Ad26.COV2.S

Vector-based vaccine against SARS-CoV2

Module Biodesign: Ways to active pharmaceutical ingredients

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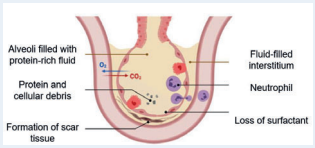
1. Introduction

The infectious disease SARS-CoV2, which was first detected in Wuhan in 2019, poses an unprecedented challenge to people worldwide. The corona virus can cause severe effects such as acute respiratory problems, fever and leads to loss of the sense for smell and/or taste. According to the WHO on 15 december, 71 million corona cases have already been confirmed, with more than 1.5 million dead casualties and counting. [1], [2]

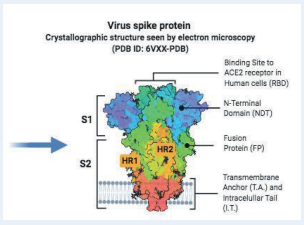


Effect of SARS-CoV 2 infection [3]

Ad26.COV2.S is a vector-based vaccine candidate from Johnson and Johnson (Janssen Pharmaceutical) against the corona virus SARS-CoV2. It is based on the harmless human rhinitis virus Adenovirus 26, to which the genetic information for the spike protein of the coronavirus SARS-CoV-2 has been added. [4]

Structure of SARS-CoV2

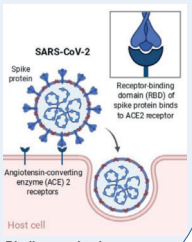
The SARS-CoV-2 virus-construct contains the code sequence of the Spike-protein (S-protein), which is necessary for the virus to bind to the ACE2-receptor of the bronchial and alveolar epithelial cells and to infect them. The S-protein contains the S1 and S2 part, responsible for recognition is the S1 complex, which contains the receptor binding domain (RBD). [5]



Structure Spike-Protein (Membran Protein SARS-CoV-2) [3]

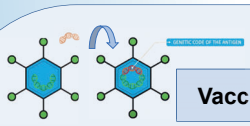
Penetration and infection

The S-protein binds to the ACE2-receptor, which leads to an infection of bronchial and alveolar epithelial cells. Additionally, a suppression of dendritic cells occurs, which results in a delayed/ reduced Interferon I and Interferon III (INF-I & INF-III) secretion, which therefore causes a delayed T-helper-cell activation. The infection can also lead to an uncontrolled and increased level of cytokines and chemokines and their combined activation of different immune cells like monocytes and neutrophils. This hyperstimulation can eventually lead to a hypercytokinemia, an acute respiratory distress syndrome. [6], [7]



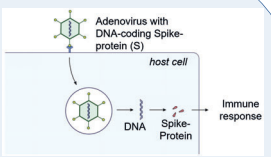
Binding mechanism to host cell [3]

2. Vaccine strategy

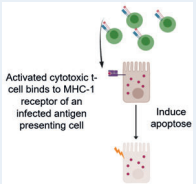


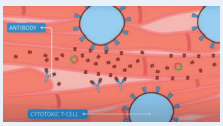
Vaccination

Adenovirus 26 is genetically modified and can no longer replicate itself. The S-Protein coded DNA (red) is implemented into the viral genome. [6], [8]

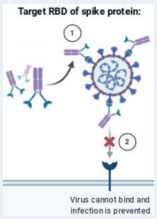


After a single-shot intra-muscular administration of the modified virus the cells get infected. The coding sequence for the Spike-protein will be transcribed and the expressed protein will get released out of the cell. [6], [8]



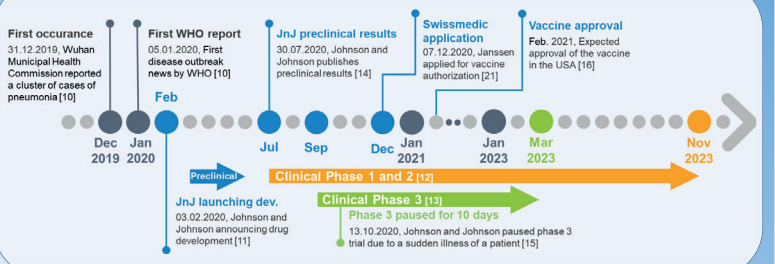


If the vaccination was successful, an infected cell presents fractions of SARS-CoV-2 virus S-protein with the MHC-1 receptor, which can be recognized by cytotoxic T-cells. The T-cells then induce intracellular apoptosis in the presenting cell. The humoral immune response will produce specific antibodies to the S-protein in order that the virus is no longer able to bind to its receptors. Additionally, opsonization (sterically restricted) will occur and lead to the inhibition and destruction of the virus [6], [9]



The humoral response include the detection of S-protein plus maturation of B-cells and T-helper cells. After differentiation of B-cells into plasma cells, the production of specific antibodies gets initiated as well as the memory cells are produced for long-term safety The cellular response include the activation of naive cytotoxic T-cells as well the production of memory cells. [8], [9]

3. Timeline

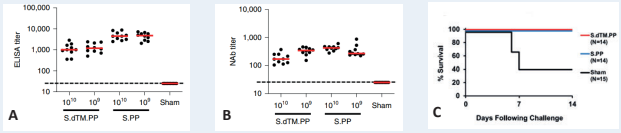


4. Preclinical Trials

52 Rhesus macaques immunized with Ad26 vectors
Results [17]:
Week 2 and 4:
- RBD-specific binding and neutralizing antibodies (NAb)

Week 6:
- Animals challenged with $1 \cdot 10^5$ TCID50 SARS.CoV2 by intranasal and intratracheal routes
- No detectable virus in bronchoalveolar lavage (BAL)
- Protection in upper and lower respiratory tract
- Robust NAb responses and complete protection in 5 of 6 animals (and near complete in 1 of 6 animals)

50 Syrian golden hamsters
Results [14]:
Week 4:
- Observation of neutralizing and RBD-specific antibodies
- 100% protection against mortality
- Single immunization of Ad26.COV2.S provides complete to near complete protection against Sars-Cov2



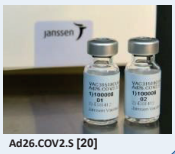
A) Humoral Immune response in vaccinated hamsters and sham control. In week 4, observation of RBD-specific binding antibodies by enzyme-linked immunosorbent assay ELISA. Red bars reflect the median responses.
B) Humoral Immune responses in vaccinated hamsters and sham control. Observation of neutralizing antibodies (NAbs) by pseudovirus neutralization assay after 4 weeks.
C) Survival Curves Studies involving all hamsters that received the $5 \cdot 10^5$ $TCID_{50}$ challenge dose and sham control. N= number of Animals. Leading vaccine in all Figures: S.PP

5. Clinical Trials / Submission

Phase 1 and 2a [8]:
- Took place in USA and Belgium
- With 1055 healthy volunteers
- Ages between 18 - 55 and 65+
- Positive interim results:
  - A complete or near complete protection
  - Good safety profile and immunogenicity

Phase 3 [4]:
- International studies
- With 60'000 volunteers
- Placebo controlled, double blind study
- Main purpose of the study:
  - Safety
  - Detect adverse side effects
  - Assess efficacy

Submission of an application for admission [21]:
7.12.2020: Submission of marketing authorisation application in
- Switzerland
- Europe
- Canada



Ad26.COV2.S [20]

6. Benefits of vector-based vaccines

Benefits compared to other strategies and companies [18]

+ Single-shot vaccination
+ No freezing for storage (≈ 1 year)
+ No adjuvants needed to boost efficacy
+ High production yield due to PER.C6® cell line

7. Comparison with previous vaccines

Vaccine [19]	Disease	Status	Adverse effects (AE)
Ad26.ZEBOV	Ebola	Approved	mild-to-moderate AE's after 1-2 days for up to 3 days light pyrexia in children
Ad26.Mos.HIV	HIV	Completed Phase 1/2a	
Ad26.CS.01	Malaria	Completed Phase 1/2a	
Ad26.RSV.preF	RSV	Completed Phase 2a	
Ad26.ZIKV.001	Zika	Completed Phase 1	

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Cuban vaccine candi- date against SARS-Cov-2:

Lena M. de León Esperón

The emergence of the highly pathogenic coronavirus SARS-CoV-2 in Wuhan and its rapid international spread has posed a serious global public-health emergency . SARS-CoV-2 binds to host cells via interactions between the Spike protein and ACE2. On Aug. 18th, 2020, the head of Epidemiology at Cuba's public health ministry announced that the Finlay Vaccine Institute in Havana would start a clinical trial on a vaccine for Covid-19. The expertise of this Institute with prior meningococcal vaccines and the manufacturing capabilities of the Centre of Molecular Immunology (CIM) with 25 years of experience in using mammal cell technology, lead to a collaboration that also included the University of Havana. The vaccine candidate Finaly-FR-1, now in clinical trials, will most likely be available in Cuba on early 2021.

I.

