Dr Gérard Schockmel

Webinar LNS 4 Feb 2021

All of the first generation vaccines teach the immune system to make antibodies against the coronavirus's spike protein. The virus uses the spike proteins on its surface to attach to the ACE-2 receptor on host cells. Neutralising antibodies against the spike protein block attachment to the ACE-2 receptor and prevent viral entry into host cells. Since SARS-CoV-1 *(2002)* and MERS-CoV that emerged a decade later *(2012*), scientists have known that neutralising antibodies against the coronavirus spike protein are protective.

Gene-based vaccines: they include mRNA, DNA and viral vector vaccines. These vaccines carry the genetic instructions for the host cells to make the antigen, which more closely mimics a natural infection. The approach isn't entirely unfamiliar. In live-attenuated vaccines, like the measles, mumps and rubella vaccines, weakened viruses incorporate their genetic instructions into host cells, causing the body to churn out viral copies that elicit antibody and T-cell responses.

Non-replicating mRNA vaccines: contain mRNA that is unable to replicate itself. The two currently approved mRNA vaccines from Pfizer-Biontech and Moderna consist of ssRNA that contains the coding sequence for the full-length coronavirus spike protein, flanked by regulatory regions on either end. The ssRNA is produced using a cell-free *in vitro* transcription *(IVT)* from the corresponding DNA template.

Modified nucleoside – the two vaccines contain a modified uridine triphosphate to make the mRNA less inflammatory for the cell. Indeed, a non-self nucleic acid in the cytosol activates the cell's innate immune system *(type I IFN, proinflammatory cytokines)*, which may cause a reduction of protein expression in case of repeat dosing. However, for vaccines, mRNA that engages the innate immune system may actually potentiate the response to the vaccine. Nevertheless, both the Pfizer-Biontech and Moderna vaccines use a nucleoside modified mRNA.

Modified spike protein: Both mRNA vaccines encode for a modified coronavirus spike protein that contains two *(consecutive)* proline substitutions *(at amino acid positions 986 and 987)* to stabilise the spike protein in its prefusion conformation for better antigenicity. This is important for a safe and robust immune response.

Purification: removal (elimination) of dsRNA impurities (contaminants) to reduce an immune-stimulatory response (RP-HPLC, or treatment with ribonuclease III).

Encapsulation: the mRNA is encapsulated in a carrier lipid nanoparticle (LNP) that protects the mRNA long enough to reach the desired tissue, preventing it from degrading too quickly in the body. The LNP may even have an immune-stimulating adjuvant effect. In addition, the LNPs help the mRNA cross the cell membrane and allow its uptake into endosomes. The mRNA is subsequently released into the cytosol, where it is translated into the spike protein. The spike protein then migrates to the cell surface.

Immunological properties: The spike protein expressed on the host cell membrane is recognised by the immune system as a foreign antigen. This elicits the production of antibodies and the activation of $CD4^+$ helper T cells. In addition, $CD8^+$ cytotoxic T cells (killer cells) are recruited through the MHC, major

histocompatibility complex, class I pathway. As you might remember from your immunology course, MHC class I molecules bind antigenic peptides and present them to T cells. The MHC/peptide complex is recognised by the TCR and its CD8 coreceptor on cytotoxic T lymphocytes. This results in the activation of the cytotoxic T lymphocytes. Of note, only viral proteins produced by the host cells themselves are expressed through the MHC class I pathway. Thus, gene-based vaccines, such as RNA, DNA and viral vector vaccines, possess the unique advantage of being able to activate $CD8^+$ cytotoxic T lymphocytes, which likely contributes to their high efficacy.

Pharmacological properties: After intramuscular injection, cells at the injection site and the draining lymph nodes take up the LNP, effectively delivering the mRNA sequence into cells, for translation into viral protein. The delivered mRNA does not enter the cellular nucleus or interact with the genome. It is expressed transiently mainly by dendritic cells and subcapsular sinus macrophages. The vaccine mRNA is destroyed after a few days at most.

Efficacy: *(prime-boost)* the 2-dose regimen of the two mRNA vaccines boosts the immune response and the generation of memory cells. For both vaccines, the interim analyses of their Phase III data, respectively published in the NEJM, indicates an efficacy of about 95% against the circulating viral strains.

