COMIRNATY FACTS

Mechanisms of action and damage potential

18. DEZEMBER 2024

Version 1.0

Created by U. Kämmerer

Contents

1. Preliminary remarks ... 4

1.1 Classification of the product Comirnaty as a genetic vaccine ... 4

Figure 1: The ... 5

1.2 Ingredients of Comirnaty: ... 7

Active ingredient: ... 7

1.3 The manufacturing process as the basis of the possible harmful potential ... 8

Figure 2 shows the comparison of Process 1 and 2 ... 9

1.3.1. production of the "sample template (DNA template) ... 9

1.3.2. amplification of the starting template, ... 10

1.3.3 RNA synthesis ... 10

Figure 3: the RNA modification ... 11

1.3.4 Various purification and filtration steps ... 11

Figure 4: Steps from RNA production to bottling ... 11

1.3.5 To produce the transfectable lipid-modRNA nanoparticles ... 11

Figure 5 for the specification of lipid nanoparticles (LNP). ... 12

1.3.6 For the end product ... 12

1.4 Production steps with hazard potential ... 12

1.4.1 The DNA template. ... 12

Figure 6: The modifications of the modRNA ... 13

1.4.2 Duplication of the DNA template as starting material for RNA production ... 13

Figure 7 shows the plasmid map ... 14

Insert for understanding: Plasmids ... 15

1.4.2.1 The special feature of the plasmid used for Comirnaty ... 15

Figure 8 for the SV40 enhancer element ... 16

1.4.2.2 Is the SV40 element contained in the manufacturing plasmid in all batches and active in principle? ... 17

Figure 9 shows the most important subsequently submitted points from the ... 18

Figure 10: Proof that the basic plasmid used with the SV40 elements originates from Pfizer's gene therapy program ... 19

1.4.3 RNA production and modification of the base uracil. ... 20

Insert for understanding: Genetic code ... 21

The wobbling, which is increasingly triggered by the Ψ ... 21

1.4.4 Purification of the spike-coding RNA and separation of the DNA template as well as plasmid and bacterial DNA residues and malformed RNA molecules ... 22

1.4.4.1 Insufficiently separated DNA and plasmid components ... 23

Figure 11 shows the detection of integrated plasmid DNA ... 24

1.4.4.2 Special hazards of the SV40 enhancer element ... 24

An insert for understanding: the 72 bp enhancer element of SV40 ... 25

1.4.4.3 Further dangers of DNA residues ... 26

To understand: Primer ... 26

1.4.4.4 Differently long RNA molecules and double-stranded RNA ... 26

1.4.5 Packaging of RNA in lipid nanoparticles ... 27

1.4.5.1 General problems of lipid packaging ... 29

1.4.5.2 The size of the lipid particles ... 29

Figure 12: Typical lipid particles from Comirnaty original bottles ... 30

1.4.5.3 The individual components of the lipid envelopes ... 30

Figure 13 Fusion of lipid envelopes with cell membranes: ... 32

1.4.5.4 Lipid quantity and distribution in the body ... 33

Figure 14: Size distribution of DNA fragments from Comirnaty ... 34

1.4.6 Final cleaning and filling ... 35

Figure 15 Production steps Comirnaty ... 36

2. The SARS-CoV-2 spike protein as a vaccine antigen ... 37

2.1 Structure of the spike protein ... 37

2.1.1 General structure ... 37

Figure 16 Spike protein and proline anchor from Comirnaty ... 38

2.1.2 Special features of the SARS-CoV-2 spike protein ... 38

2.1.2.1 Unusual structural elements: ... 38

Figure 17 shows the HIV receptor binding sites in the spike protein ... 38

2.1.2.2 The conspicuous furin cleavage site ... 39

Figure 18 on the furin cleavage site: ... 39

2.1.2.3 Possible neurotoxic properties: ... 40

2.1.2.4 A master pattern of prions ... 40

Figure 19 shows the prion sequence found ... 41

2.1.2.5 Receptor binding domains in the spike molecule. ... 41

Figure 20 on the function of the receptor binding domains ... 41

Figure 21 of the unusual receptor binding sites, ... 42

Figure 22 shows binding partners at the blood-brain barrier ... 43

2.1.2.6 Summarizing the structure 43
2.2 Special effects of the spike protein in the body 44
2.2.1 The S2 subunit triggers cell fusions 44
Figure 23 shows the formation of syncytia by the S2 subunit of the spike protein 45
2.2.2 The S1 subunit can spread throughout the body and persist for a long time 45
Figure 24: Spike staining on blood vessels 46
2.2.2.1 The S1 subunit triggers inflammation in macrophages 47
Figure 25: Sketch of the connection between spike and inflammation 48
2.2.2.2 S1 as a cause of vascular inflammation and damage 48
Figure 26: Thrombosis induction via the S1 subunit 49
2.2.2.3 Summary of spike and thrombosis/embolism due to vascular damage 50
2.2.2.4 S1 subunit is neuroinflammatory and can trigger ME/CSF in the brain 50
3. Disruption of the immune system at several central sites 51
3.1 Link between genetic vaccinations and infections 51
3.1.1 The more genetic vaccinations, the more Covid-19 diseases. 51
Figure 27 on the increasing risk of Covid-19 disease after vaccination. 52
Figure 28 on the accumulation of Covid-19 in three genetic vaccinations 53
3.2 Dendritic cells, not muscle cells, are the target of transfection 53
Figure 29 shows Comirnaty's target cell: 54
Insert for understanding: dendritic cells 54
3.2 Spike expression on dendritic cells other than physiological 55
Figure 30: Theoretical effect of Comirnaty on dendritic cells 56
Figure 31 of the effect of modRNA on dendritic cells 57
3.3 Antibody class change as a sign of an immune problem: IgG4 58
Insert for understanding: Antibodies and Comirnaty 58
Figure 32 shows the class change of the antibodies 59
Figure 33 on the lack of mucosal immunity after vaccination. 60
4. Conclusion from points 1-3 61
Figure 34 summarizes the most important points of the Comirnaty effect from the preliminary remarks. 61
5. Bibliography 62

1. Preliminary remarks

1.1 Classification of the product Comirnaty as a genetic vaccine

The medical product Comirnaty (BNT162b2, BioNTech/Pfizer, "Covid-19 vaccine", active ingredient: tozinameran, modified mRNA for the spike protein of SARS-CoV-2) to be assessed is not a classic vaccine as defined before 2019, but represents a new mode of action, a "***novel mRNA technology***" (regulations on gene-based vaccines, German Bundestag: WD-9-116-20-pdf-data.pdf).

Classic vaccines contain either attenuated or killed pathogens or the antigen/several antigens, i.e. components of pathogens against which the vaccinated person's body is supposed to develop an immune reaction. Vaccines with killed pathogens or pure antigens are called inactivated vaccines (e.g. tetanus, diphtheria, hepatitis B), those with attenuated pathogens are called live vaccines (e.g. measles, mumps, rubella). In order to strengthen the immune response, adjuvants are added to classic vaccines, which stimulate the innate immune system to produce cytokines and thus strengthen the response to the vaccine. In modRNA-based vaccines such as Comirnaty, the components of the lipid particles themselves serve as adjuvants.

According to "Impfen-Info.de" from the Federal Centre for Health Education, Comirnaty falls into the new class of **gene-based vaccines**. "*With these vaccines, it is not the pathogen itself or components of the pathogen that are inoculated**, but the genetic information, i.e. the blueprint for components of the pathogen**. This leads to body cells "reading" this blueprint and producing these parts of the pathogen themselves. The genetic material is the blueprint for one or more antigens of the pathogen. Antigens are components of the pathogen to which the immune system reacts and develops a defense. **Gene-based vaccines include mRNA vaccines, vector vaccines and DNA vaccines**."*

The three principles of the vaccine classes are defined by the Paul Ehrlich Institute in a diagram as follows: (https://www.pei.de/DE/newsroom/hp-meldungen/2022/220221-covid-19-pandemie-impfstoffe-im-fokus.html) Here, the gene-based vaccines are even referred to as "**genetic vaccines**". A term that is used below for Comirnaty.