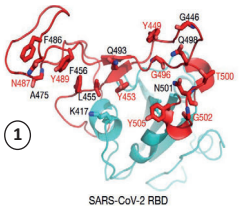
Protein subunit vaccine

Basic components:

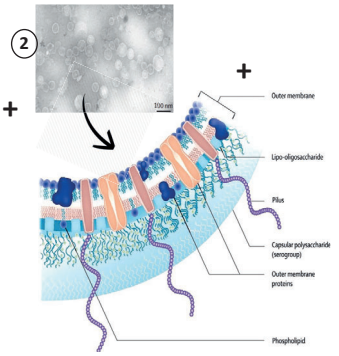
1. Dimeric RBD domain of spike (S) protein of SARS-CoV-2

2. Outer Membrane Vesicule (OMV) of meningococcus b (from VAMENGO-BC® vaccine)

3. Aluminum hydroxide (Al $(OH)_3$**) gel**



Recombinant RBD spontaneously forms dimers (S-S bonds)



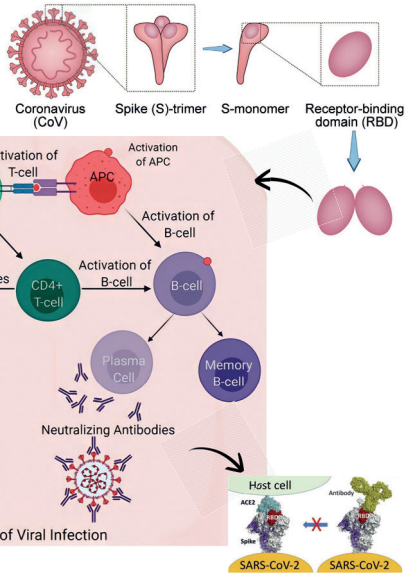
OMVs contain immune stimulators (e.g., LPS, proteins, etc) that trigger maturation as well as activation signals for the immune system

Alum potentiates immune system by:

- Depot effect
- Induction of inflammation
- Conversion of soluble antigen into a particulate form

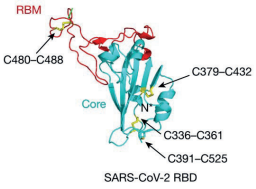
II. Immunological principle

Generation of antibodies specific for the RBD domain of the S protein, able to prevent binding of SARS-CoV-2 to ACE positive host cells ; activation of specific CD8+ T cells



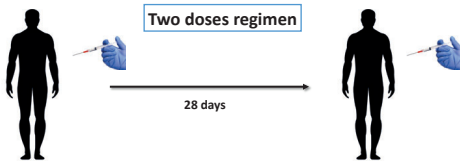
III. Antigen

SARS-CoV-2 RBD domain (dimer)



- Contains five antiparallel β-sheets forming the core, and a motif (RBM) with most of the contact residues participating in the interaction with ACE-2
- 9 Cys residues, 8 of which forming pairs of S-S bonds (marked in arrows)
- Recombinantly expressed in mammalian cells

IV. Vaccination regimen



- **Intramuscular injection**
- **10 or 20 ug dose depending on age**

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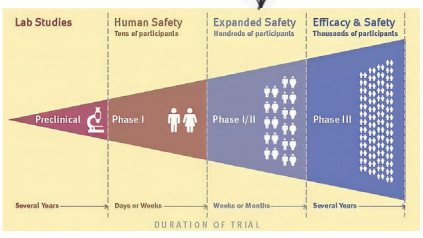
- **10 or 20 ug dose depending on age**

V. State of development



Phase I/II Clinical Trial "Soberana-01"

- Randomized, controlled, double-blinded
- 676 healthy Cuban adults (19-80 years old), both genders
- Goal: to determine safety, reactogenicity and immunogenicity of the candidate
- Parameters to evaluate: 1) seroconversion and 2) neutralizing antibodies titre

Phase III of clinical trials should start in January, and depending on results, it should be approved by the end of the first trimester of 2021 for its use in Cuba. Clinical trials in other countries should also start then.

VI. Benefits and risks

Benefits:

- The safety and immunogenicity of these OMVs has been tested, as it is part of an approved Cuban vaccine on the market for more than 30 years in over 15 countries (VAMENGO-BC®)
- Alum as adjuvant constitutes a long-term successful component of countless approved vaccines
- Subunit vaccines based on SARS-CoV RBD have been extensively explored and proven safe in their initial development phases
- This kind of vaccine usually induces a strong neutralizing antibodies response
- There is no risk of pre-existing antivector immunity

Risks/Disadvantages

- This platform is generally unsuitable for respiratory mucosal vaccination.
- The overall immunogenicity of subunit vaccines is weak, with poor activation of CD8+ T cell responses and requires adjuvants and repeated vaccination.

VII. Comparison to similar approved vaccines

Name	Pathology	Components	Vaccination regimen	Immunological pathways involved	Efficacy (%)
Recombivax-HB®	Hepatitis B	Recombinant hepatitis B virus surface antigen (HBsAg) (antigen) Aluminum hydrophosphate sulphate (adjuvant)	Intramuscular injection, time and dose depending on age	Induction of a B-cell response that generates HBsAg-specific antibodies; T cell specific response	>90
Shingrix®	Shingles (varicella zoster virus)	Recombinant Zoster virus Glycoprotein E (gE) (antigen) AS01B Suspension (adjuvant)	Intramuscular injection, 2 doses (2 to 6 months apart) Adults > 50 years old	Induction of a B-cell response that generates gE-specific antibodies; T cell specific response	>90

Other recombinant protein subunit vaccines for viral diseases, wit the same immunological principle and vaccination route show efficacy levels higher than 90 %

VIII. Refer-

BNT162b2 – The real solution?

Introduction

In 2020, the world is facing the most common infection leading to a pandemic. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results to the corona virus disease 2019 (COVID-19) and has already caused around 1'600'000 casualties up to now[1]. It is widely believed that pre-pandemic normalcy will only return upon an effective vaccination[2]. Due to this, 2020 has been an exceptional year in which pharmaceutical manufacturing companies worldwide worked with high pressure to find effective and safe vaccines, with a main focus on the never before market approved RNA-based vaccines, as they are allegedly faster and easier to produce compared to classical cell-based production systems[3]. One promising vaccine candidate is BNT162b2, a lipid nanoparticle (LNP) formulated, nucleoside-modified mRNA vaccine, developed by BioNTech and Pfizer[4].

Vaccine strategy

Vaccine platforms are differentiated into six different groups, with the group nucleic acid based vaccines including both, DNA and RNA based vaccines. Out of the 52 vaccines in clinical trials and the 162 in pre-clinical, nearly a quarter fall in this category[5].

mRNA based vaccines can then be further differentiated into non-replicating (NRM) and self-replicating mRNA (SAM) constructs, both carrying the coding sequence in the middle (see Fig.1)[3]. These SAM constructs yield the advantage of requiring lower doses, but carry a viral conponent[6]. Additionally, the mRNA can be modified to modRNA by replacing nucleosides like uridine with pseudouridines (ψ), to reduce the innate immune response and enhance transcription efficiency[7].

Similar to the various vaccine platforms, there are various methods to deliver the mRNA construct into the cell. They range from naked or protamine coupled mRNA molecules, to different carriers like dendrimers, nanoemulsion, polysaccharides, and differently modified lipid nanoparticles (LNPs). The LNPs have become the most commonly used nanocarriers for mRNA delivery, as their self assembly and endosomal release of mRNA into the cytoplasm, are clear advantages (see Fig. 2)[8]. After mRNA take-up by the host cells, it is immediately translated in the cytosol. In case of the SAM the mRNA self-replicates at this point. The expressed protein undergoes subsequently post translational modifications and the final antigen gets presented (see Fig. 3)[3].

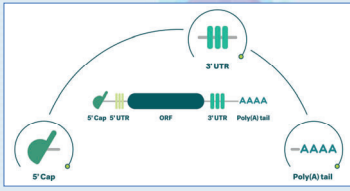


Figure 1: mRNA construct for RNA vaccines[13]
Containing a cap structure a the 5' end, untranslated regions (UTR) regulating translational efficiency on both sides, the coding sequence (CDS) in the middle, and a 3' poly-A-tail for protection of the mRNA molecule.

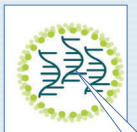


Figure 2: LNP-mRNA construct[13]

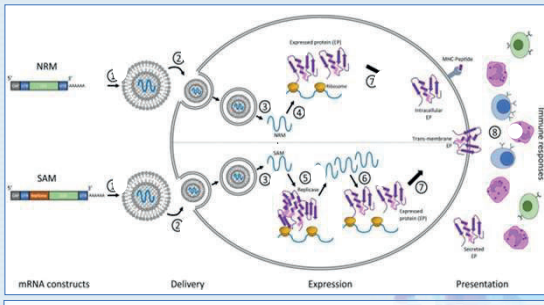


Figure 3: Molecular Mechanism of mRNA constructs[3]
For both mRNA constructs, the mRNA enclosed in a delivery system is taken up by the host cell via endocytic pathways. Subsequent endosomal escape releases the mRNA, where it is immediately translated by host's ribosomes to produce the protein of interest. The SAM construct self-replicates its mRNA at this point. The expressed protein undergoes post-translational modifications before it gets either presented intracellularly, secreted, membrane bound, or presented on an MHC peptide to activate the immune response.

