukaryotic cells. This is a particular sequence post transcriptional gene silencing that degrades RNA by double standard RNA precursor which are often small RNA (21-23 nucleotides known as si-RNAs. The process of RNAi requires ATP and is mediated by proteins which are dsRNA specific RNAs-III type nucleases. A dsRNA is introduced into cytoplasm of cell to activate the process of RNAi. Dicer enzyme along with other co-factors generates the pool of siRNA, by processing the dsRNA. The generate a siRNA are double standard RNA-21 base pairs in length and have 2 nucleotides overhangs at both 3`end [31].

Transfection of synthetic siRNA can stimulate RNAi mediated virus inhibition. Although siRNA is an effective therapeutic against viral infection but still important impediments including stability of RNA molecule and off-target effects which are resulted from the suppression of m RNA other than target m RNA. These effects are off target and related to toxicity and immune effect intrinsic to RNAi itself, interference with endogenous mi-ARN machinery and delivery vehicle.

These obstacles require to be considered before using the siRNA as a drug against viral infections. Both vector based and chemical synthesized siRNA have several downsides before being used as therapeutics. IFN responses had been examined to determine the blocking of gene expression by siRNAs. One of limit siRNA is the over-production of siRNAs that can influence machinery necessary for the cell regulation [32].

SARS virus is a member of Nido-viral family and has a positive-stranded RNA genome which is known for its repetitive mutation and because of that it is difficult to develop a vaccine against it. Specific siRNA has been applied to target viral spike NP-RdRP envelope protein and leader sequence to interrupt the SARS-CoV-2 replication. These siRNA restrained the viral gene expression and consequently it can be proposed that RNAi therapy could be an effective option for the prevention and treatment of SARS [33].

New perspectives: Applying the newest techniques to cultivate and sequence the genome of tobacco plant, researchers participating in the European funded New Project reported they are capable to produce tailor-made molecules to combat disease. By injecting other DNA into tobacco leaves, its genes can be changed for creating specific pharmaceutical products in very large quantities. Hopes are high, the technique which harnesses the plant's cells and sap, could thereafter help facilitate the production of a COVID-19 vaccine. Researchers claim the approach has many advantages. It can produce large amounts of the mandatory protein and is also affordable compared to other methods [34].

In Valencia, at the Institute for Plant Molecular and Cellular Biology, a team of top researchers are aiming to harness the power of nature. This procedure is done by introducing genetic material into their genome. This contains information that allows the production of medicines such as antibodies, vaccines and other products. The extract of tobacco contain the acid nicotinic with stimulate NADH mitochondrial of chain receptors, accelerated mitochondrial cellular to be stronger in invasion of viruses, increasing the resistant cells to viral invasion [35].

The experts have suggested nicotine attaches to the ACE2 receptors, through preventing the virus from attaching and potentially reducing the amount of virus that can infiltrate into lung cells. The experts are now planning a randomized trial involving nicotine patches to test their hypothesis, though the French health authorities didn't approve it yet.

World Health Organization (WHO) is ceaselessly evaluating new research, considering all researches including who examines connection between tobacco use, nicotine used and COVID-19 and

desire the researchers, scientists and the media to be careful about amplifying unproven claims that tobacco or nicotine could reduce the risk of COVID-19 disease. Until now there is currently insufficient information to confirm any link between tobacco and nicotine in the prevention or treatment of COVID-19 [36].

Known unknowns: The scientific community is still scratching the surface of its understanding of SARS-CoV-2. Key unknowns include the number of ACE2 receptors needed to bind to each spike protein; when exactly the S2 site is cleaved by TMPRSS2; and the number of spikes needed for virus–cell membrane fusion and that's just for entry [37].

Also, at the least cases, in the lysosomal storage disorders, (deficiency of lysosomal enzymes, ex. Beta-Galactosidase), mRNA Messenger of covid vaccine could be escape from lysosome before to be destroy and can have an interference with m-RNA of cells.

Conclusion

The risks and benefits for immunocompromised patients receiving a SARS-CoV-2 vaccine should be weighed on a case-by-case basis, with consideration of the incidence of infection in the community. Vaccines are not a perfect solution. Other measures should be still needed to be practice like social distancing, hand washing, wearing a mask until public health.

