Special Issue: Where is Science Going?

Vol. 5, No. 2 (2022)
ISSN: 2532-5876

DOI: 10.13133/2532-5876/17641

RNA-based Vaccines against SARS-CoV-2: A Word of Caution and an Analysis of Potential Long-term Adverse Events

Carlo Gambacorti-Passerini[a*] *& Andrea Aroldi*[a]

[a] *University of Milano-Bicocca, Department of Medicine and Surgery, Hematology Division, ASST Monza, Italy*

***Corresponding author:** Carlo Gambacorti-Passerini, Email: carlo.gambacorti@unimib.it

Abstract

This paper briefly discusses the mechanism and potential of still unknown side-effects of RNA-based vaccines against SARS-CoV-2.

Keywords: RNA-based vaccines, SARS-CoV-2, LNP, S protein

Citation: Gambacorti-Passerbi, C & Aroldi, A 2022, "RNA-based Vaccines against SARS-CoV-2: A Word of Caution and an Analysis of Potential Long-term Adverse Events", *Organisms: Journal of Biological Sciences*, vol. 5, no. 2. DOI: 10.13133/2532-5876/17641

Introduction

The COVID-19 pandemic saw the introduction of new types of vaccines, i.e. DNA- and RNA-based products. These vaccines were developed and released based on an emergency authorization, where many steps related to the development of a vaccine or of a drug were shortened or completely eliminated. The urgent need to make them available to be public justified this expedited approach. However, such an approach risks to become an excuse to omit a much needed surveillance activity.

We will not deal with DNA-based vaccines. In fact, their use is dwindling because of Vaccine Induced Thrombocytopenic Thromboses (VITT), a rare (1/100,000 event) but often fatal adverse event (AE) (Pavord *et al.* 2021).

RNA-based vaccines are instead gaining widespread use to prevent SARS-CoV-2 infection, and offer an excellent tool to prevent serious COVID-19 signs and related deaths (Public Health England 2021; Evans & Jewell 2021).

Vaccinated people enjoy a high degree of protection against serious COVID-19, Intensive Care Unit (ICU) admission, and COVID-19 related death. They however can become infected and transmit SARS-CoV-2, although with reduced viral loads.

RNA-based vaccines are formed by injecting the RNA coding for the S protein (or part of it) admixed with lipid nanoparticles (LNP) that protect it from degradation and increase its cellular uptake. This in turn lead to RNA transcription and production of the S protein. The S protein then induces an immune response consisting of both a cellular (T-based) and long-lived response, and in a humoral (B-based) short-lived one with the production of anti-S antibodies (Dan *et al.* 2021).

Upon intramuscular injection, the RNA gets distributed to the injection site, the draining lymph node(s), and the liver. The presence of the RNA usually lasts 48-72 hours (European Medicines Agency 2021).

The S protein of SARS-CoV-2 was chosen because it mediates virus entry into cells through the binding of Angiotensin Converting Enzyme 2 (ACE2), present on the membrane of epithelial and endothelial cells

(Guney & Akar 2021). Therefore, antibodies against the S protein should be able to block the fusion of the virus with a cell membrane, thereby blocking its entry and subsequent viral replication and cytolytic effects.

RNA-based vaccines represent also a new type of medical tool, as traditional vaccines contain the entire microbiological agent (either attenuated or inactivated) or proteins derived from it.

Because of the global emergency linked to COVID-19, no long-term safety analysis of RNA-based vaccines was undertaken. Hence, caution should be exercised, as for any new medical treatment.

Three items need to be considered when examining the potential AEs linked to the use of RNA-based vaccines: the lipid nanoparticles (LNP), the RNA, and the protein being produced.

1. LNP

Lipid nanoparticles make the RNA able to resist degradation, and allow them to be picked up by cells in an endosome and finally be released into the cell cytoplasm for translation (Schoenmaker *et al.* 2021). They are composed by cationic (ionizable) lipids whose positive charges bind to the negatively charged backbone of mRNA, pegylated lipids that help stabilize the particle, and phospholipids as well as cholesterol molecules that contribute to the particle's structure. Cationic and pegylated lipids have showed safety problems as they could accumulate in the liver and cause hepatotoxicity. They could also elicit an innate or conventional immune response (Ndeupen *et al.* 2021).

Indeed, some generalized inflammatory response, including myocarditis, were observed with a prevalence of approximately 1-5/100,000 and are being monitored (Haaf *et al.* 2021). The cause might be the LNP, alone or combined with RNA.