Target antigen and Vaccination route

The herein presented BNT162b2 vaccine is a N-methylpseudouridine nucleoside-modified mRNA encapsulated in a lipid nanoparticle (LNP)[4].

The mRNA is encoding a modified Spike S Protein, called P2 S [9]. The modification consists of two point mutations, namely position 986 and 987, which were each replaced by proline (see Fig. 4). This mutation results in the protein remaining in the „pre-fusion" conformation - no membrane-fusion will take place with host cells expressing receptors exploitable for viral entry [9].

In case of the BNT162b2's LNP, the NRM is taken up by the host cell via endocytic pathways. Subsequent endosomal escape releases the mRNA, where it is immediately translated by host's ribosomes to produce the protein of interest: the Spike S protein. It further undergoes post-translational modifications3 and finally gets integrated into the host cell membrane to excert its function: induction of humoral and cellular adaptive immunity (see Fig. 3)[10].

Digression:
Three host key players are needed for infection with the native SARS-CoV-2: ACE2 (Angiotensin-converting enzyme 2), Furin (ubiquitously expressed type I transmembrane serine-protease) and Transmembrane Serine Protease 2 (TMPRSS2). Upon binding onto ACE2, Furin cleaves Spike S Protein into S1 and S2 subunits at the exposed cleavage site. S2 subunit is further cleaved at the S2' position, finally exposing fusion peptide, promoting endocytic viral entry[3].

P2 S has a native Furin-cleavage site resulting in cleavage of S1 and S2 without said membrane fusion[9]. P2 S is able to bind to ACE2, shows alternative states of 'RBD up' and 'RBD down' formations and induces SARS-CoV-2 S-specific neutralising antibodies, which are the first line of defense [9]. Target antigen(s) : P2S and its cleaved sub-units S1/S2.

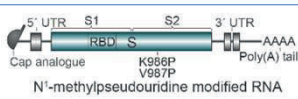


Figure 4: mod-RNA of BNT162b2[9]

The membrane-anchored P2S was reported to induce a coordinated immune response involving SARS-CoV-2 S-specific neutralising antibodies (S1 specific IgGs), S-specific CD4+ T-cells and cytotoxic S-specific CD8+ T-cells[10]

Vaccination route:
Intra-muscular, two doses (30 µg each) within 21 day interval[11].
Muscles contain a large network of blood vessels, in which the recruitment and recirculation of different immune cell types is facilitated, such as infiltrating APCs and B-cells[12]. This route has been shown to favour stability of the mRNA, as a radiolabeled mRNA was detected at the site of injection and draining lymph node even 28h post injection[12]. Even though intra-dermal injection was shown to have preferential access to immune cells and lymphoid organs than intra-muscular injection, ID has been limited by its small injection volume and a higher risk of severe local adverse events as compared to IM delivery[12].

Benefits

- CD4+ and antibody activation, additionally CD8+ and cytotoxic cell response which is important for virus destruction[14]
- mRNA vaccines like BNT162b2 act non-integrating to the DNA and poses no risk for insertional mutagenesis[2]
- Synthesised by *in vitro* transcription free from microbial molecules[2]
  - non-infectious when entering the body
- Efficacy and safety profile in Phase I/II was confirmed[15]
- 95% vaccination protection without previous SARS-CoV-2 infection[16]
- Low incidence of severe or serious events and non-clinically concerning[15,16]

Risks

- Preclinical studies showed disparities in magnitude and longevity of immune response[2]
  - Protective efficacy in humans and their amenability to respiratory mucosal delivery remains questionable
- Phase I/II studies: Mostly mild or moderate reactogenicity[15,16]
  - general disorder or administration site conditions (injection site pain, fatigue, pyrexia, chills)
  - Musculoskeletal and connective disorder (myalgia and arthralgia)
  - nervous system disorder (includes headaches)
- Phase III studies: Serious risk of allergic reactions in people who have had severe allergies to vaccines or drug in the past[16]

The BNT162b2 vaccine is mainly investigated in preclinical studies. Therefore, long-term safety, speed, stability and scalability remains in question and the risk in clinical development is significantly higher. The vaccine is still in its starting phase, so although it shows predominantly positive effects in human, real adverse side effects will probably become apparent much later.

Current development and Outlook

January 2020
Start of Project Lightspeed to find a vaccine for SARS-CoV-2 [17]

March 2020
WHO declared the SARS-CoV-2 outbreak a pandemic[18]

April 2020
Start of Phase I/II clinical trials[17]

June 2020
Listed for Operation Warp Speed[19]

December 2020
01.12. „Rapid temporary regulatory approval" in UK[20]
11.12 Approval for Emergency Use Authorization by FDA[21]

In „rolling review" by EMEA, results expected for 21th December[22]

Swissmedic currently reviews BNT162b2 data by „rolling submission" [23]

January 2021
First vaccinations probably to begin in January:
Particularly at-risk persons[24]

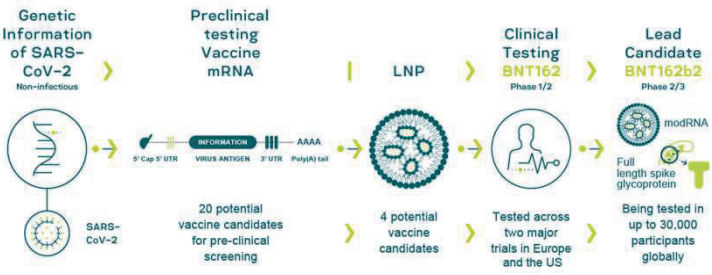


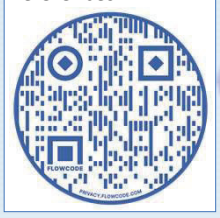
Figure 5: Timeline for Project Lightspeed by BioNTech[17]
The research for a candidate vaccine against SARS-CoV-19 started after the genetic information of the virus was available. Afterwards 20 potential vaccine candidates entered pre-clinical screening with 4 of them being formulated as LNP delivered systems. Clinical testing took place across Europe and the US with all 4 candidates, resulting in BNT162b2 being the lead candidate entering phase 2/3 testing and now being approved for emergency use across the globe.

Comparison to existing vaccines

Before December 2020, there were no approved mRNA vaccines. The herein presented BNT162b2 vaccine by BioNTech/Pfizer was the first mRNA vaccine to be approved for use in humans through emergency use authorisation as a COVID-19 vaccine[20]. The mRNA-1273 vaccine by Moderna could be the second nucleic acid based vaccine to receive emergency use authorisation[25].
However, there are several promising mRNA vaccine candidates in development for diseases like Influenza, Rabies, Cancer, and the Zika virus[8].

By Chiara Braun, Jannika Finger, and Dafina Ismaili
Master of Life Sciences, specialisation in Pharmaceutical Biotechnology
Module Biodesign

References



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NVX-CoV2373 – a Recombinant Spike Nanoparticle Vaccine by NOVAVAX

Chuundattu, Alexia; Ledergerber, Bettina; Pastieriková, Ivana

Introduction

SARS-CoV-2 is responsible for the 2019 COVID-19 outbreak and it was first reported in Wuhan, China. The structural proteins presented on the surface of SARS-CoV-2 virus include: S protein, N protein, M protein and E protein. Among these SARS-CoV-2 spike (S) glycoprotein is vital for the receptor binding, fusion, virus entry, and a target of host immune defence [13].

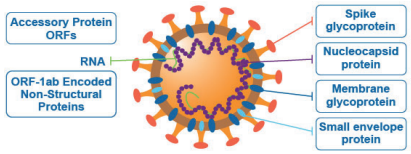


Figure 1: Surface representation of SARS-CoV2 [15].

Novavax is an American biotechnology company developing innovative vaccines for infectious diseases. One of its latest vaccine candidates is NVX-CoV-2373. The vaccine contains SARS-CoV-2 spike (S) glycoprotein, which is the primary target for the human angiotensin-converting enzyme 2 (hACE2) receptor. Furthermore, it includes the MATRIX-M1™ adjuvant which enhances the immune response and increases the production of neutralizing antibodies [14]. Additionally, NVX-CoV-2373 is a liquid formulation which allows for an easier vaccine distribution at 2 °C to 8 °C [16].

Current development and vaccination route

To evaluate the safety, immunogenicity and efficacy of SARS-CoV-2 nanoparticle vaccine, a randomized, observer-blinded and placebo-control clinical trials (phase I, II and III) were performed. The immunogenicity results were compared with convalescent serum samples from patients with COVID-19 [1, 2, 16].

Like other platforms (e. g. nucleic acid or viral vectors), a repeated homologous vaccination regimen is required to be effective [13].

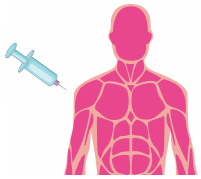
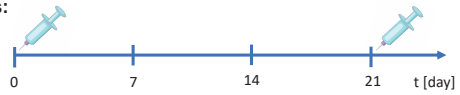

[6]

Table 1: Description and vaccination route of Phase I, II and III trials [1, 2, 16].