References

1. Ignătescu M. The mRNA vaccine, how does it work? Danivers 12-01-2020.
2. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase 1/2 study of a COVID-19 RNA vaccine BNT162b1. Nature. 2020.
3. American Medical Association (AMA). COVID-19 VACCINES. Vaccine safety and the speed of vaccine development.
4. Goodman JL, Grabenstein JD, Braun MM. Answering key questions about COVID-19 vaccines. JAMA. 2020.
5. Xia S, Duan K, Zhang Y, Zhao D, Zhang H, Xie Z, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: Interim analysis of 2 randomized clinical trials. JAMA. 2020;324(10):951-60.
6. Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, et al. Phase 1-2 Trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. N Engl J Med. 2020;383:2320-32.
7. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Bellamy D, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: A preliminary report of a phase 1/2, single-blind, randomized controlled trial. Lancet. 2020;396(1024):467-78.
8. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou L. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: A randomized, double-blind, placebo-controlled, phase 2 trial. Lancet. 2020;396:479-88.
9. Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med. 2020;383:2427-38.
10. Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two rna-based COVID-19 vaccine candidates. N Engl J Med. 2020;383(25):2439-50.
11. EMA's human medicines committee (CHMP). EMA starts rolling review of Janssen's COVID-19 vaccine Ad26.COV2. S.
12. Diamond MS, Pierson TC. The challenges of vaccine development against a new virus during a pandemic". Cell Host and Microbe. 2020;27(5):699–703.
13. Le T, Cramer JP, Chen R, Mayhew S. Evolution of the COVID-19 vaccine development landscape. Nat Rev Drug Discov. 2020;19(10):667-8.
14. Auletta J, Chemaly R, Khawaja F, Papanicolaou G, Hill J. ASH-ASTCT COVID-19 and Vaccines: Frequently asked questions.
15. Dagnew AF, Ilhan O, Lee WS. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in adults with haematological malignancies: a phase 3, randomised, clinical trial and post-hoc efficacy analysis. Lancet Infect Dis. 2019;19:988-1000.
16. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018;17:261-79.
17. Bolke E, Matuschek C, Fischer JC. Loss of anti-SARS-CoV-2 antibodies in Mild COVID-19. N Engl J Med. 2020;383:1694-5.
18. Diego OrzáezTerpos E, Mentis A, Dimopoulos MA. Loss of anti-SARS-CoV-2 antibodies in mild COVID-19 the production of medicines such as antibodies, vaccines and other products. N Engl J Med. 2020;383:1695.
19. YangK, Yang J, Yi J. Nucleolar stress: Hallmarks, sensing mechanism and diseases. Cell stress. 2018;2:125–40.
20. Mrakovcic M, FröhlichLF. p53-mediated molecular control of autophagy in tumor cells. Biomolecules. 2018;1:18-14.
21. Katagiri N, Kuroda, T Kishimoto H, Hayashi Y, Kumazawa T, KimuraK. The nucleolar protein nucleophosmin is essential for autophagy induced by inhibiting Pol I transcription. Sci Rep. 2015;5:8903.
22. Tasdemir E, Maiuri MC, Galluzzi, L, Vitale I, Djavaheri-Mergny M, D'AmelioM, et al. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol. 2008;10(6):676–87.
23. Ding WX, Ni HM, Gao W, Hou YF, Melan MA, Chen X, et al. Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. J Biol Chem. 2007;282(7):4702–10.
24. Du Y, Yang M, Du Z, Feng C, Xing C, Wu Y, et al. CX-5461-loaded nucleolus-targeting nano-platform for cancer therapy through induction of pro-death autophagy. Acta Biomater. 2018;79;317–30.
25. Pasquier B. Autophagy inhibitors. Cell Mol Life Sci. 2016;73(5):985–1001.
26. Rosmino C. Powerful properties: how tobacco is being used to fight COVID-19. Plant Molecular and Cellular Biology in Valencia, a team of top scientists are attempting to harness the power of nature, last updated: 27/05/2020.
27. Klein U, Lia M, Crespo M, Siegel R, Shen Q, Ambesi-Impiombato A, et al. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Proc Natl Acad Sci. 2002;99(24):15524-9.
28. Rehei AL, Zhang L, Fu YX, Mu WB, Yang DS, Liu Y, et al. MicroRNA-214 functions as an oncogene in human osteosarcoma by targeting TRAF3. Eur Rev Med Pharmacol Sci. 2018;22(16):5156-64.
29. Laxman N, Rubin CJ, Mallmin H, Nilsson O, Pastinen T, Grundberg E, et al. Global miRNA expression and correlation with mRNA levels in primary human bone cells. RNA. 2015;21(8):1433-43.
30. Boija A, Klein IA, Young RA. Biomolecular Condensates and Cancer. Cancer Cell. 2021;39(2):174-92.
31. Ashfang UA, Yousaf MZ, Aslam M, Ejaz R, Jahan S, Ullah O. siRNA: Potential therapeutic agent agains hepatitis C virus. Virol J. 2011;8-274.
32. Bridge AJ, Pebernard S, Ducraux A, Nicoulaz AL, Iggo R. Instruction of Interferon response by RNAi vectors in mammalian cells. Nat Genet. 2003;34(3):263-4.
33. Meng B, Lui Y, Meng S, Cao C, Hu Y. Identification of effective siRNA blocking the expression of SARS viral envelope E and RDRP genes. Mol Biotechnol. 2006;33(2);141-48.
34. Rosmino C. Powerful properties: How tobacco is being used to fight COVID-19. Plant Molecular and Cellular Biology in Valencia, a team of

top scientists are attempting to harness the power of nature. 2020.

35. Pecoraro A, Pagano M, Russo G, Russo A. Role of autophagy in cancer cell response to nucleolar and endoplasmic reticulum stress. Int J Mol Sci. 2020;21(19):7334.

36. WHO statement: Tobacco use and COVID-19. WHO Official Updates - Coronavirus Disease 2019.

37. Scudellari M. Scientists are unpicking SARS-CoV-2's life cycle. Nature. 2021;595:1-7.