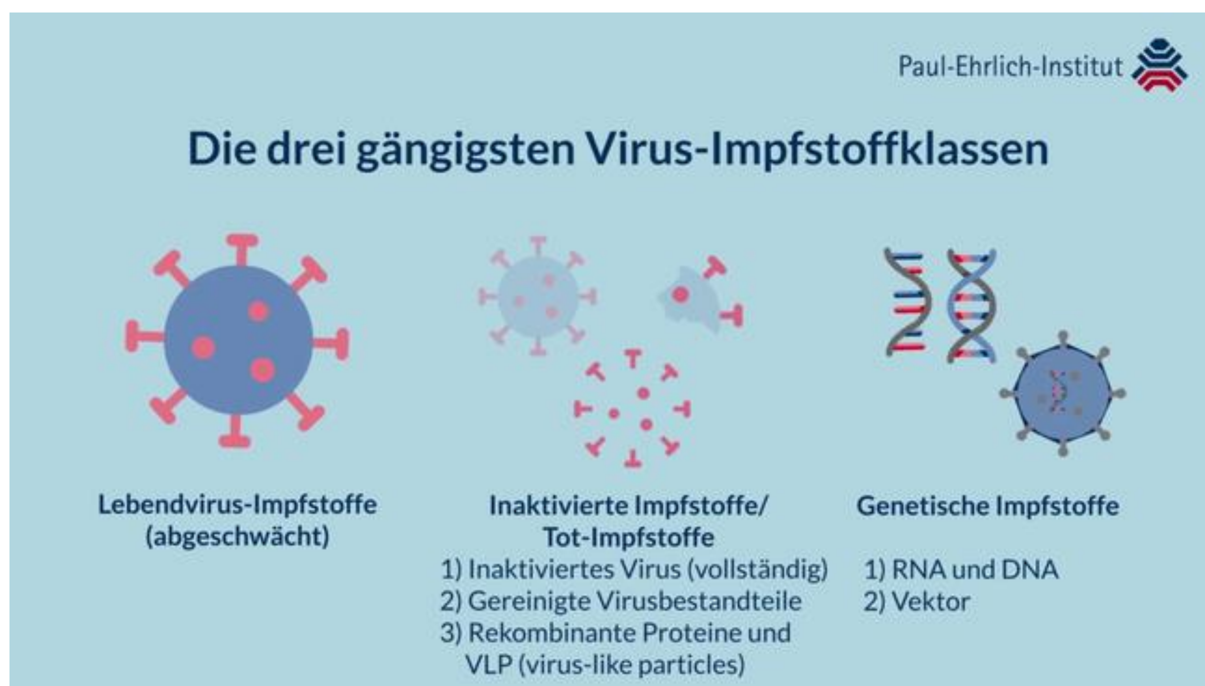


Figure 1: The Paul-Ehrlich-Institut's **vaccine class definition** (Q: Messages - COVID-19 pandemic - Vaccines in focus - Paul-Ehrlich-Institut)

According to the scientific service of the German Bundestag (Regulations on gene-based vaccines, Dt. Bundestag: WD-9-116-20-pdf-data.pdf), Comirnaty as a genetic RNA vaccine ("novel mRNA technology") does not fall under the term gene therapy. This corresponds to the definition of gene therapy as amended on June 20, 2019 (Annex I, Part IV, Section 2.1 of Directive 2001/83/EC, cited in Regulations on gene-based vaccines, German Bundestag: WD-9-116-20-pdf-data.pdf), which pre-emptively excluded genetic vaccines from the group of gene therapy medicinal products. ("*Medicinal products containing mRNA, which are vaccines against infectious diseases, are* ***not*** *classified* ***as gene therapy medicinal products*** *and therefore not as ATMPs in accordance with Annex I, Part IV, Section 2.1 of Directive 2001/83/EC.")*. The EMA also confirms on a dedicated information page on its website that "mRNA vaccines" are not gene therapy medicinal products, but are classified as "biotechnology medicinal products" (COVID-19 vaccines: key facts | European Medicines Agency (EMA) (europa.eu))

Technically speaking, Comirnaty's mechanism of action corresponds most closely to a **transfection** due to the packaging of the gene information (here: RNA) in a lipid envelope, the properties of which are used to introduce the RNA into the cell, because: "***Transfection*** *is the introduction of foreign DNA or RNA into a cell. Transfection typically leads to the stimulation of the host cell's protein synthesis apparatus with the formation of the gene product*." (Transfection - DocCheck Flexikon).

Comirnaty (as well as comparable products characterized by the nucleic acid packaged in lipid envelopes) can therefore technically be seen as an **effective transfection system**. With this system, foreign genetic material (here: RNA which codes for the spike protein of the SARS-CoV-2 virus) is introduced into potentially all body cells with the help of so-called cationic lipids (the predominantly used lipids of the lipid nanoparticles) in order to stimulate the production of the spike protein as a viral, foreign protein. This spike protein should then be presented on the cell surface by the transfected cells in order to stimulate the human immune system to react against this foreign protein (antigen).

In contrast to the comparable group of classic antigen vaccines, in which the vaccine antigen of a pathogen (e.g. HBsAg from hepatitis B) is genetically engineered in transfected cell cultures (so-called recombinant antigen) and inoculated after purification, genetic RNA vaccines such as Comirnaty **involve the transfection and genetic engineering of the recombinant antigen directly in the body of the vaccinated person.** This eliminates the need for costly and contamination-prone cell cultures and turns the vaccinated people themselves into the antigen production site.

In principle, this transfection by vaccination with Comirnaty is a classic genetic engineering application as defined by the Genetic Engineering Act (umwelt-online: Gesetz zur Neuordnung des Gentechnikrechts (1) GenTG §3 GenTG, Begriffsbestimmungen - 3a*):* Methods *of modifying genetic material in this sense are in particular*: b) "*methods in which* ***genetic material is introduced directly into an organism*** *which has been produced outside the organism and does not occur naturally therein, including microinjection, macroinjection and microencapsulation*."

In relation to Comirnaty: by vaccinating the lipid-encapsulated RNA, genetic material is actually introduced directly into the organism (of the vaccinated person), because the genome (genetic material) of the corona virus SARS-CoV-2 consists of RNA (unlike the human genome, for example, which consists of DNA). The RNA was produced *in vitro* at Pfizer or BioNTech, i.e. outside the organism. The gene for the SARS-CoV-2 spike protein does not occur naturally in human cells and the lipid nanoparticles fulfil the aspect of microencapsulation.

According to the old GenTG, every recipient of Comirnaty would thus become a "genetically modified organism" (GMO). However, this was changed in the amendment of 21.12.2004 to the effect that genetic techniques on humans no longer lead to a GMO, because in the current version of the GenTG (https://www.umwelt-online.de/regelwerk/cgi-bin/suchausgabe.cgi?pfad=/gefstoff/gen_tech/zz05.htm&such=Umweltaktuellen version) a GMO is "*an organism,* ***with the exception of humans****, whose genetic material has been modified in a way that does not occur under natural conditions through crossing or natural recombination (...*)".

The advantage of this "novel mRNA technology", the significantly simpler, faster and cheaper production of the genetic RNA vaccine compared to the more complex production of classic vaccines, is offset by a number of fundamental disadvantages and risks when vaccinating substances such as Comirnaty, the most important of which are presented below:

1) The number and type of cells transfected and thus genetically transformed into spike producers within the body cannot be predicted or verified. *In vitro*, in cell culture (e.g. yeast cells in the production of the hepatitis B antigen), this process is defined and easy to monitor, but not in a complex organism such as humans.
2) The production of the desired antigen from the transfected genetic information depends on many environmental conditions and the intracellular environment of the transfected cells. This is also unpredictable in the complex organ and tissue system of the vaccinated person and corresponds to a "black box". Consequently, the quality and quantity of the antigen produced *in vivo* cannot be controlled.
3) It is known from the transfection of cell cultures that with this technique many cells lose their integrity and perish due to the fusion of the lipid particles with the cell membrane. This is minimized by even and diluted distribution of the transfection solutions over the culture. In

the case of vaccination, however, the transfection substance is injected in a concentrated form at one location, meaning that possible cell damage or problems with high "on-site concentrations" are possible.

4) As this is a novel technique, which was used for the first time on a large scale as part of the anti-Covid vaccination campaign, little is known about the effects of the individual components of the very elaborately produced active ingredient and the accompanying and auxiliary substances in the body of the "vaccinees".
5) The manufacturing process involves a large number of process steps using genetic engineering methods. The chemical reactions in these methods with enzymes and building blocks are not reliably controlled, even on a laboratory scale, and therefore almost inevitably lead to end products that are not precisely defined and not highly pure on a large technical scale. Production process "2" using bacteria and plasmids, which is discussed in detail below, should be emphasized here.

Further product-specific effects (including special features of the spike protein of SARS-CoV-2) of Comirnaty in connection with possible damage to the organism are dealt with in detail under points 2.1-2.2 .

Despite these genetic engineering mechanisms, for the sake of simplicity, the naturalized term vaccination is largely retained here with the addition of "genetic" (genetic vaccination), although this novel mRNA technology is correctly a transfection.

1.2 Ingredients of Comirnaty:

Summary of ingredients is seen on (https://www.vfa.de/de/arzneimittel-forschung/coronavirus/corona-impfstoffe-herstellung)

See also Table 1 under point 1.4.5.3

Active ingredient:

Tozinameran = modified mRNA for the spike protein of SARS-CoV-2 (the name Tozinameran has been proposed but not yet authorized by the WHO).

For a more detailed description of the active substance, see the EMA assessment report (Comirnaty, INN-tozinameran, tozinameran/riltozinameran, tozinameran/famtozinameran (europa.eu);

Point 2.2.2 Active Substance - General Information (Translated):

"The active substance consists of a ***single-stranded, 5'-capped mRNA that is translated into a codon-optimized sequence encoding the spike antigen of SARS-CoV-2****. The vaccine is based on the spike glycoprotein (S) of SARS-CoV-2. The sequence was chosen based on the sequence for the "Severe acute respiratory syndrome corona virus 2 isolate Wuhan-Hu-1". The protein sequence contains two proline mutations, which ensures antigenically optimal prefusion confirmation (P2 S). The RNA contains no uridine;* ***instead of uridine, the modified N1-methylpseudouridine is used in the RNA synthesis****."*

Lipids:

- ((4-Hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315)
- 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159)
- 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC = colfosceril stearate)
- Cholesterol

Substances for adjusting the pH value and other salts:

- Potassium chloride
- Potassium dihydrogen phosphate
- Sodium chloride
- Sodium monohydrogen phosphate 2H O_2

Other ingredients:

- Sucrose
- Water for injection purposes

1.3 The manufacturing process as the basis of the possible harmful potential

Sources considered:

Description under point 2.2.2 Active Substance in the EMA Assessment Report (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf) and point 7.1 (Manufacturing Process) in the Presubmission Meeting Briefing Document in the BioNTech presentation Australia (FOI 2389 document 3-1 (tga.gov.au)) as well as in a publication (Rosa SS 2021).