	Phase I	Phase II	Phase III
Description (exp. cohort)	5 µg or 25 µg of rSARS-CoV-2 with or w/o MATRIX-M1™ adjuvant	5 µg of rSARS-CoV-2 with MATRIX-M1™ adjuvant	5 µg of rSARS-CoV-2 with MATRIX-M1™ adjuvant
Participants	131, age 18-59	1500, age 18-84	Up to 15'000, age 18-84
Placebo group	X (0.9% normal saline)		
Vaccination route	2 intramuscular injections of vaccine in the deltoid muscle		

Vaccination regimen in clinical trials:

2 doses of vaccine candidate, 21 days apart



Risk and Benefits

Side effects of NVX-Cov-2373:

- Injection site reactogenicity
- Tenderness and pain
- Headache
- Fatigue
- myalgia
- Most of the symptoms are mild with average duration < 2 days
- No serious adverse events were reported [1, 14]

Benefits:

- NVX-CoV2373 induces high titer anti-S IgG
- Elicits CD4+ and CD8+ T-cell responses.
- Induces Tfh cell and GC B-cell development [1, 14]
- Vaccine provided protection from infection and disease (preclinical phase) [16]

Vaccine Strategy, platform and immunogen

NVX-CoV2373 is a recombinant nanoparticle composed of a trimeric SARS-CoV-2-spike-glycoprotein and a MATRIX-M1™ adjuvant [1]. The spike-glycoprotein is responsible for the entry of the coronavirus into the host cell, which is the key target for the development of antibodies and vaccines [1]. It contains the two subunits S1, which binds to the human angiotensin-converting enzyme 2 (hACE2) on the host cell surface and S2, which mediates membrane fusion [1, 3].

NVX-CoV2373 is based on full-length S protein (from residues 1-1273). It includes the transmembrane (TM) and cytoplasmatic tail (CT). The final construct carries modified S1/S2 polybasic cleavage sites (682-QQAQ-685) and also 2 proline substitutions at residues K986P/V987P. These modifications enhance the stability of the protein against protease [4].

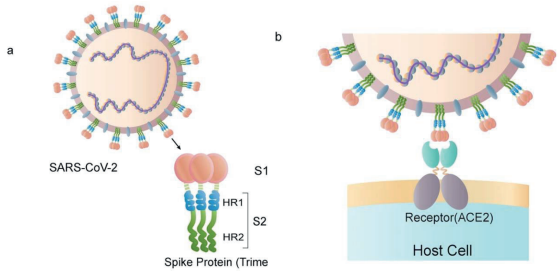


Figure 2: schematic structure of the spike protein of SARS-CoV-2 (a) and the binding of the S1 subunit to the ACE2 receptor [3].

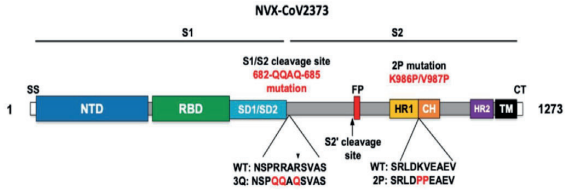
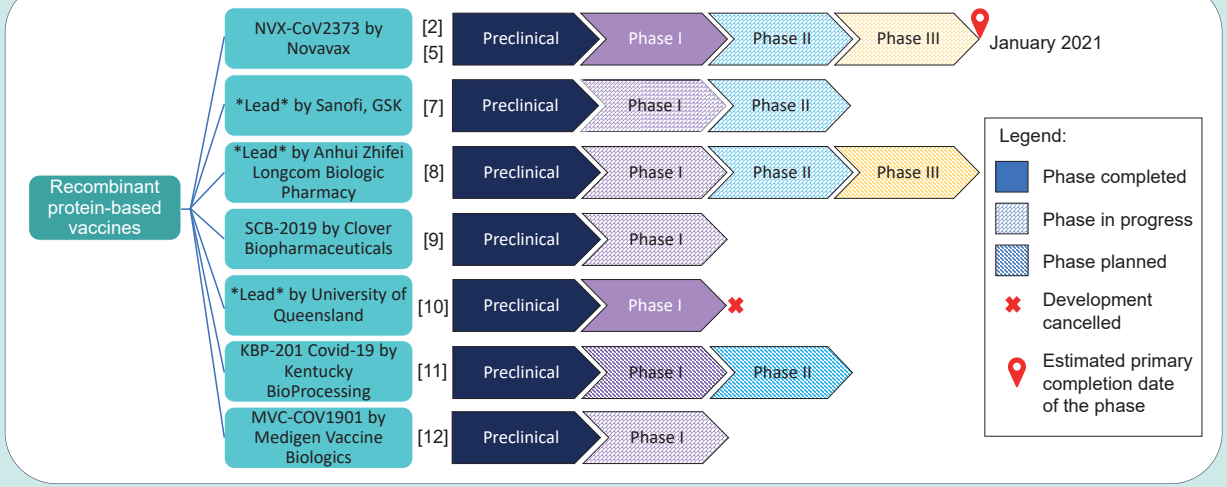


Figure 3: Full length spike-protein with the mutations on the S1/S2 cleavage site [4].

Comparison of protein-based vaccines & outlook for NVX-CoV2373



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COVID-19 Vaccine Medicago, Laval University

A virus-like particle vaccine

Maria Fernanda Blättler, Aline Bettelini, Deniz Türkcan

Vaccine strategy

The plant-based manufacturing technology used for the COVID-19 vaccine was developed by the company Medicago with the partnership of the University of Laval in March 2020. Their technology uses virus-like particles (VLP) to develop protein-based vaccines. The plant *Nicotiana bethamiana* was transfected with a viral vector from *Agrobacterium tumefaciens* and the vaccine was recovered from the transfected plants in the form of VLP [8] .

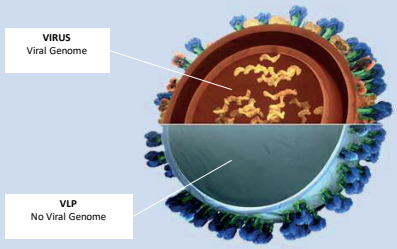


Figure 1: Virus compared to VLP. [Image adapted from https://www.medicago.com/en/technologies/]

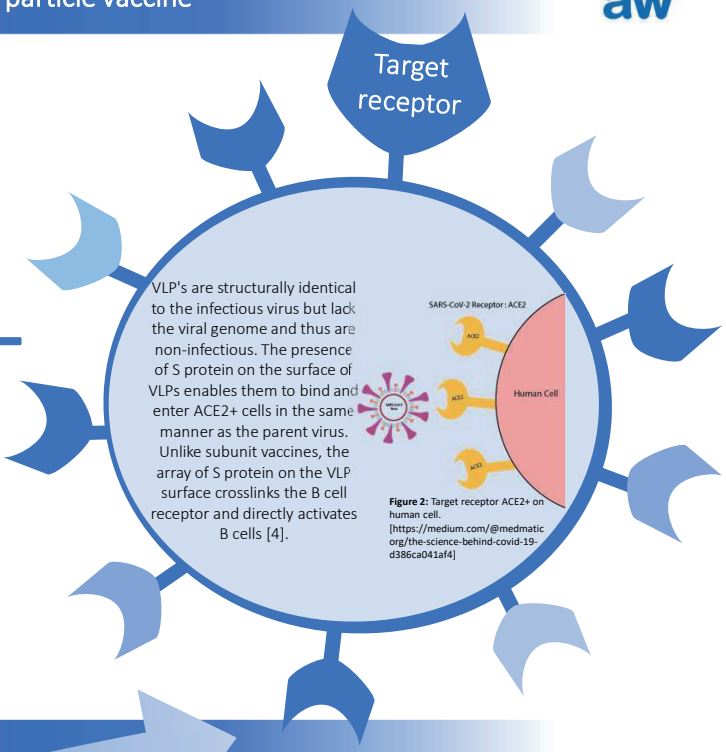
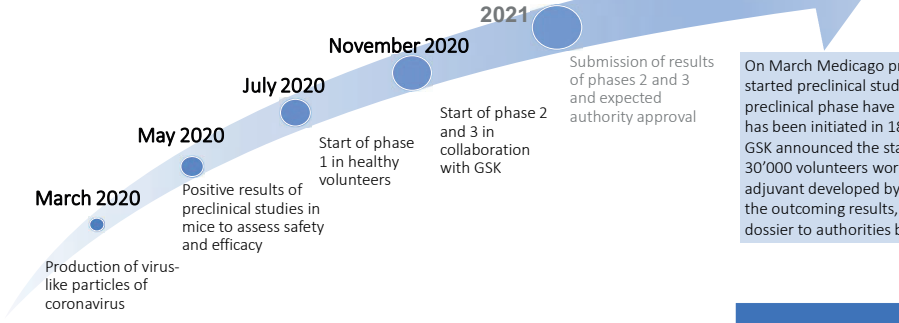
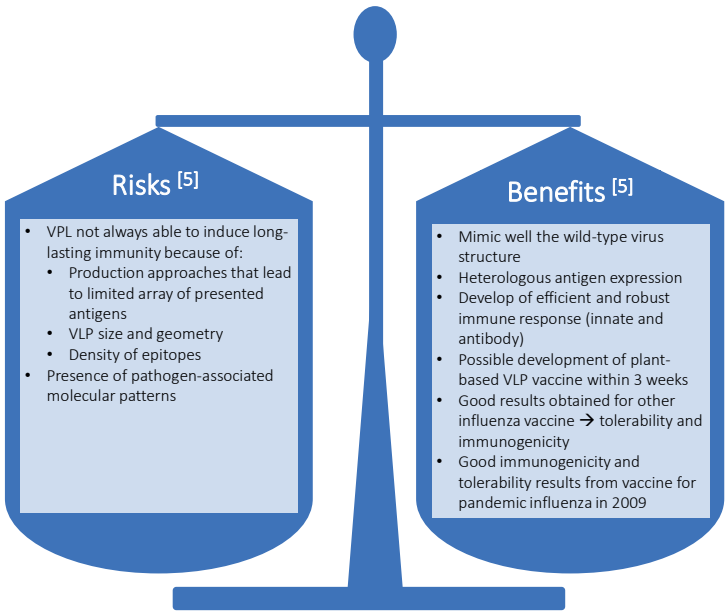