2. RNA

It constitutes the core of the vaccine, as it is translated into the S protein, the real immunogen in an RNA-based vaccine. RNA itself can be toxic upon injection in cells because it can activate Toll-Like Receptors (TLR), usually in late endosome, thus initiating a cytokine storm (Dalpke & Helm 2012). It appears that the conversion of uridine into pseudouridine in the RNA strand reduces this risk (Dolgin 2021).

Another risk linked to the intracellular presence of RNA is its ability to form DNA and to integrate into cellular DNA. Although conventional wisdom indicates that the RNA –> DNA direction is not possible in cells, we need to remember that our cells contain many retrotransposons (or class I transposons). Under certain circumstances, these can activate and produce reverse transcriptase and catalyse the reverse transcription of RNA into DNA. This, in turn, can anneal to homologous sequences in the genome and cause genetic damage (Pray 2008). This fact was proven recently in vitro for SARS-CoV-2 (Zhang *et al.* 2021), and proposed as the mechanism by which patients clinically cured from COVID-19 can remain positive by SARS-CoV-2 PCR assay for months (Zhang *et al.* 2021). Indeed, this hypothesis was recently questioned (Smits *et al.* 2021). In addition, it was demonstrated in vitro that the use of RNA to modulate the transcriptome profile of cells for producing pluripotent stem cells was the safest tool available, but was still associated with the development of some genomic alterations in transduced cells (Steichen *et al.* 2014).

It can be argued that the virus itself can induce a similar phenomenon during a natural infection as well as through the spontaneous activation of transposons themselves. This is certainly true but pertains to two different types of contexts: virus infection and transposon activation occur naturally, while the injection of a vaccine is a human activity, which requires an informed consent.

The most common disease following insertional mutagenesis is represented by the development of a malignancy, which requires a minimum of 2-3 years to become clinically detectable. In this case, we lack information simply because an insufficient amount of time elapsed. Most possibly, such a risk will be absent (included in the "background noise" of present cancer rates) or very low. However, it is essential that we put in place an adequate and efficient monitoring system for it.

3. S Protein

A safety analysis must consider the protein produced by the injected RNA, although it is not yet

present in the RNA-based vaccine. In the case of SARS-CoV-2, the S protein plays an important pathogenetic mechanism in determining COVID-19.

The S protein, in fact, binds to ACE2 and cause its down-regulation. ACE2 plays an important role in cell homeostasis: its product (mainly angiotensin 1-7) has anti-inflammatory, vasodilation and anti-thrombotic effects that balance the opposite effect of angiotensin II, i.e. the product of ACE1 in physiological conditions. This unbalance produces important effects typical of severe COVID-19, such as the cytokine storm and the production of sFLT1, which causes endothelial damage and coagulation activation (Giardini *et al.* 2020).

A work performed with SARS-CoV-1 elegantly demonstrated that injecting the S protein (more precisely, the ACE2 binding part of it) in animals caused the pathogenetic changes typical of lung and endothelium viral infections (Imai *et al.* 2005). Therefore, one could argue that the RNA-based vaccines that drive the production of S protein could cause similar effects in vaccinated people.

Two lines of evidence are against this hypothesis:

1. The injection site of the vaccine involves a minimal part of the body. Usually, the injection site involves a volume of a few ml and 30-100 micrograms of RNA. It is true that in the case of RNA-based vaccines it is impossible to predict how many micrograms of protein will be produced. However, these figures pale in comparison with an entire lung involved by SARS-CoV-2 infection. Even in the aforementioned animal model, it took tens of milligrams of S protein to cause COVID-like pathological changes.

2. The S protein is produced in cells transduced by the RNA-based vaccine. It contains a single stop-transfer, membrane-spanning sequence located at the C terminus, which prevents it from being fully released into the lumen of the ER and subsequent infected cells' secretion. Consequently, it remains stuck in the cell membrane, similarly to the S protein produced during an infection. However, the mature virus formed inside the infected cells is released into the circulation upon cell lysis. When cells are transduced by the RNA-based vaccine, the S protein is not released into the circulation and remain in the cell, where it is subsequently degraded into peptides and presented to CD4 lymphocytes. These then initiate the immune response. The final development of the immune response leads to the production of anti-S antibodies.

The above-mentioned data strongly object to the possibility that the S protein can be released into the blood stream in significant amounts and damage cells.

Conclusions

RNA-mediated vaccines offer a new but not fully tested vaccination tool. Some of the possible AEs require closer monitoring. Any toxicity of the LNP and the S protein itself would manifest in the form of acute AEs. Thus, short-term follow up programs would be able to spot them timely.

On the contrary, any insertional mutagenesis operated by the retrotrascription and genomic integration of the RNA itself would require a substantial amount of time (2 to 6 years) to manifest as an increased incidence of neoplasias (mostly lymphomas, sarcomas, and leukemias). This effect remains hypothetical at present, but cannot be discounted *a priori*.