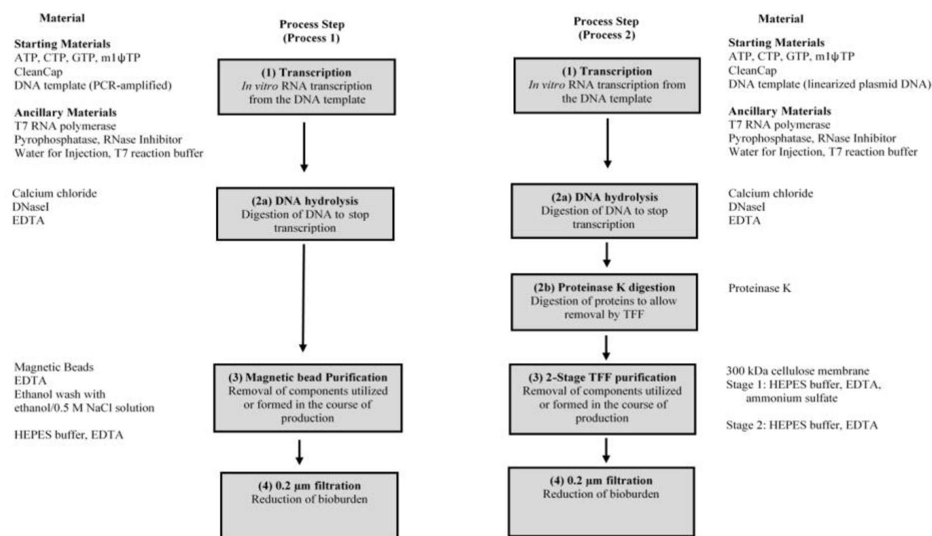
According to the report, the process described applies both to the production site Wyeth BioPharma Division, Andover in the United States (Pfizer) and to the two German production sites BioNTech Manufacturing GmbH, Mainz, and Rentschler Biopharma SE, Laupheim.

In general, when assessing Comirnaty, it is important to note that there were two different manufacturing processes, the so-called "Process 1", in which the DNA starting material for RNA synthesis was produced using PCR (Figure 2, left column) and "Process 2", in which the DNA template is a "linearized plasmid DNA" (Figure 2, right column).

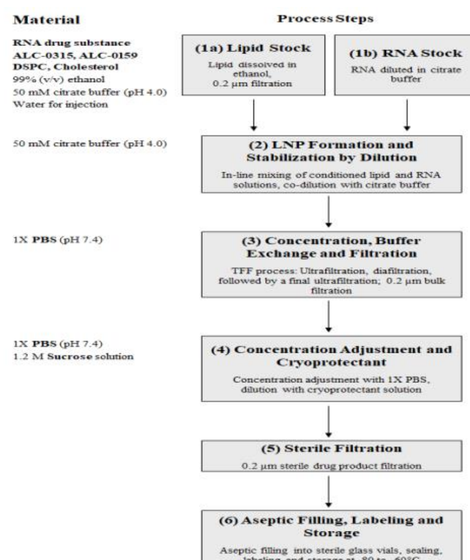
Process 1 generated RNA was used for preclinical and clinical studies, Process 2 generated RNA for global vaccination.

Comparison of Representative Messenger RNA Vaccine Drug Substance Manufacturing Process (Process 1 vs Process 2)

Document 3 Part 1



Pfizer Confidential 23



Note: Drug substance and excipients are depicted in bold.

Pfizer Confidential 24

Figure 2 shows the comparison of Process 1 and 2 during production (Q: FOI 2389 Document 3-1) Process 1 with the DNA template from PCR products on the top left and Process 2 with the DNA template from a literaturized plasmid on the right.

According to the manufacturer, the manufacturing process for the active ingredient BNT162b2 comprises various main steps, which can be summarized as follows:

1.3.1. production of the "sample template (DNA template)" as a double-stranded DNA molecule with the desired sequence. Here: coding for the spike protein of SARS-CoV-2 Wuhan 1 variant, supplemented by the incorporation of two amino acids proline instead of the original amino acids lysine and valine - the latter with the idea of stabilizing the spike. This DNA template is optimized based on the known sequence information in the computer for the human genome apparatus and with

regard to the desired RNA properties (i.e. changed from the viral codon signature to the signature common in human cells, see also point 1.4.1. and also the flanking ends of the resulting RNA are changed for greater stability and then produced as synthetic DNA).

1.3.2. amplification of the starting template, in order to have sufficient DNA molecules available for RNA production. Two processes were used for this purpose - for preclinical and clinical testing, the DNA template was amplified using the polymerase chain reaction (PCR) (**so-called process 1, according to the EMA: "clinical trial material"**). For clinical use in the vaccination campaign, the DNA template was cloned into a plasmid (see explanation under point 3.2.2.). The corresponding genetically engineered plasmid was introduced into E. coli bacteria by transfection and these were amplified in suitable cultures in the fermenter. After purification of the plasmids from the bacteria, the desired genetic material is enzymatically excised from the plasmids and is then available as a replicated DNA template for RNA production (**process 2, according to EMA "commercial process")**

See also Figure 2 and fact check Pharmaz. Zeit. (Fact check: mRNA vaccines contaminated with DNA)

"*A key difference between the two processes lies in the **origin of the DNA**: in process 1, it is produced cell-free in a test tube using a polymerase chain reaction (PCR); in **process 2, it is obtained as plasmid DNA from bacteria**.*"

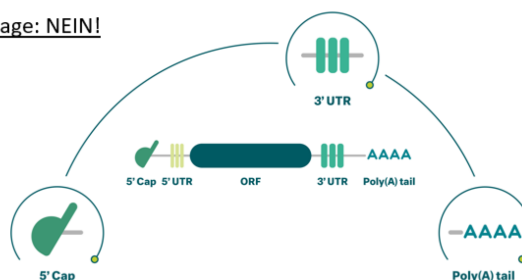
1.3.3 RNA synthesis. RNA is synthesized from the purified linear DNA template using the enzyme T7 RNA polymerase via an in-vitro transcription step (translation of DNA into RNA). The modified 1N-methyluridine is incorporated instead of the nucleotide uridine (shown in the nucleic acid sequences with the symbol: Ψ). The product is the modified RNA (modRNA), the actual active substance. Furthermore, the RNA-ends are also modified to make the modRNA more stable and longer-lived overall. See Figure 2 with the corresponding information on the BioNTech website. (https://biontech.de/de/how-we-translate/mrna-therapeutics

The slightly modified current version of the relevant information on BioNTech's website is shown in Figure 3 with comments.

Enthält die "Vaccine" normale mRNA?- Von der Biontech Webpage: NEIN!

Our mRNAs all contain basic structural elements that we believe are critical for successful development:

5' cap: Incorporation of a unique cap analogue into the mRNA helps to achieve superior translational performance by stabilizing the mRNA molecule and directing the immune response.
3' untranslated region: The composition and structure of the 3' untranslated regions of the mRNA molecule are important determinants of the intracellular stability of mRNA.
Poly(A) tail: We have performed extensive research on the structure of the poly(A) tail and the translational performance of mRNA and customized our template design accordingly.



Non-immunogenic vector
Strong antibody responses
Therapeutic protein delivery

We have profound expertise in incorporating naturally-occurring modified nucleosides into our therapeutic mRNAs. We have demonstrated that the presence of a variety of modified nucleosides in the manufactured mRNA suppresses its intrinsic immune activation, while leading to superior protein production for long duration. Deimmunizing mRNA by incorporating modified nucleosides helps to avoid production of anti-drug antibodies and broadens the therapeutic application of these types of mRNA drugs.

Figure 3: the RNA modification as advertised by BioNTech (Q: Modified from BioNTech webpage)

1.3.4 Various purification and filtration steps are intended to separate the modRNA of the correct length from by-products such as DNA residues, RNA fragments that are too short or too long, or RNA/RNA or RNA/DNA hybrids. For this purpose, the DNA is digested via hydrolysis using the enzyme DNAseI and the amplified modRNA is freed from DNA residues and protein residues using magnetic beads in process 1 and tangential flow filtration and proteinase K digestion in process 2.

According to BioNTech itself (Annual Report 2020, to be found here: Annual Reports | BioNTech) page 45 "...*around 50,000 steps are required from the production of the mRNA to the final bulk vaccine*"

Figure 4: Steps from RNA production to bottling as presented in BioNTech's 2020 Annual Report.

1.3.5 To produce the transfectable lipid-modRNA nanoparticles, the purified modRNA is mixed with the lipid mixture in a controlled manner (Figure 3, point 3). According to the manufacturer and approval documents, the aim is to produce particles with a diameter of less than 100 nm.