Figure 2: Target receptor ACE2+ on human cell. [https://medium.com/@medmatic org/the-science-behind-covid-19-d386ca041af4]

State of current development



On March Medicago produced virus-like particles of coronavirus and after that, they started preclinical studies in mice to assess safety and efficacy. Positive results of the preclinical phase have been announced in May and in July the phase 1 of human trial has been initiated in 180 healthy volunteers. In November Medicago together with GSK announced the start of phases 2 and 3 clinical trials, where they will enrol over 30'000 volunteers worldwide. The COVID-19 vaccine will be administered with an adjuvant developed by GSK. Depending on discussions with regulatory bodies about the outcoming results, Medicago plans to complete the clinical phases and to submit a dossier to authorities by the end of 2021. [1, 3]



Comparison with other approved VLP vaccines

	COVID-19 vaccine	Recombivax HB [6]	Gardasil [5]
Company	Medicago Laval University	Merck	Merck
Indication	Sars-cov-2	Hepatitis B	Human Papillomavirus (HPV)
Production	Plant	Recombinant yeast (*Saccharomyces cerevisiae*)	Recombinant yeast (*Saccharomyces cerevisiae*)
Viral protein in vaccine	Recombinant spike (S) glycoprotein	Surface antigen HBsAg of hepatitis B	recombinant major capsid (L1) protein of HPV Types 6, 11, 16, and 18
Adjuvens	CpG 1018 or AS03	amorphous aluminum hydroxyphosphate sulfate	amorphous aluminum hydroxyphosphate sulfate
Administration	Intramuscular injection	Intramuscular injection	Intramuscular injection

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18.12.2020

mRNA-1273 Vaccine Moderna

Course: Biodesign

Authors: Alexandra Eberle, Seyedehsara Shafieechashmi

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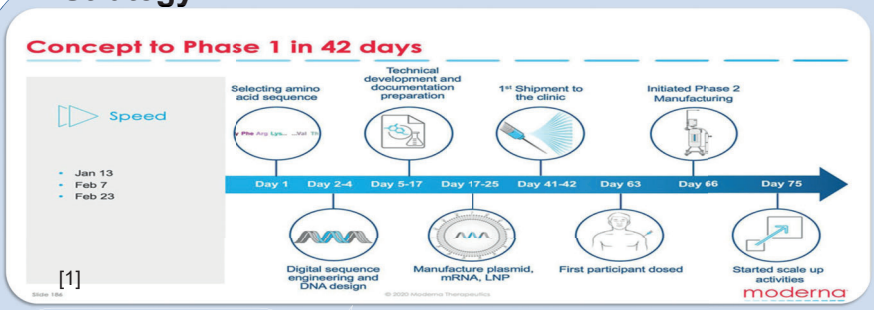
Strategy



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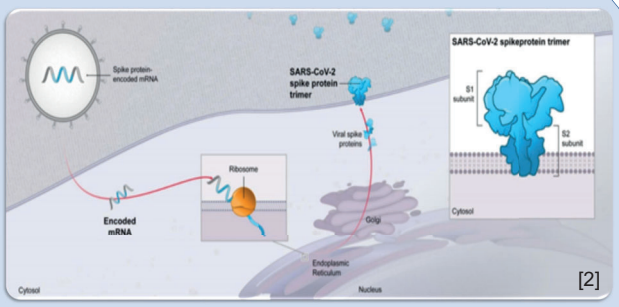

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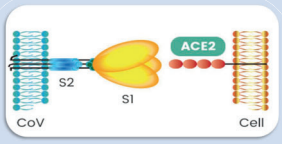
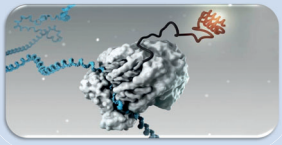


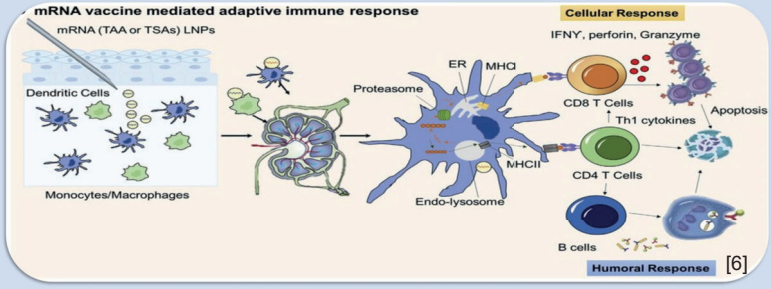


[3], [4]

Vaccine Platform	Antigen	Country	Route of Vaccination	Immunogenicity
mRNA-1273 mRNA in Lipid-nanoparticle	Spike protein (S-2P)	USA-based	• Parenteral (IM) • 18-55, 56+ years • 12-17 years old in clinical trial	• Repeated deliver: 2 • 2 doses (0-28 day) • Midlevel dose: 100µg [5]

Moderna uses information from the virus, by extracting mRNA information against viral spike protein and making mRNA-based-vaccine encapsulated by LNP. Following by IM administration, people make their own vaccines. mRNA enters cells and is used to produce viral antigen proteins improving B and T cell responses that include natural, and post-translational modifications [5].

Antigen Response



[6]

Neutralizing Antibody Response	T-cell Response	Other Attributes
Unimpeded due to lack of pre- existing Anti-vector Immunity	• Th1 response • Lung Trm cells cannot be induced by parental route	• Adjuvant required • Unclear whether it is amenable to RM vaccination [5]

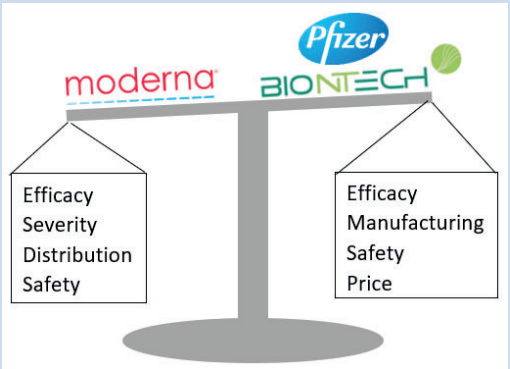
Benefits

- Protein translation and post-translational modification [5]
- Non-infectious, free of microbial molecules [5]
- Potential was shown in different virus infections in animals [5]
- Ease of production, scalability [5]
- Efficacy of 94.5 % [10]
- No potential risk of genomic integration [11]

Risks

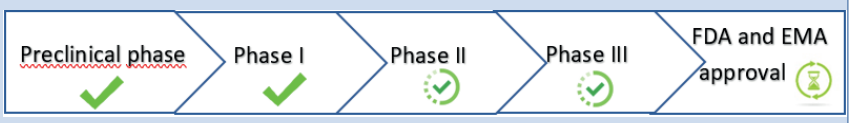
- The first mRNA vaccine was licensed for human use, at the beginning of December 2020 [5, 9]
- Storage and Distribution [11]

Comparison to other mRNA vaccines



The first mRNA vaccine against COVID-19 from BioNTech/Pfizer was licensed by the MHAR and FDA at the beginning of December 2020. Next to Moderna and Pfizer/BioNTech, CureVac also produces a mRNA vaccine against COVID-19 [5, 9, 10].

State of current development & outlook



Moderna is currently in phase II & III and applied to the FDA and EMA for a conditional marketing authorisation, whereas the FDA committee recommended the approval of the vaccine on 17 December 2020 [7, 9]. Earliest EMA approval is on 12 January 2021 [8].