Safety: In vaccine development, there are some general caveats to be aware of, in particular the possibility for an excessive or misguided immune response in vaccine recipients, and the possibility of vaccine-enhanced disease upon encounter with the coronavirus. In terms of tolerability *(reactogenicity)*, both mRNA vaccines cause local and systemic adverse events. Local adverse events include pain at the injection site, swelling, and redness. Systemic adverse events include fatigue, headache, nausea, arthralgia, myalgia, and a raised body temperature. Anaphylaxis remains rare, but appears to be more frequent with the mRNA vaccines than with the flu vaccine. Anaphylactic reactions may be attributable to one of the fat components contained in the LNP, that is, to polyethylene glycol (PEG). PEG is a molecule found in products such as cosmetics and laxatives. *(impurities from IVT)*.

Self-replicating (amplifying) mRNA vaccines: contain mRNA that is able to replicate itself. Self-replicating mRNA vaccines are currently explored by Biontech, Imperial College London and other vaccine developers. With a conventional mRNA vaccine, a lower dose means lower potency. Self-replicating vaccine candidates, however, include instructions for the RNA to copy itself: they contain a replicase gene (RDRP, RNA-dependent RNA polymerase). The self-replicating mRNA vaccines should allow to lower the dose. Also, these vaccines more closely mimic a natural infection – triggering a stronger, broader immune response. This, in turn, might allow for single-dose inoculation regimens.

DNA vaccines: DNA is a very stable molecule due to the double helix, and this stability presents important advantages for vaccine storage and transportation. DNA vaccines must enter the host cell nucleus. From there mRNA is created, which travels out of the nucleus into the cytoplasm, where protein is formed from it. Thus, DNA vaccine technology faces two important challenges: (1) the vaccine must cross the cell membrane, and (2) the DNA must be delivered into the cell nucleus.

The delivery of the vaccine DNA into the cell is achieved with an electroporation device, applying electric pulses to the cell membrane to make it become more permeable. For delivery into the cell nucleus, the vaccine DNA might include genetic information encoding for proteins that facilitate entry into the cell nucleus. Otherwise, the process may be inefficient. A potential risk associated with DNA vaccines, is that vaccine DNA might be integrated into the genome of the cell.

Non-replicating viral vector vaccines: contain vector viruses that are unable to produce new viral particles. There is the AstraZeneca vaccine (used as a two-dose regimen), the Johnson & Johnson vaccine (used as a single shot, with studies exploring a two-dose regimen underway), and the Russian Sputnik 5 vaccine. All these vaccines use a common cold virus of the family of adenoviruses to transport the genetic instructions to build the coronavirus spike protein into host cells. Adenoviruses are non-enveloped viruses that contain a dsDNA genome.

The vaccine's adenovirus DNA has been modified, both to be replication-deficient and to contain the genetic instructions for the synthesis of the full-length coronavirus spike protein. These genetic instructions are inserted in the form of dsDNA. The dsDNA fragment encoding for the spike protein is translated in the cell nucleus into mRNA. The mRNA is released into the cytosol, where it is translated into the spike protein. The spike protein then migrates to the cell surface, where it is recognised by the immune system as a foreign antigen. This elicits the production of antibodies, and the activation of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells (killer cells).

A potential drawback of adenovirus-based viral vector vaccines, is the risk of pre-existing immunity, such as neutralising antibodies, against the viral vector, which can limit effectiveness. If the immune system clears the vector before it can get into cells, that is a big problem. A pre-existing immunity against the viral vector can be more common in some geographical areas than others, rendering a vectored vaccine more or less effective depending on the region. To avoid the risk of pre-existing immunity, the AstraZeneca vaccine *(ChAdOx1)* uses an adenovirus that infects chimpanzees instead of humans. However, even with a chimpanzee adenovirus, there might still be cross-reacting pre-existing immunity to human adenoviruses. Immunity against the vector virus can also develop after the first shot in a two-dose regimen. For this reason, the Russian Sputnik 5 vaccine uses an Ad26 for the first dose, and an Ad5 for the second dose. AstraZeneca and the Gamaleya Institute in Russia plan to run a study exploring a first dose with ChadOx1 and a second dose with Ad26, and vice versa. The Johnson & Johnson vaccine uses an adenovirus 26 strain.

In interim analyses of their Phase III data respectively published in the Lancet, the efficacy of the AstraZeneca vaccine used in a 2-dose regimen was about 60 %*, and the efficacy of the two-dose regimen of the Sputnik 5 vaccine was about 91 %. Interestingly, the AstraZeneca seems to work better when the dosing interval is longer (between 4 and 12 weeks)*. Johnson & Johnson recently announced in a press release, that according to an interim analysis of their Phase III study in the US, the efficacy of their Ad26 based vaccine administered as a single dose was estimated at 72 %. Overall, the vaccine safety of the viral vector vaccines currently appears comparable to that of the mRNA vaccines.