BNT-162 LNP

Lipid nanoparticle (LNP) formulation

The BNT162 vaccine candidate RNA is encapsulated into LNPs, which protect the RNA from degradation and enable transfection of the RNA into host cells after IM injection. The same LNP formulation is used for all of the BNT162 vaccine candidates (Figure 1).

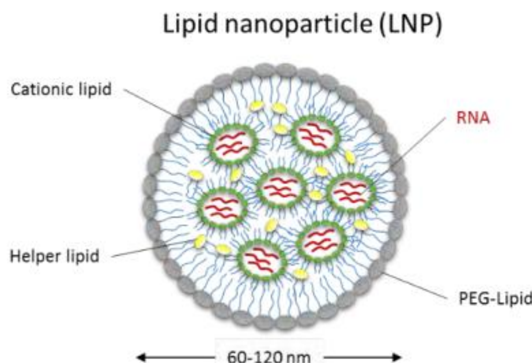


Figure 1: Schematic overview of a LNP

Pfizer Confidential

Figure 5 for the specification of lipid nanoparticles (LNP). It should be noted that an acceptable size in the range of 60-120 nm is specified here. (Q: Q: FOI 2389 Document 3-1, page 20)

1.3.6 For the end product, the transfectable lipid nanoparticles are then purified in various steps and adjusted to the desired final concentration before being filled into the medication vials after sterile filtration. (Figure 2 from step 3 in the flow chart)

1.4 Production steps with hazard potential

1.4.1 The DNA template.

DNA with the corresponding gene sequence is required as starting material for the production of large quantities of RNA. This is where the signature of the subsequent RNA is determined. The most important problem in designing the DNA template of the gene for the SARS-CoV-2 spike protein is that the original gene from the active virus was not used for the vaccine. Instead, various modifications were made to the natural sequence in the computer to make the subsequent RNA more effective, stable and long-lasting. These modifications mainly involved codon optimization. In the genetic code, several different base triplets often code for the same amino acid, and the frequency of triplets varies from organism to organism. Furthermore, rather rare triplets mean that the production process of the resulting protein on the ribosomes is slowed down (it takes longer to find the right transfer RNA with the amino acid that can bind). This delay is important because it gives the molecule time to fold

spatially - a crucial prerequisite for its subsequent correct function. As part of the codon optimization applied by BioNTech, the base triplets, which each code for one amino acid, were modified in such a way that they work as effectively as possible, i.e. with high throughput - albeit artificially "tuned". From the manufacturer's point of view, this serves to improve the yield of proteins due to the greater stability and durability of the codon-optimized RNA in the host cells. On the other hand, the mRNA is adapted as closely as possible to the codon patterns of the intended host cells (humans) and typical "viral" codon patterns (of the SARS-CoV-2 original RNA) are eliminated. This is necessary because viral codon patterns from the original gene sequence would cause the host cell to recognize the RNA as foreign and infectious with the help of special recognition sensors (receptors) and "sound the alarm". This would immediately trigger immune activation against the transfected cells, which is undesirable (Sahin U 2014). However, this codon optimization obviously did not take into account the important function of slowing down protein synthesis for the correct folding of the three-dimensional final structure, so that problems with the correct spatial structure and corresponding malfunctions are to be expected here.

The intended RNA sequence was also designed in the DNA in such a way that particularly effective start sites (at the 5' end of the RNA) for protein production and end pieces on both sides (at the 5' and 3' end) of the RNA for better longevity (primarily prevents the degradation of the RNA in the cell) were incorporated into the DNA matrix. This principle "*structural modifications for tuning mRNA pharmacokinetics*" was already published in 2014 in the seminal publication by Comirnaty developers Sahin, Kariko and Tureci (Sahin U 2014).

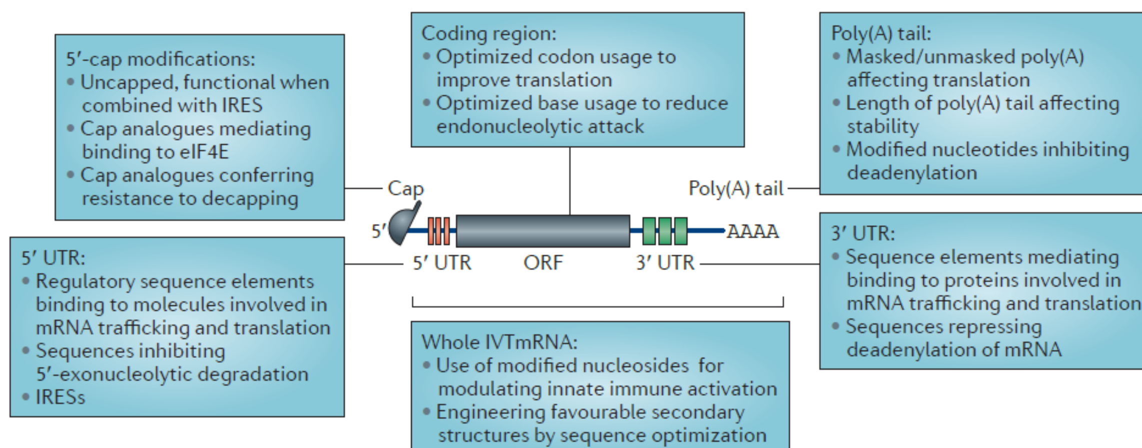


Figure 6: The modifications of the modRNA are shown and described in a publication by Sahin, Kariko and Türeci in Nature Drug Discovery from 2014 Figure 3a.

1.4.2 Duplication of the DNA template as starting material for RNA production

Only process 2 will be discussed here, as this is used for the commercialized use of the product, i.e. the vaccination campaign, and is relevant for possible vaccination damage caused by Comirnaty.

How this process is optimally designed was described very well and vividly back in 2021 in an article in the New York Times (https://www.nytimes.com/interactive/2021/health/pfizer-coronavirus-vaccine.html), which is now behind a paywall but is still available.

In patent GB2594365A dated 16.04.2021 (GB2594365A - Coronavirus vaccine - Google Patents), the use of the first step of vaccine production is described as follows: "*Briefly, human codon optimized SARS-COV-2 spike (GenBank: MN908947.3) was synthesized (Genscript) and cloned into an expression plasmid. SARS-CoV2 complete genome sequences were downloaded from GISAID Nucleotide database (https://www.gisaid.org)*"

The nature of the so-called expression vector, a plasmid in which the gene for the spike protein is genetically inserted (cloned), is decisive in assessing the potential danger. In the first patent on "Coronavirus vaccines and methods of use" dated March 19, 2020 from BioNTech (EP4121104A2 - Coronavirus vaccines and methods of use - Google Patents), a number of different plasmids are listed under point [000195]. By sequencing the plasmid then selected (gene bank no. PP544445 from the finished RNA injection solution and by analysing the sequence of the plasmid found by Pfizer in the submitted approval documents, several structures with hazard potential can be identified, of which the critical SV40 promoter/enhancer (point 1.4.4.2 more on this) was interestingly not specified in the approval documents (Rolling Review, Comirnaty | European Medicines Agency (EMA) page 24).

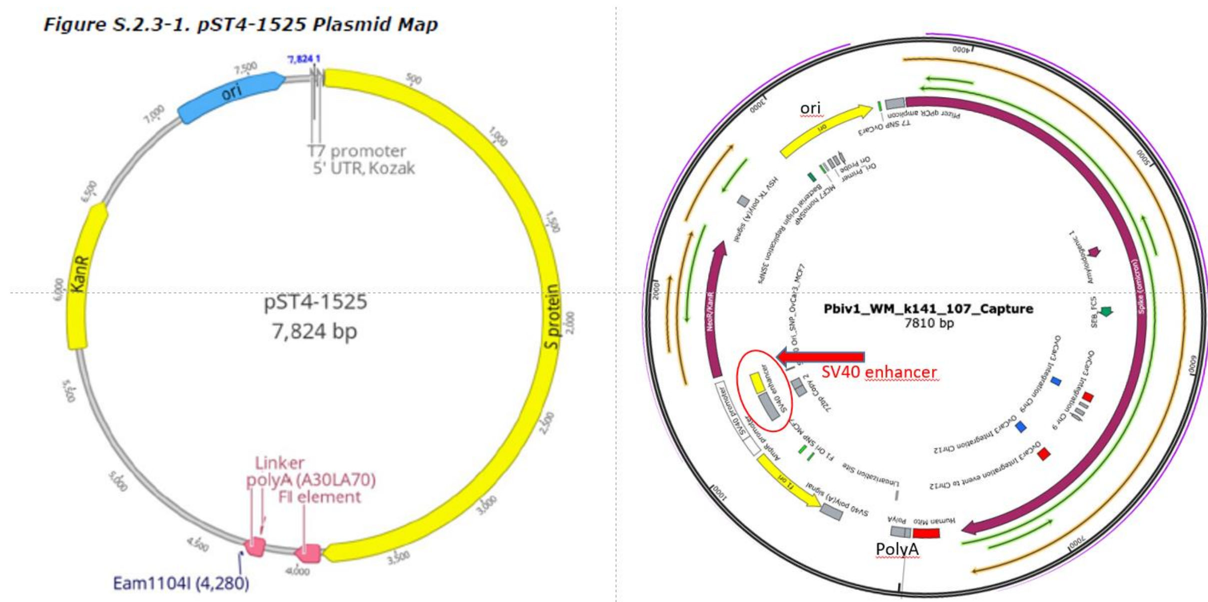


Figure 7 shows the plasmid map as specified in the approval documents (Comirnaty | European Medicines Agency (EMA) 24) (left), on the right the map determined on the basis of the sequencing data (Kevin McKernan, Medical Genomics). In the map on the right, the coding regions for kanamycin and the spike protein (yellow in the approval) are shown in red, and the Ori for E. coli (blue in the approval map) in yellow.