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COVID-19 Vaccine V590 by Merck & IAVI

Module Biodesign: Ways to active pharmaceutical ingredients
Ramona Müller, Roman Senn, Thierry Wüthrich

1. Introduction

Merck & IAVI use a replication-competent recombinant Vesicular Stomatitis Virus (rVSV) for their vaccine called V590. While the native VSV can cause infections in animals, it only rarely causes disease in humans (Yahalom-Ronen et al., 2020). Therefore, this virus is used to achieve an immune response against a later SARS-CoV-2 infection. For this purpose, the G-protein (glycoprotein) of VSV is replaced by the spike (S) proteins of SARS-CoV-2. The organism immunized by a vaccination with rVSV generates antibodies against the spike proteins of SARS-CoV-2 (Yahalom-Ronen et al., 2020) and can thereby fight against a later COVID disease.

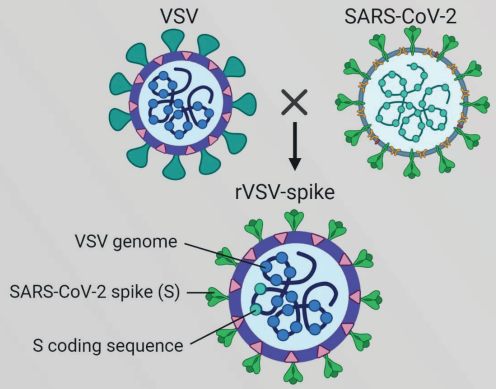


Figure 1: Method to produce the rVSV-spike virion. The G protein (glycoprotein) of VSV is replaced by the spike (S) proteins of SARS-CoV-2. (Created with BioRender.com)

2. Target and Route of Application

As already mentioned, the targets of the V590 vaccine are the spike molecules on the surface of the SARS-CoV-2 virus. The spikes mediate the receptor binding and membrane fusion steps of viral entry. Since the S proteins are the primary target of neutralizing antibodies and can elicit CD4+ and CD8+ T cell responses (Grifoni et al., 2020) they are a promising target. After animal tests in mice (Case et al., 2020) and hamster (Yahalom-Ronen et al., 2020), it is expected that a single dose of V590 administered intramuscular is sufficient for an immunisation.

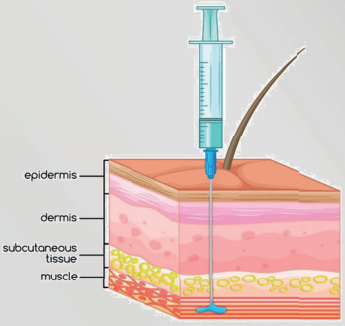


Figure 2: The vaccine V590 is administered intramuscular as a single dose. (Graphic source: colourbox.com)

3. Current State of Development

Pre-clinic	Phase I	Phase II	Phase III	Market approval
• Done in mice and hamsters • Tested in animals ○ Efficacy ○ Safety assessment ○ Well tolerated ○ Provides protection against SARS-CoV-2	• **Current** • First-in-human • Assess the: ○ Safety ○ Tolerability ○ Immunogenicity	• Planned for early 2021 • Could be expected 2-3 months after Phase I studies	• Planned for early 2021 • Could be expected 2-3 months after Phase II studies	• Not yet known • Could be expected 4-6 months after Phase III studies

Figure 3: State of current development phases with an outlook on earliest possible time point for market approval (Sources: Case et. al. 2020; ClinicalTrials.gov, 2020; Yahalom-Ronen et al., 2020)

4. Risk and Benefits

Currently, the vaccine is only in Phase I clinical trials, so no bene-fits and risks are yet known. Due to the fact that the Covid-19 V590 vaccine and the Ebola Zaire vaccine (Ervebo®, Merck) share the same vesicular stomatitis virus, the benefits and risks could therefore also be similar or even identical. (EMA, 2019; IAVI, 2020)

Benefits	Risks
Based on rVSV vector used to develop the Ebola vaccine that was 100 % efficacious in a trial in Guinea	Pain Muscle pain Joint pain
Relative quick immunity	Swelling and redness at the injection site
Potential single-dose formulation	Headache
Oral (not investigated yet) and intramuscular administration being tested	Fever
	Fatigue

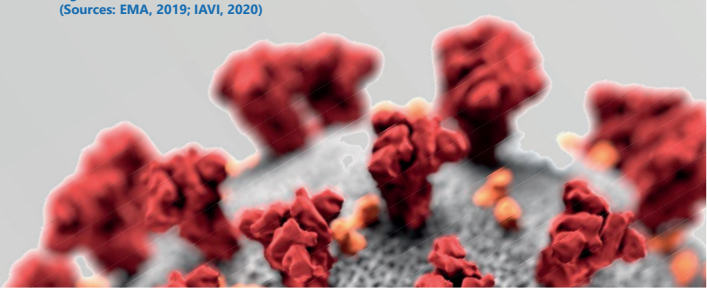
Figure 4: Possible benefits and risks of V590 vaccine based on Ebola Zaire vaccine (Ervebo®, Merck) (Sources: EMA, 2019; IAVI, 2020)

5. Analogy to existing Vaccines

The previously mentioned Ebola Zaire vaccine (Ervebo®, Merck) is the only vaccine approved to date that uses the principle of VSV-based immunization. Nevertheless, the introduction of this vaccine was a major breakthrough in its time and showed, that the usage of VSV-based drugs could be a leading technology in the future. Back in 2008, Kapadia et al. found, that the VSV based vaccine against SARS-CoV can achieve specific antibody expression withing a single-dose cycle in rats, using the same strategy. Other *in vivo* tests, described in Cobleigh et al. (2013) showed, that this technique can also be applied to fight the Hepatitis B virus, where they inserted a surface envelope glycoprotein (MS) into the VSV vector and achieved a specific antibody expression in transgenic mice.

All used Literature can be found withing here:

https://cdn.dtxcloud.com/prodassets/Literaturverzeichnis_Poster_Br79zOC.pdf



moderna

Biodesign 2020: Become familiar with the major vaccine strategies, under current development to fight COVID19

Zurich University of Applied Sciences
zh aw Life Sciences and Facility Management

HOW READY IS THE MODERNA mRNA-1273 VACCINE KNIGHT TO FIGHT THE COVID-19 DRAGON?

INTRODUCTION

SARS-CoV-2 virus is a novel Coronavirus first identified in humans in December 2019 and is causing COVID-19 [1].
Disease burden was globally estimated to up to 72 million confirmed cases and 1 600 000 deaths [2].
In Switzerland, up to 384 000 cases and 6151 lethal cases were reported [3].

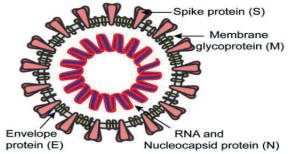


Fig. 1: Schematic of the SARS-CoV-2 structure [6].

The risk of mortality increases with increasing age and comorbidity factors [4].
Currently, there are several vaccine candidates for COVID-19, in clinical evaluation and nucleic acid vaccine platform had proved its efficacy [5].

mRNA-1273™: A PILOT ANTI- SARS COV-2 NUCLEIC ACID PLATFORM BASED VACCINE

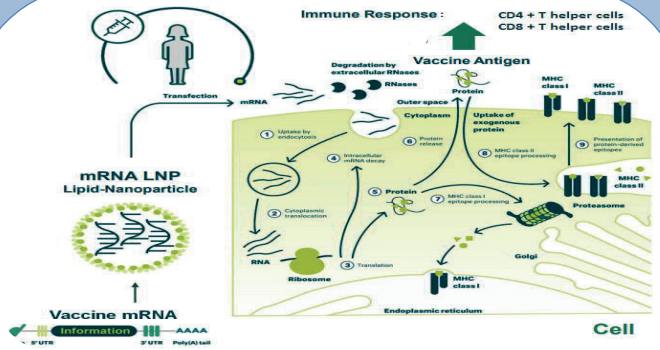


Fig. 2: Mechanism of action of mRNA vaccines [6] .

The Moderna mRNA-1273 ™ vaccine candidate, encodes the S-2P antigen, packaged in a lipid nanoparticle [7]. Multiple steps are required to acquire the immunogenicity:

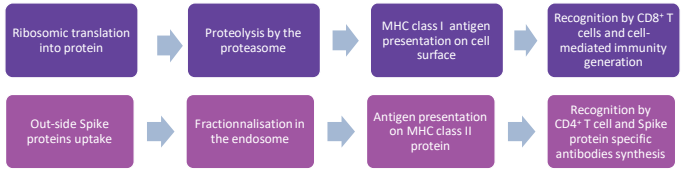


Fig. 3: Cell mediated (violet) and humoral (pink) immunity mecanisms induced by the mRNA vaccine candidate [8] .

In most trial participants, mRNA-1273 ™ induces [7] :

- A strong S protein-specific antibody response.
- A primarily CD4+ cell, response.
- A CD8+ T cell response.

TARGETED ANTIGEN

The Moderna mRNA-1273™ vaccine candidate targets the Spike protein, activated during viral infection by TMPRSS2 after being bound to the host cell receptor ACE2 and subsequently cleaved S1 and S2 subunits [10], mediating the mechanism of the viral cell entry [11].

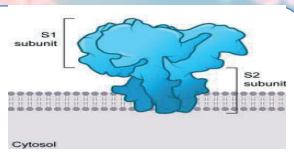


Fig 4. : SARS-CoV-2 Spike protein trimer [12].