In our opinion, a successful immunization strategy needs to convince, not coerce people. Many have suggested that we should bar these arguments from public view and knowledge, in order "not to scare people away from vaccines." We think this is a short sighted and counterproductive view. In the long term, transparency, reliability, and science-based opinions win people's trust.

It is essential however that the separation of powers be maintained even when COVID-19 pandemic is concerned. Scientists must retain their independence from politicians, similarly as judiciary must remain separated and independent from legislative and executive powers.

Acknowledgments

We gratefully acknowledge the help of Federica Poggi, M. Sc. for editorial management.

Funded in part from AIRC grant IG 2017 Id.20112 project – P.I. Gambacorti-Passerini Carlo.

References

Dalpke, A & Helm M 2012, "RNA mediated Toll-like receptor stimulation in health and disease", *RNA Biol.*, vol. 9, no. 6, pp. 828-42. doi: 10.4161/rna.20206

Dan JM, Mateus J, Kato Y, *et al.* 2021, "Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection", *Science*, vol. 371, no. 6529, pp. eabf4063. doi: 10.1126/science.abf4063

Dolgin E 2021, "The tangled history of mRNA vaccines", *Nature*, vol. 597, no. 7876, pp. 318-324. doi: 10.1038/d41586-021-02483-w

European Medicines Agency, Science medicines Health February 19, 2021, "Assessment report". Retrieved from:

https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

Evans SJW & Jewell NP 2021, "Vaccine effectiveness studies in the field", *N Engl J Med.*, vol. 385, no. 7, pp. 650-651. doi: 10.1056/NEJMe2110605

Giardini V, Carrer A, Casati M, *et al.* 2020, "Increased sFLT-1/PlGF ratio in COVID-19: A novel link to angiotensin II-mediated endothelial dysfunction", *Am J Hematol*, vol. 95, no. 8, pp. E188-E191. doi: 10.1002/ajh.25882

Guney C & Akar F 2021, "Epithelial and endothelial expressions of ACE2: SARS-CoV-2 entry routes", *J Pharm Pharm Sci.*, vol 24, pp. 84-93. doi: 10.18433/jpps31455

Haaf P, Kuster GM, Mueller C *et al.* 2021, "The very low risk of myocarditis and pericarditis after mRNA COVID-19 vaccination should not discourage vaccination", *Swiss Med Wkly.*, vol. 151, pp. w30087. doi: 10.4414/smw.2021.w30087

Imai Y, Kuba K, Rao S *et al.* 2005, "Angiotensin-converting enzyme 2 protects from severe acute lung failure", *Nature*, vol. 436, no. 7047, pp. 112-116. doi: 10.1038/nature03712

Ndeupen S, Qin Z, Jacobsen S *et al.* 2021, "The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory", *iScience*, vol. 24, no. 12, pp. 103479. doi: 10.1016/j.isci.2021.103479

Pavord S, Scully M, Hunt Beverley J *et al.* 2021, "Clinical features of vaccine-induced immune thrombocytopenia and thrombosis", *N Engl J Med*, vol. 385, no. 18, pp. 1680-1689. doi: 10.1056/NEJMoa2109908

Pray L. 2008, "Transposons: the jumping genes", *Nature Education*, vol. 1, no. 1, p. 204.

Public Health England June 28, 2021, "Direct and indirect impact of the vaccination programme on COVID-19 infections and mortality". Retrieved from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/997495/Impact_of_COVID-19_vaccine_on_infection_and_mortality.pdf

Schoenmaker L, Witzigmann D, Kulkarni JA *et al.* 2021, "mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability", *Int J Pharm*, vol. 601, p. 120586. doi: 10.1016/j.ijpharm.2021.120586

Smits N, Rasmussen J, Bodea GO *et al.* 2021, "No evidence of human genome integration of SARS-CoV-2 found by long-read DNA sequencing", *Cell Rep*, vol. 36, no. 7, p. 109530. doi: 10.1016/j.celrep.2021.109530

Steichen C, Luce E, Maluenda J *et al.* 2014, "Messenger RNA- versus retrovirus-based induced pluripotent stem cell reprogramming strategies: analysis of genomic integrity", *Stem Cells Transl Med*, vol. 3, no. 6, pp. 686-69. doi: 10.5966/sctm.2013-0158

Zhang L, Richards A, Barrasa MI *et al.* 2021, "Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues", *Proc Natl Acad Sci USA*, vol. 118, no. 21, p. e2105968118. doi: 10.1073/pnas.2105968118