Insert for understanding: Plasmids

A plasmid is a small, ring-shaped DNA molecule that many bacteria have in addition to the main chromosome. Plasmids can replicate independently within bacteria and can be exchanged between different bacteria. Bacteria use plasmids to transfer special additional properties such as antibiotic resistance to "colleagues", which is a major problem in the treatment of infections and the development of multi-resistant germs.

Plasmids are THE key tool in molecular biology and are indispensable for the manipulation, research and production of genes with desired properties. Molecular cloning, a technique for producing many identical copies of genes, is inextricably linked to the use of plasmids. Plasmids serve as vehicles for the multiplication of desired (foreign) genes in bacterial cells. In genetic engineering drug production, which also includes the production of RNA vaccines, genes that code for a desired protein (e.g. insulin or spike protein) are inserted into a plasmid using molecular genetic techniques. In order for these plasmids with the additional gene to be accepted and replicated by the host bacteria, these plasmids, also known as expression vectors, must have some additional properties that have also been artificially incorporated. Nowadays, genetic engineering laboratories (or companies) can order such plasmids in all possible variants from catalogues of specialized companies or even have them custom-made.

Bacteria that are supposed to reproduce the artificial plasmids do not normally accept any additional gene ballast - but the artificial plasmid represents such a ballast. Therefore, all expression systems contain one or more genes for enzymes that make the host bacterium resistant to antibiotics. The corresponding antibiotics are used in the bacterial culture. Consequently, only those bacteria that accept the plasmid and can therefore multiply in the antibiotic-containing culture medium can survive here.

Depending on the planned use of the gene integrated into a plasmid and propagated in the bacteria, additional control elements must be integrated (cloned) into the plasmid in addition to the antibiotic resistance.

If the gene is "only" to be replicated, a simple starting point for the DNA replicating enzyme (polymerase) of the host bacterium, the so-called ori (origin of replication), is sufficient.

If the gene in the bacteria is to be converted into an RNA, the cell machinery of the bacteria also needs a starting point for special DNA-dependent RNA polymerases so that the DNA is converted into an RNA. These starting points are called promoters. In the case of the plasmid for the production of Comirnaty, this promoter is recognized by the so-called T7 polymerase and is therefore described on the genetic maps as the T7 promoter. The T7 polymerase originates from a bacteriophage, a virus that can infect bacteria. In nature, this T7 promoter is therefore only active in bacteria that are infected with the corresponding T7 bacteriophage. Human cells do not have this T7 polymerase. They would not be able to produce RNA from the plasmid if it entered the body with only the T7 promoter.

With the help of the T7 promoter and the pure T7 polymerase enzyme, the RNA molecules can then be produced *in vitro*, i.e. in the laboratory, from the plasmid DNA.

1.4.2.1 The special feature of the plasmid used for Comirnaty, however, is that not only the T7 promoter (which cannot be controlled by human cells) is used, but also a partial element from the

promoter of a virus that can infect primates (and thus also humans). This promoter component is the "enhancer" from the promoter region (Dean DA 1999) of the SV40 virus ("Simian Virus 40"). The detection of this SV40 element results in several safety-relevant problems, which are discussed in detail in a new publication (Kämmerer U 2024). It should be noted here, that the enhancer element used obviously has exactly the 72-base-long sequence that can effectively transport an attached DNA into the nucleus of human cells (Dean DA 1999) and is not necessary for the actual production or function of the modRNA vaccine. This element is classically used in plasmids for gene therapy. Here, the introduction of the therapeutic gene(s) into the genome is desired in order to repair existing genetic defects.

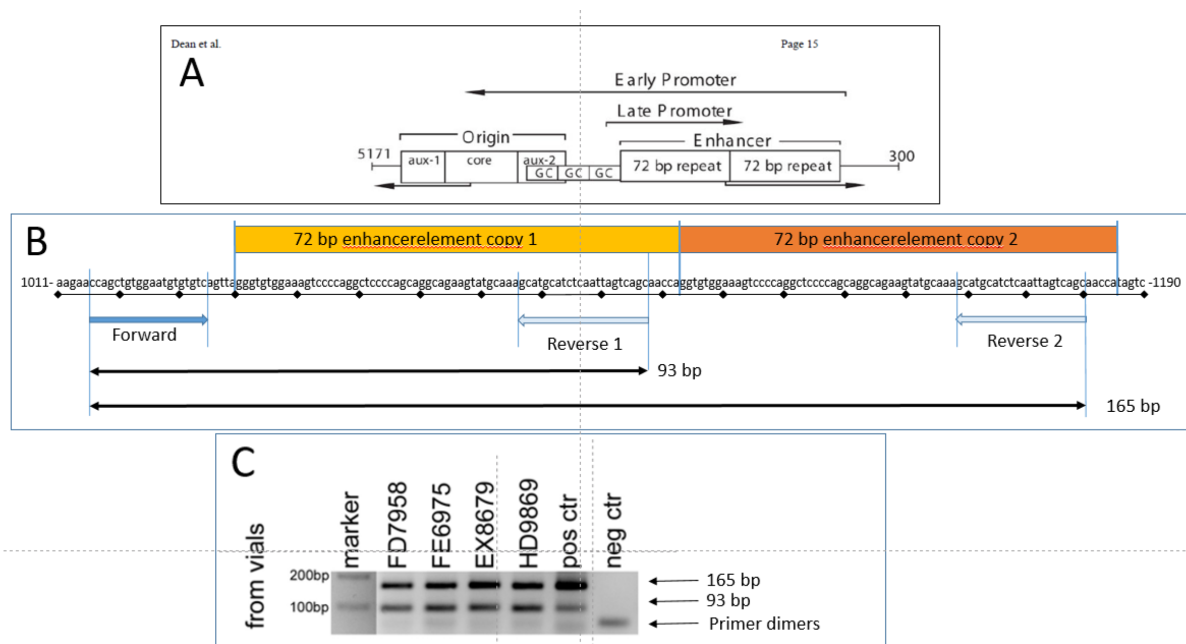


Figure 8 for the SV40 enhancer element. A) The SV40 enhancer element identified in the publication by Dean DA 1999 as critical for the nuclear transport of DNA (here as a particularly effective duplex cassette of two 72 bp elements) is found in the sequence of the plasmid for Comirnaty production (Genbank: PP544445; B). C) The existence of the critical SV40 enhancer double cassette in several exemplary batches of Comirnaty could be proven by a suitable PCR (primer position in B) (From Kämmerer U, 2024).

See also the following statement by Prof. F. Weber in a “Fact check” "mRNA vaccines against corona: What does DNA have to do with it? *“Or, if we take the **plasmids used by Pfizer/BioNtech, they contain a sequence called SV-40 enhancer**. It goes back to Simian virus 40, a polyomavirus that can infect monkeys and humans. The virus has the aforementioned **enhancer sequence** in its genome**, which helps the virus to bring its genome into the cell nucleus**. **This enhancer is in turn contained in the plasmids for vaccine production**. It wouldn't necessarily be needed, **but it is frequently used**.*"

What is missing from "is frequently used" is the fact that the "aforementioned enhancer sequence" is actually frequently used - albeit in vectors for gene therapy. Fittingly, a publication by Pfizer scientists with the beautiful title "*The journey of a lifetime - development of Pfizer's COVID-19 vaccine*" (Thorn CR, 2022) explains what type of plasmids Pfizer/BioNTech is dealing with: "*One critical starting material for mRNA manufacturing is the DNA template encoding the antigen [15]. At Pfizer, **we utilized prior***

plasmid DNA (pDNA) manufacturing technology expertize from Pfizer's Gene Therapy Program." This directly confirms in the Pfizer publication that the spike gene was simply cloned into an existing plasmid from the gene therapy program for the production of Comirnaty.

1.4.2.2 Is the SV40 element contained in the manufacturing plasmid in all batches and active in principle?

It can be assumed that all batches were produced with the corresponding identical plasmid as a template, because only this one plasmid was submitted to the regulatory authorities as the basic plasmid for Comirnaty in the documents, but without declaring the critical SV40 enhancer range in the initial submission. The production of the "master cell bank" with this plasmid (pst4-1525) is also described here:

Preparation of pST4-1525 Master Cell Bank

The plasmid pST4-1525 pre-Master Cell Bank (pre-MCB) was generated by transforming Escherichia coli DH10B competent cells with pST4-1525. A pure culture of transformed cells was produced by growth on selective medium. A single colony isolate was then grown in liquid culture and aliquots were taken and frozen to generate pre-MCB pST4-1525_preMCB_DH10B_20Apr2020.