FORMULATION, VACCINATION ROUTE AND REGIMEN

The Moderna, mRNA-1273™ vaccine candidate, encodes the S-2P antigen, consisting of the SARS-CoV-2 glycoprotein with a transmembrane anchor and an intact S1–S2 cleavage site [7]. The lipid nanoparticle capsule composed of four lipids (X ,Cholesterol, DSPC and PEG2000DMG) was formulated in a fixed ratio of mRNA and lipids. mRNA-1273 has a lipid content of 9.7 mg/mL and is formulated at a concentration of 0.5 mg/mL [9]. The mRNA-1273™ is provided as a sterile liquid for injection and is administered through 0.5 mL intramuscular injection in the deltoid muscle on days 1 and 29 at a concentration of 100 µg per dose [7] .

CURRENT DEVELOPMENT

Actually, the Phase 3 of the COVE study is in progress [13] :

- Vaccine efficacy: 94.1% .
- Vaccine efficacy against severe COVID-19: 100% .

Sollicitation of Moderna strategic partners Lonza and Rovi to deliver up to 1 billion doses per year [14].
The first adolescent participants have been dosed in the Phase 2/3 study [14].

EXPECTED MARKET APPROVAL DATE

- Aug.2020: Canada exercises increased option for 20 million doses .
- Sep.2020: Supply agreement with U.S. for initial 100 M. doses . Discussions with E.U. commission for a 80 M. doses supply .
- Oct.2020: Japan partnership to supply 50 M. doses . Supply agreement with Qatar . Confirmation of eligibility for submission of marketing authorization application to the European Medicines Agency . Initiation of rolling submission to Health Canada .
- Nov.2020: Application for FDA authorization . EU secures 80 million doses of mRNA vaccine .
- Dec.2020: Switzerland exercises increased option for 7.5 M. doses . Scheduled FDA meeting on December, 17, 2020 . **Emergency use nod for mRNA vaccine expected shortly after December, 17, 2020 .**

Fig. 5: Market approval process timeline [14] .

RISKS AND BENEFITS

- Severe AEs in 20% of vaccinee in case of high doses [7].
- $ 69 per patient : $32 and $37 per dose [15].

- Rapid and robust immunogenicity [7] .
- Durability of responses [19] .
- Efficient antigen production [7].
- High-quality and high-magnitude antibody responses [7] .
- Safety [7] .
- Affordable storage conditions [9] .

Fig.5: Risks and benefits of the Moderna mRNA-1273 vaccine candidate .

COMPETITIVE LANDSCAPE

Efficacy over 94% [17] .
$ 50 per patient or $ 25 a dose [16] .

Possibly lethal cases reported in trials [18].

Pfizer BNT162b1 ™
Temporary authorization from the UK

Drastic storage conditions and problems in distribution [17,20] .

Severe side effects [17] .

Fig.6: Risks and benefits of BNT162b1 ™ vaccine candidate

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CVnCoV (CureVAc)

mRNA based Vaccine for COVID-19

Module Biodesign: Ways to active pharmaceutical ingredient

Zürcher Hochschule für Angewandte Wissenschaften
zh aw Life Sciences und Facility Management

Overview of vaccine

The SARS-CoV-2 pandemic requires rapid development and production of a vaccine on a large scale. The mRNA vaccine CVnCoV codes for the trimeric S protein of the virus and is encapsulated in a lipid nanoparticle. The formulation allows the mRNA to be endocytosed by the target cell so that its post-translational protein is presented on the cell surface. The vaccination with CVnCoV triggered a strong humoral immune response with high virus neutralizing titers in mice and hamsters. Furthermore, stable CD4+ and CD8+ T cell responses were induced in mice. Based on the pre-clinical phase, a safe vaccine candidate could be delivered for the vaccination for the time being. (Status 8. 12. 2020)

Mode of action

4 Receptor-mediated endocytosis allows the mRNA molecules to be taken up by the lysosomes. Most mRNA is degraded, but only those mRNA molecules that interact with eIF4E proteins escape from the endosomes and bind to ribosomes [7]. Figure 1 shows the total amount of intra- and extracellular S-Protein to analyse S expression.

5 The mRNA recognized by Toll-like receptors (TLRs) then triggers a series of signalling cascades that exert a regulatory function on pro inflammatory cytokines. These include interferon cytokines (IFNs), which directly activate T cells and indirectly induce the transcription of interferon-stimulated genes (ISGs) involved in the maturation process of dendritic cells (DCs). IFNs also supports antiviral enzymes that promote mRNA degradation and inhibit mRNA translation [7].

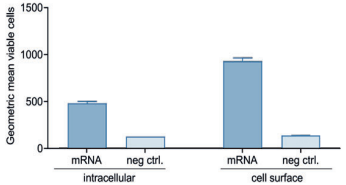


Diagramm 1: S Protein expression from CVnCOV in HeLa. The cells were transfected with 2 µg mRNA and the amount of intracellular and cell-surface bound S Protein expression detected afterwards [2].

Risk and Benefit

mRNA vaccines are non-infectious, free of microbial molecules and showed no insertional mutagenesis[4].

Moreover they showed of safety, efficacy and antivectorial immunity profiles[1].

Phase I study of CVnCoV show good tolerability, induction of antibodies and signs of T-cell activation[6,9].

Benefit

Risk

Currently no mRNA-based vaccine is approved, which poses a risk due to the lack of long-term studies [2].

mRNA is very unstable in vivo and is rapidly degraded by the RNase and only storable at low temperatures [3,4].

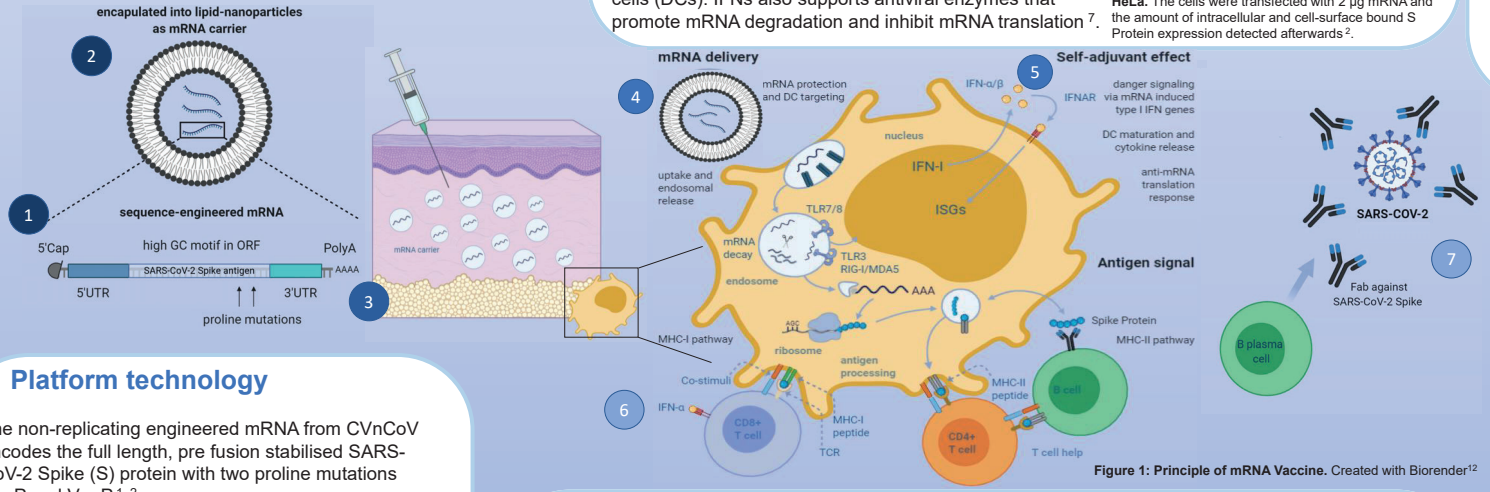
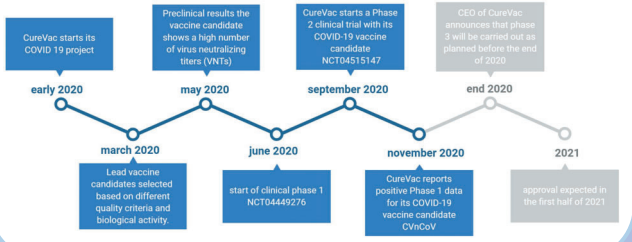


Figure 1: Principle of mRNA Vaccine. Created with Biorender[12]

Current development & Timeline of Approval



Platform technology

The non-replicating engineered mRNA from CVnCoV encodes the full length, pre fusion stabilised SARS-CoV-2 Spike (S) protein with two proline mutations $K_{986}P$ and $V_{987}P$ [1, 2].

1 Based on the RNActive® platform, the mRNA includes an optimised open reading frame (ORF) with a high GC content as a 5' cap and a 3' UTR, polyA tail improving stability and translation efficiency [3]. The in vitro transcription can be performed using a linearized plasmid DNA [4].