Protein-based vaccines: they include whole-inactivated (killed) vaccines, as in the flu shots, subunit vaccines, as used in the hepatitis B vaccine, and virus-like particles *(VLPs)* vaccines, such as the human papilloma virus vaccine.

Inactivated (killed) coronavirus vaccines are produced by the state-owned Chinese company Sinopharm and by the private Chinese company Sinovac. Inactivation of the coronaviruses is achieved by applying a chemical, called beta-propiolactone, to the viral surface. The Russians are also developing an inactivated virus vaccine.

A protein-based vaccine for which phase III data are currently available is the vaccine produced by the US-based company Novavax. The Novavax vaccine uses stable, prefusion coronavirus spike proteins assembled into nanoparticles, also called virus-like particles *(VLPs)*. The vaccine is administered in two doses and contains an adjuvant *(Matrix-M)* for an increased immunostimulatory effect. (*Expression system: baculovirus, moth cells)*

In a press release, Novavax announced that according to an interim analysis of their UK Phase III study, the efficacy of their vaccine was estimated at 90 %.

Emerging variants (strains) of SARS-CoV-2: are found in Britain, Brazil, South Africa, Japan, and other countries. Recent *in vitro* studies carried out by Pfizer-Biontech and Moderna suggest that their mRNA vaccines preserve their efficacy against all the coronavirus variants tested.

The Moderna - NIH study reports results from *in vitro* neutralisation studies of sera from individuals vaccinated with the Moderna vaccine (preprint). The results show that the vaccination produced neutralising titers against all key emerging viruses, including variants B.1.1.7 and B.1.351, first identified in the UK and in the Republic of South Africa, respectively. However, the SA variant represents an important challenge, since it carries 10 mutations in the spike protein, with 3 mutations located in the RBD, among which, a key antibody escape mutation in position 484 (E484K, E - glutamic acid, K - lysine). Thus, a six-fold reduction in neutralising titers was observed with the SA variant relative to other variants. The SA variant was, however, fully neutralised *("cushion effect"),* albeit at lower dilutions of sera (1/300 versus 1/1852 for other viral strains).

Despite this reduction, neutralising titers with the SA variant remain above levels that are expected to be protective *(cross-protection with Wuhan strain based S-protein)*. The existing mRNA vaccines are likely to still prevent serious illness, but it may be more difficult to prevent milder disease and transmission.

However, the lower neutralising titers observed in these studies may suggest a potential risk of earlier waning of immunity to the new SA strains. To address this risk, an additional third, booster dose may be considered beyond the primary 2-dose regimen to further increase neutralising titers against emerging strains. The third dose may be either identical to the initial vaccine, or else customised for the SA variant. Moderna is advancing its vaccine candidate specific to the SA variant into preclinical studies and a Phase I study in the US. Since the study will be performed in the US, this suggests that they will be looking at *in vitro* data from neutralisation studies.

Meanwhile, Pfizer-Biontech posted on the preprint server bioRxiv the results of an *in vitro* study testing sera from 20 vaccinated people for neutralisation against the British and South African variants. They noted that for one third of the serum samples, neutralisation titers were half as high for the SA variant than for wild-type virus. This corresponds to a small reduction of neutralisation titers for the SA variant.

So far for *in vitro* studies, but what about real-world data? In small vaccine trials conducted in South Africa, the US-based companies Novavax with their protein-based vaccine, and Johnson & Johnson with their adenovirus 26 based viral vector vaccine have provided real-world data on how their vaccines perform against the new variant there. In a press release, Novavax reported an efficacy of 49% for their vaccine candidate in South Africa, with most vaccine failures attributed to the SA variant, compared to a 90% efficacy in their UK study (49% vs 90%). As to Johnson & Johnson, they reported in a press release that their AD26 vaccine candidate administered as a single dose was 57% effective in South Africa, compared to an efficacy of 72% in the US (57% vs 72%).

Fortunately, the gene-based vaccines, i.e. RNA, DNA, and viral vector vaccines, are "relatively straightforward to redesign for a new variant". Both Pfizer-Biontech and Moderna have announced that it takes them about six weeks to develop a modified vaccine. This, however, does not take into account the amount of time it takes for the conduct of safety trials *(preclinical, Phase I)* and the approval of the modified vaccine by health authorities.

***** A new, subgroup analysis of the currently available clinical trial data on the AstraZeneca vaccine suggests that there is a vaccine efficacy of about 80%, provided that the dosing interval between doses 1 and 2 has a duration of 12 weeks.