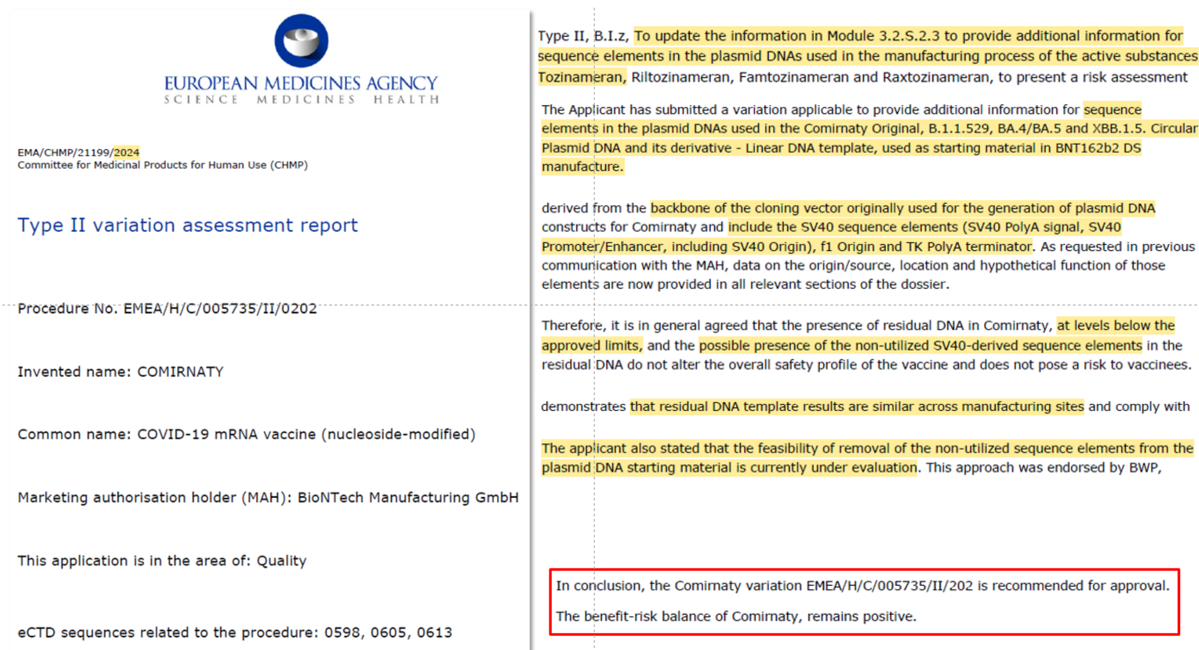
Vials from pre-MCB pST4-1525_preMCB_DH10B_20Apr2020 were thawed to inoculate shake flasks containing LB Broth, additional yeast extract and kanamycin to a final concentration of 50 µg/mL. The flasks were placed in a shaker incubator (200 rpm) and incubated at 32 ± 2°C, for a maximum of 10 hours. The cultures were stopped once the optical density (OD) at 600 nm (OD600) reached a value of ≥ 2.0. Sterile glycerol was added to the cell culture to a final concentration of 20% (v/v). Aliquots of the formulated cell culture were dispensed into screw-cap cryovials, each containing approximately 1.5 mL of cell suspension. The vials were frozen using a controlled rate freezer and then transferred to storage in the vapor phase of liquid nitrogen freezers.

MCB DW8968 vials are stored at -125°C or colder. Storage is in the vapor phase of liquid nitrogen in validated freezers, with temperature and alarm monitoring. The freezers are in controlled access storage areas at multiple sites as a precaution against loss due to catastrophic events."

The evidence that the plasmid or components thereof or components of it are present in all vaccine batches is now available thanks to a "subsequent submission" by BioNTech to the EMA in February 2024 (EMA/CHMP/21199/2024 - Type II variation assessment report, on Comirnaty, see Figure 9) This document is not available directly from the EMA, but was submitted to the court in a civil action by BioNTech, documented at Pfizer admits the SV40 promoter in EMA documents) and is also not negated by the regulatory authorities ("*Evaluation of 236 batches manufactured between 2020 and 2023 at three commercial manufacturing sites (Pfizer Global Supply (PGS) Andover, PGS Grange Castle, and BioNTech Marburg) and encompassing four unique variants (Wildtype/Original and Omicron BA.1, BA.4/BA.5, and XBB.1.5) demonstrates that residual DNA template results are similar across manufacturing sites*": Evaluation of 236 batches manufactured between 2020 and 2023 at three commercial manufacturing sites (Pfizer Global Supply (PGS) Andover, PGS Grange Castle, and BioNTech Marburg) and including four unique variants (Wildtype/Original and Omicron BA.1, BA.4/BA.5, and XBB.1.5) demonstrates that residual DNA template results are similar across manufacturing sites, but only indicated that official limits are not exceeded.

As an important side aspect, it should be noted that this subsequent notification is made to BioNTech Manufacturing GmbH and not to the actual company, BioNTech SE nor by Pfizer.

In this subsequently filed report from the beginning of 2024 (within the BioNTech documents under seal, presented in court in a civil lawsuit, available via attorney Tobias Ulbrich or here Pfizer gives the SV40 promoter in EMA documents to view) the presence of SV40 elements is now also mentioned, with the addition that these are "unused".



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/CHMP/21199/2024
Committee for Medicinal Products for Human Use (CHMP)

Type II variation assessment report

Procedure No. EMEA/H/C/005735/II/0202

Invented name: COMIRNATY

Common name: COVID-19 mRNA vaccine (nucleoside-modified)

Marketing authorisation holder (MAH): BioNTech Manufacturing GmbH

This application is in the area of: Quality

eCTD sequences related to the procedure: 0598, 0605, 0613

Type II, B.I.z, To update the information in Module 3.2.S.2.3 to provide additional information for sequence elements in the plasmid DNAs used in the manufacturing process of the active substances Tozinameran, Riltozinameran, Famtozinameran and Raxtozinameran, to present a risk assessment

The Applicant has submitted a variation applicable to provide additional information for sequence elements in the plasmid DNAs used in the Comirnaty Original, B.1.1.529, BA.4/BA.5 and XBB.1.5. Circular Plasmid DNA and its derivative - Linear DNA template, used as starting material in BNT162b2 DS manufacture.

derived from the backbone of the cloning vector originally used for the generation of plasmid DNA constructs for Comirnaty and include the SV40 sequence elements (SV40 PolyA signal, SV40 Promoter/Enhancer, including SV40 Origin), f1 Origin and TK PolyA terminator. As requested in previous communication with the MAH, data on the origin/source, location and hypothetical function of those elements are now provided in all relevant sections of the dossier.

Therefore, it is in general agreed that the presence of residual DNA in Comirnaty, at levels below the approved limits, and the possible presence of the non-utilized SV40-derived sequence elements in the residual DNA do not alter the overall safety profile of the vaccine and does not pose a risk to vaccinees.

demonstrates that residual DNA template results are similar across manufacturing sites and comply with

The applicant also stated that the feasibility of removal of the non-utilized sequence elements from the plasmid DNA starting material is currently under evaluation. This approach was endorsed by BWP,

In conclusion, the Comirnaty variation EMEA/H/C/005735/II/202 is recommended for approval.

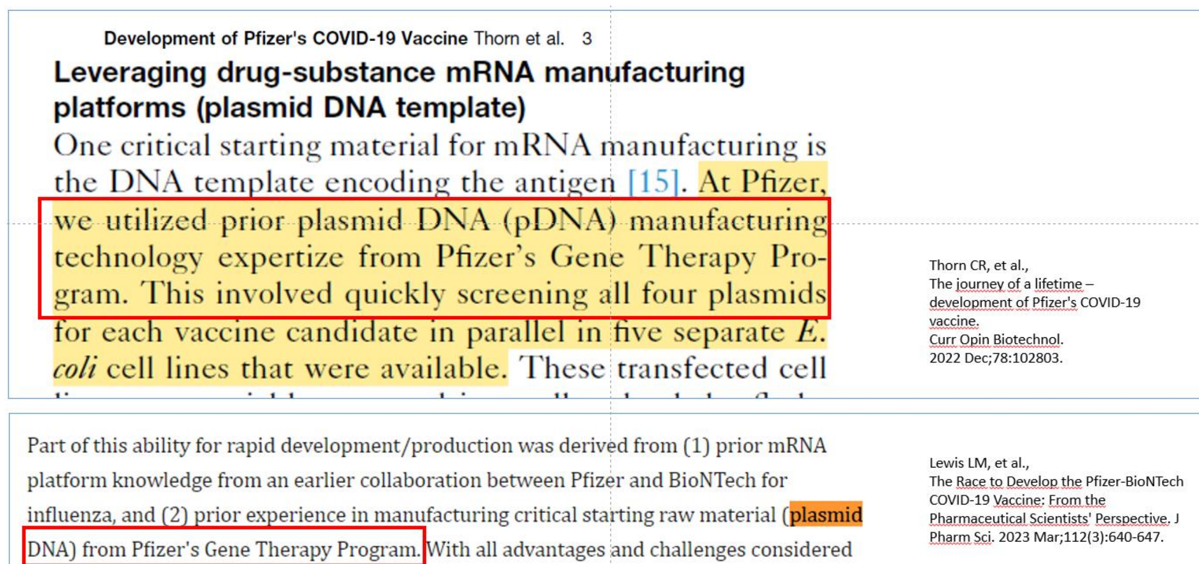
The benefit-risk balance of Comirnaty, remains positive.