2 To improve stability, efficacy and delivery to the translational machinery, the mRNA sequence is encapsulated into Lipid nanoparticle, consisting of ionizable amino lipid, phospholipid, cholesterol and PEGylated lipid [2,5].

Route of Application

3 The optimal effect of the mRNA-based vaccine should be achieved by parenteral administration and the resulting systematic immunity. The encapsulated mRNA is administered i.m., i.v. or s.c. and, due to the lipid nanoparticle carrier, is delivered into the cytoplasm where protein synthesis can take place [1,2].

Extended Mode of Action-Immunological Response

6 Balb/c mice, were vaccinated with different schemes i.m. with 2 µg of CVnCoV. After a balanced immune response of the innate immune system (results not shown), a short peptide is presented on the cell surface of the dendritic cell via MHC-I and MHC-II. This activates the specific cellular and humoral response.

7 After one week, robust SARS-CoV-2 S-specific IgG1 (see figure 2 left hand side) and IgG2a antibodies were detected by ELISA[2]. Median endpoint titres increased to 3.9 x 10^6 for both IgG1 and IgG2a on day 28. The induction of virus neutralising titres (VNTs) in mouse sera was tested in a cytopathic effect (CPE)-based assay using wild-type SARS-CoV-2. Detectable titers were measured 4 weeks after the 1st injection. The 2nd injection resulted in a significant increase in VTN levels (see figure 2 right hand side)[2]. On day 49 the highest VNT were able to neutralize 50 % of the virus at a dilution of 1:5120. The neutralizing antibodies block its uptake into a cell without recruiting further immune cells and thus contributes to vaccine protection [8].

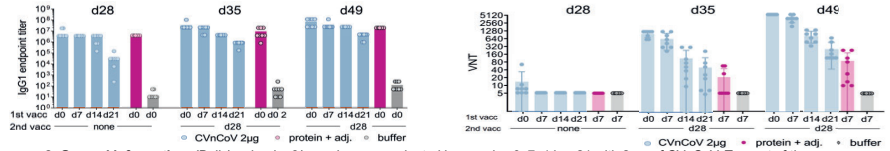


Diagramm 2: General information: (Balb/c mice (n=8/group) were vaccinated i.m. on day 0, 7, 14 or 21 with 2 µg of CVnCoV. Two out of three groups received a second immunization on day 28. Positive control: 1.5µg of Album adjuvanted SARS-CoV-2 S ectodomain (SECD) protein, negative control: 0.9% NaCl admin. on day 0 and day 28 [2] **SECD protein specific binding antibodies, displayed as endpoint titres for IgG1 (left hand side).** maximum endpoint titers were reached on day 28. **CPE-based virus neutralising titres in serum (right hand side).** The highest VNT measured was able to neutralise 50 % of the SARS-CoV-2 with a dilution of 1:5120 in cell culture [2].

Comparison

CVnCoV is among the 25 % most developed vaccines out of a total of 231 candidates worldwide. Compared to the top three vaccines Biontech, Moderna, Astrazeneca, the vaccine is lagging behind with its Phase III [10].

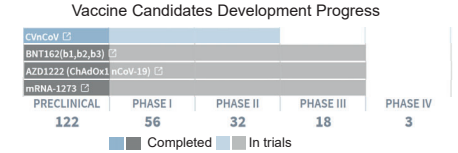


Figure 3: Comparison of CVnCoV worldwide and against the three most developed vaccines [10, 11].

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Brunner Kathrin, Défayes Raphael, Weiss Noémi

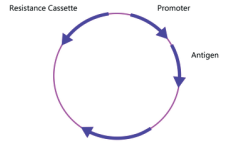
COVID-19 pDNA Vaccination by AnGes, Inc.

Introduction of AG0301-, and AG0302-COVID

Joëlle Inderbitzin, Tobias Wyser, Msc Biodesign 2020

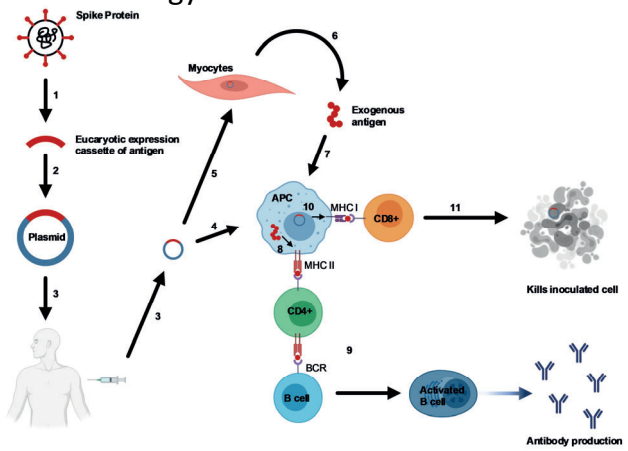
Introduction

AnGes, Inc. Is a Japanese biopharmaceutical company that focuses on the development of gene medicines. It was founded in 1999 because of a discovery of researchers at Osaka University and has had their first product released in 2019 on the Japanese market. With AG0301- and AG0302-COVID AnGes, Inc has currently two clinical trials running for DNA vaccines based on their experience in gene medicines. [3][4] The first experiments done with DNA vaccination have been done in 1990, however so far, no vaccination has been released for use in human. Due to the highly versatile plasmid platform, pDNA vaccination strategy is often brought up in pandemic scenarios such as H1N1, Zika, or Ebola. [6] The challenge yet to overcome is to create a reliable immunity in human. [8]



Possible DNA Plasmid for vaccination [15]

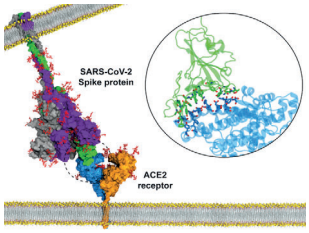
Vaccine strategy



Mechanism of pDNA vaccination. By administration of pDNA a cellular (4, 10, 11) or humoral (5, 6, 7, 8, 9) immune response can be induced [9]. Adapted from [9] and created with biorender.com

Target and Administration

The target of the vaccination is the spike glycoprotein. It plays a crucial role in infecting the hosting cell and occurs as a homotrimer complex on the virus surface. The spike complex interacts with the host cell surface receptor ACE2. [1] Due to its integral nature in viral transmission and exposed position, the spike protein offers an ideal target for the vaccine since the infectiousness of the virus relies on a functioning spike protein complex [2].



Homotrimer Spike Complex targeted by the vaccine interacting with ACE2 receptor. [5]

The vaccine is administered by intramuscular injections. Both studies consists of multiple groups of healthy individuals receiving different doses and intervals. [3][4]
AG0302-COVID is currently being tested on 500 healthy volunteers with 2mg dosage in 2, respectively 4, week intervals.
Parenteral administration may fail to induce the important IgA and T-cells in the respiratory mucosa – the area of first contact [2], which may become a hurdle during the COVID-19 trials.

AG0301-COVID	**Start Phase I/II**	**Est. Primary Completion**	**Est. Study Completion**
	June 29, 2020	**September 26, 2020**	**July 31, 2021**
AG0302-COVID	**August 31, 2020**	**November 23, 2020**	**March 31, 2022**
	Start Phase I/II	**Start Phase II/III**	**Est. Study Completion**

Approved pDNA Vaccines

Target	Description
Veterinary Vaccines (approved)	
IHN vaccine for fish [10]	• Prevention of Infectious Hematopoietic Necrosis Virus in fish • Administration: Single intramuscular injection
West Nile virus in horses [11]	• Antigen: premembrane (prM) envelope gene (E) • Administration: Single intramuscular injection
Human Vaccines (not approved)	
Zika Virus VRC 705 (Cl. Phase II) [12]	• Antigen: prM and E-Protein from Zika virus wildtype • Administration: 3-doses of intramuscular injection with needle-free device
Ebola Virus INO-4212 (Cl. Phase I) [14]	• Antigen: GP EBOV pre 2013 & EBOV 2014, IL-12 (3 vectors) • Administration: Electroporation and intradermal or intramuscular 2-3 doses

Benefits

- High degree of versatility and flexible production [6]
- Well tolerated in patients [6]
- Immunogen presented by both histocompatibility complexes (class I & II) [7]
- Mimics protein synthesis during viral infection including glycosylation patterns [6]

Risks

- Failed trials due low immune responses [6]
- Long term persistence of DNA plasmids upon injection and risk of genomic integration resulting in threat of mutagenesis and oncogenesis [6]
- High DNA doses required for intermuscular injection since vaccine must cross multiple barriers [7]
- Co-stimulatory adjuvant to enhance immunogenicity may lead to adverse effects like allergic reactions [6]
- Potential of DNA detecting antibodies [6]
- Parenteral administration may fail to induce the important IgA and T-cells in the respiratory mucosa [2]
- Use of Kanamycin selective markers for replication in bacteria [6]

Conclusion

The current status of AG0301 is unclear since the study has not been updated for months. AG0302 just reached clinical phase II/III. The slow progress raises critical voices within Japan which are denouncing Japans slow progress in development and its vaccination policy in general as government funding is lacking. While certain countries have started their COVID vaccination, a Japanese vaccine is not expected before 2022. [13]

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