Figure 9 shows the most important subsequently submitted points from the Assessment Report submitted to with the most important passages in which reference is also made to the plasmid and the SV40 control elements, including the EMA's decision that "the benefit-risk ratio of Comirnaty" is nevertheless positive and approval is still recommended.

All Pfizer and BioNTech batches analyzed to date by RT-PCR and sequencing by independent scientists show the 72 bp long element of the SV40 enhancer/promoter as a double cassette.

However, the fact that this SC40 promoter/enhancer element was not shown in the original plasmid disclosure in the papers for the EMA (Figure 7 under point 1.4.2.), although in two scientific publications by Pfizer employees (Thorn C 2022; Lewis LM 2023) it was explicitly emphasized that plasmids from the Pfizer gene therapy program were used (see Figure 10), suggests that these crucial sequence components were deliberately omitted. As can now be seen in the subsequently submitted report, this SV40 enhancer element was described as inactive, contrary to the clear literature (point 1.4.2. in the preliminary remarks).

Particularly in view of the fact that the basic plasmid used originates from Pfizer's gene therapy program, i.e. the SV40 elements were (and are) actively used by Pfizer in the plasmid in order to introduce genes into the genome of patients as part of gene therapy, the question arises as to whether either these plasmids are suitable for gene therapy or not, whether either these plasmids are unsuitable for gene therapy due to "inactive" SV40 elements (which would call Pfizer's gene therapy program into question) or, in the case of Comirnaty's production, whether they do contain active SV40 elements which - if they enter the cells - can in principle serve their original function as gene shuttles into the genome.


Development of Pfizer's COVID-19 Vaccine Thorn et al. 3

Leveraging drug-substance mRNA manufacturing platforms (plasmid DNA template)

One critical starting material for mRNA manufacturing is the DNA template encoding the antigen [15]. At Pfizer, we utilized prior plasmid DNA (pDNA) manufacturing technology expertize from Pfizer's Gene Therapy Program. This involved quickly screening all four plasmids for each vaccine candidate in parallel in five separate *E. coli* cell lines that were available. These transfected cell

Thorn CR, et al., The journey of a lifetime – development of Pfizer's COVID-19 vaccine. Curr Opin Biotechnol. 2022 Dec;78:102803.

Part of this ability for rapid development/production was derived from (1) prior mRNA platform knowledge from an earlier collaboration between Pfizer and BioNTech for influenza, and (2) prior experience in manufacturing critical starting raw material (plasmid DNA) from Pfizer's Gene Therapy Program. With all advantages and challenges considered

Lewis LM, et al., The Race to Develop the Pfizer-BioNTech COVID-19 Vaccine: From the Pharmaceutical Scientists' Perspective. J Pharm Sci. 2023 Mar;112(3):640-647.

Figure 10: Proof that the basic plasmid used with the SV40 elements originates from Pfizer's gene therapy program. All authors of the two publications are listed as employees of Pfizer.

A request to the British regulatory authority (FW: FOI 24/212 DNA contamination present in the mRNA COVID vaccines, SV40 promoter sequence etc - MHRA Customer Services - Outlook) shows that various authorities are also aware that the SV40 promoter is contained in Comirnaty, but that it is also dismissed here as "inactive".

"The presence of the SV40 promoter enhancer sequence is not the same as the presence of the whole virus itself. The SV40 promoter enhancer sequence was found to be a residual DNA fragment in Pfizer-BioNTech COVID-19 vaccine. The fragment is inactive, has no functional role, and was measured to be consistently below the limit required by regulators."

Much earlier, there is an inquiry on "Frag den Staat" Questions regarding the Rapporteur's Rolling Review assessment report of 19.11.2020 - FragDenStaat to the EMA about the disclosure of the SV40 element in the plasmid. Here it can be seen that BioNTech did not declare the SV40 element to the EMA because it was assessed as "non-functional".

"*While the full DNA sequence of the plasmid starting material was provided in the initial marketing authorization application for Comirnaty,* ***the applicant did not specifically highlight the SV40 sequence, as it was considered to be a non-functional part of the plasmid.*** *They have since clarified this information in response to questions raised by EMA.*"

Against the background of the "Preliminary remarks" under points 1.4.1. and 1.4.2., Figure 10 and the functions of this element as a bidirectionally effective promoter and decisive for the nuclear transport of attached nucleic acids, this is very surprising and indicates that the manufacturer did not consider it necessary to carry out quality controls with regard to the SV40 element and that the regulatory authorities did not carry out corresponding independent and autonomous sequence and literature analyses.

Since the EMA also confirms in this inquiry that the plasmid sequence once submitted cannot be changed without a new authorization procedure ("*As stated above, the full sequence was provided with the application for marketing authorization.* ***Companies cannot make changes to their products***

***when placing them on the market** without submitting the necessary applications to amend or extend the marketing authorization."),* it can be assumed that the original plasmid with the SV-40 promoter/enhancer sequence was used as starting material for all batches from process 2 production on the market and that **this SV-40 element and the other elements subsequently notified to the EMA by BioNTech (see Figure (9)) were therefore not completely separated. elements are contained as not completely separated residues in all Comirnaty batches**.

1.4.3 RNA production and modification of the base uracil.

In the next step, the RNA can be produced *in vitro* from the purified DNA. For this purpose, the gene coding for the "tuned" RNA is enzymatically cut out of the plasmids, the plasmid residues - at least that is the idea - are completely separated, and the pure drug-coding DNA starting from the T7 promoter to the end of the spike gene can be used as a template for RNA production. This is done by adding the enzyme T7 polymerase and the RNA building blocks in a suitable buffer system.

Normally, a healthy cell recognizes foreign RNA with the help of special receptors and classifies this as a viral infection. The reaction to this is a signalling cascade, as a result of which surrounding cells are "warned" via messenger substances (cytokines) that a virus has attacked the organism in order to increase their resistance. In addition, the infected cell often undergoes apoptosis, i.e. programmed cell death, which stops the invading virus from multiplying. Kathalin Karikó and Drew Weissman discovered how to switch off the intracellular receptors for foreign RNA (Karikó K 2005). For this, Karikó and Weissman were honoured with the Nobel Prize in Medicine in 2023 (https://www.nobelprize.org/prizes/medicine/2023/kariko/facts/ Prize motivation: "*for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19*"). This knowledge was directly available during the production of Comirnaty, as Katalin Karikó was employed as Vice President at BioNTech from 2013-2022 and thus, alongside the company founders Sahin/Türeci, was jointly responsible for the development of genetic RNA vaccines. If the uracil base in the RNA is replaced by 1N-methyluridine (Ψ), the synthetic RNA is no longer recognized as foreign and the cell's own receptors (called Toll like receptors - TLRs) are specifically switched off. This helps to ensure that the modified RNA introduced is accepted by the cell as its "own" messenger RNA (mRNA) and the encoded protein is produced without triggering an immune response. The disadvantage is that the silencing of TLRs in immune cells and especially in dendritic cells (see point 3.1: Target of modRNA are dendritic cells) disrupts a coordinated and effective immune response against pathogens and tumors. BioNTech itself describes the mechanism as "deimmunizing", or "the modified mRNA no longer induced any immune-stimulatory effect" (Sahin U 2014).

This means that the modification with Ψ results in a general immunosuppression in favour of accepting the introduced foreign RNA. This is very likely one of the fundamental mechanisms that leads to increased susceptibility to infections, the frequent flare-up of diseases that are typical of immunosuppression such as shingles (herpes zoster) and unexpected reactivations of tumours that have already been successfully treated (late recurrences, extremely rapid recurrences), as is frequently observed in people after injection of genetic RNA vaccines such as Comirnaty.

However, the incorporation of the base Ψ gives rise to other problems with genetic RNA vaccines that were also known before Comirnaty was developed (Xia X 2021). In natural, unmodified mRNA, the genetic code is very stable. This ensures that the genetically determined gene sequence is also converted into the exact matching protein. However, the integrated 1N-methyl-pseudouridine (Ψ) can lead to so-called "wobbling"

Insert for understanding: Genetic code

Basis of the genetic code: DNA is made up of four building blocks, the so-called bases: Adenine (A), cytosine (C), guanine (G) and thymine (T). Three bases form a triplet, each of which codes for a specific amino acid. Some amino acids are defined by several triplets, so that the amino acid proline, for example, is even coded by four triplets (CCA, CCC, CCG and CCT). The genetic information of the DNA - the sequence of the four bases A, C, G, T in many combinations - is ultimately converted into the amino acids, the building blocks of proteins, in these triplets. The messenger RNA serves as an intermediate product, a kind of information mediator from DNA to protein. The mRNA for a desired protein is read (transcribed) from the DNA in the cell nucleus. However, the base thymine is replaced by the RNA-typical base uracil. The matching small so-called tRNA molecules are selected on the ribosomes for the triplets in the long base chain on the mRNA. These have the corresponding three bases of the desired triplet on one side and the appropriate amino acid on the other side. The three bases of the tRNA are complementary to those of the mRNA. This means that each RNA base (i.e. A, C, G and U) can form a bond with a complementary base. However, and this is the basis of the genetic code, only one A with one U and one C with one G. So if the triplet on the mRNA is CGU, then the matching tRNA must have the three bases GCA in order to be able to bind. This accuracy of fit of the complementary base pairs ensures the exact translation of the genetic triplet into a specific amino acid and then of all triplets of a gene into the corresponding desired protein.

The wobbling, which is increasingly triggered by the Ψ , means that the strict rule that only complementary bases bind to each other is no longer observed. Thus, Ψ can also bind with G, C and U in addition to A (U could only bind with A) and therefore all possible triplets of tRNA molecules match the triplets on the mRNA containing the Ψ . According to the patent (US11878055B1 - Coronavirus vaccine - Google Patents), each uridine was replaced by Ψ in BioNTech's composition "*(ii) a modified uridine in place of each uridine. 2. The composition of claim 1, wherein the modified uridine is N1-methyl-pseudouridine."*

This means that not only one amino acid is possible for each codon, but that incorrect amino acids can be selected that are not actually provided for in the genetic code of the template - the resulting protein no longer corresponds to the desired one and this process is also arbitrary and cannot be controlled. In addition, the important stop codons on the mRNA, which signal to the ribosome that the protein is finished and no further amino acids should be added, also contain a U (UAA, UAG, UGA). If the Ψ is inserted here instead of the natural "U" - as in Comirnaty - this can lead to the wobble effect, which means that instead of the "stop" signal, another incorrect amino acid is simply selected and the protein chain is extended. Summarized in: Xia X 2021.

This means that the incorporation of 1N-methyluridine (Ψ) instead of the natural uridine in the modRNA of Comirnaty not only has an immunosuppressive effect, but also has an impact on the correct

translation of the genetic code into the protein. Thanks to the baseΨ , the SARS-CoV-2 spike protein encoded in the modRNA can therefore give rise to a large number of different, wildly composed proteins. These can also have different lengths than the actual spike protein due to the possibly over-read stop signals. This leads to unpredictable new proteins *with potentially deleterious effects* (Xia X 2021).

1.4.4 Purification of the spike-coding RNA and separation of the DNA template as well as plasmid and bacterial DNA residues and malformed RNA molecules

Ultimately, all DNA components that have been incorporated into the RNA production process should be reliably separated from the active ingredient, the desired RNA molecule (here coding for the spike gene), except for a small residue of 10 ng/dose (according to EMA COVID-19 vaccines: key facts | European Medicines Agency (EMA) (europa.eu) and Drucksache 20/9412 (bundestag.de)).

Quote from the EMA webpage: "Under the heading "Is there DNA in mRNA vaccines?":

*"The mRNA in Comirnaty and Spikevax is manufactured using **plasmid DNA**.*

Plasmids occur naturally in bacterial cells. They are used as a template to produce the mRNA of the vaccines.

*Once the mRNA is produced, the manufacturing process includes steps to **break down and remove the plasmid DNA** as it is no longer needed.*

*The plasmid DNA is not intended to be part of the final mRNA vaccines. However, very small amounts of DNA fragments may remain. EMA has seen no evidence linking the residual DNA to **side effects**.*

*EMA has set limits for the level of broken-down DNA in mRNA vaccines. The manufacturing process is carefully designed and controlled to ensure **safe and acceptable levels**, and the vaccines' quality is routinely checked.*"

Note: 10 ng residual DNA/dose would still correspond to 50 billion DNA fragments, assuming an average fragment length of 200 bases.

In addition to residues of the actual DNA template, this DNA in Comirnaty also consists of residues of the plasmid and genome components of the bacteria into which the plasmids were propagated. Bacterial genome components regularly occur unintentionally in the nucleic acid isolate during the general extraction of DNA for plasmid production. This frequent phenomenon impairs the quality of plasmid purifications even in the field of basic research, which is why it is often discussed in scientific forums (e.g. How to solve genomic DNA contamination in plasmid extraction? | ResearchGate). According to the manufacturer, DNA digestion in Comirnaty is carried out using the enzyme DNAse I. The competitor Moderna has patented this process (US10077439B2 - Removal of DNA fragments in mRNA production process - Google Patents). However, this enzyme is only partially reliable and does not completely degrade the DNA, but also leaves larger fragments as residues in the reaction mixture.

From the above-mentioned Moderna patent: "*DNase I is an endonuclease that cleaves DNA by breaking phosphodiester bonds and produces smaller DNA fragments and/or di-, tri- and*

oligonucleotides [....] However, it is challenging to quantitatively determine the DNase I digestion efficiency [....] "

The fact that proof of reliable DNA degradation (into its individual base components) could not be provided by the producers was already criticized as not very reliable in the EMA assessment report on Comirnaty dated February 19, 2021 (EMA/707383/2020 Corr.2*[1,2]). Page 17: "*The robustness of the DNase digestion step is not considered comprehensively demonstrated although there is routine control of residual DNA impurities at the active substance level."* This point was also mentioned in a printed matter of the German Bundestag (printed matter 20/9412 (bundestag.de))

1.4.4.1 Insufficiently separated DNA and plasmid components in Comirnaty harbour a high problem potential. In the meantime, scientific publications (König B 2024; Kämmerer U, 2024; Speicher DJ; 2023, Raoult D, 2024) have experimentally demonstrated that the DNA residues in some batches of Comirnaty clearly exceed the upper limits defined by the EMA. In addition, the complete plasmids (including the SV40 elements) from the manufacturing process can still be found in the tested batches (Speicher DJ 2023; Kämmerer U 2024), which are packaged together with the RNA in the lipid envelopes and can therefore demonstrably enter cells (Plasmid DNA replication in BNT162b2 vaccinated cell lines).

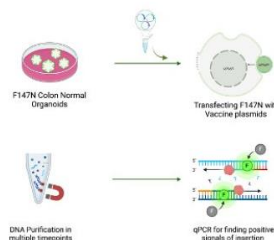
The fact that, in principle, non-degraded plasmids packaged in lipid nanoparticles can functionally enter cells is even being exploited in the development of "nanoparticle-based DNA vaccines" (Guimaraes LG 2024; Aida V, 2021).

The individual elements of the plasmids used result in potentially high genetic hazard potentials if they functionally enter the human cells of the vaccinated persons.

On the one hand, this is a possible non-specific interaction of the small DNA fragments (even in the maximum permitted quantity) with the gene apparatus of the transfected cells, as these DNA fragments can enter the cell nucleus by diffusion and integrate randomly into the genome. For the most part, this will certainly have minor consequences, as the integration areas will most likely stochastically affect non-coding areas of the genome. However, if the interaction leads to the inhibition of tumour suppressor genes, this could result in tumour growth, as is known from many malignancies. Phillip Buckhaults (Professor of Tumour Genetics) presented findings from his laboratory on Twitter (x), where DNA from genetic RNA vaccinations has been shown to integrate into the genome of cells:

Proof Plasmid DNA in mRNA vaccine modifies human genome. "Vaccination" of a normal colon organoid avatar

- **Vaccination of normal colon epithelial cells with mRNA vaccine (we just added it to the media).**
- **Growth for one month with three washings and replating.**
- **Isolated genomic DNA and perform PCR to detect presence of plasmid DNA**



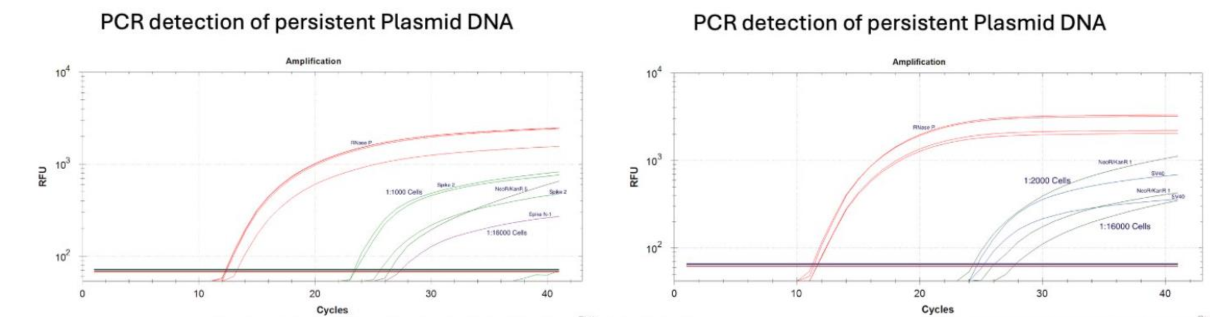


Figure 11 shows the detection of integrated plasmid DNA from the modRNA vaccine in the genome of cultured human epithelial cells. (Q: Phillip Buckhaults on X: https://x.com/P_J_Buckhaults/status/1861083163868672204)

In his X Post, Phillip Buckhaults explains:

"the plasmid DNA that is contained within mRNA vaccines can integrate into the genome of normal cells. i knew this could happen, but some were unconvinced, so we took the time to prove this in the lab. we grow normal human epithelial stem cells in my lab. its part of our normal job (cancer research). they are called organoids. these are not cancer cells, they are just the normal stem cells that make up the human colon. we "vaccinated" some of these normal cells and grew them for a month and saw pieces of the plasmid DNA persisting in the genomic DNA of the "vaccinated" cells. we detected the plasmid DNA with our qPCR protocol that was posted to X several months ago. [....] this does not mean that the integration is happening in real vaccinated humans (those experiments are ongoing) but it does prove that the DNA can get into normal cells just fine, as i told everyone a year ago. "[...]

If longer gene sequences in combination with control elements from the plasmids enter the cells for transcription (e.g. in association with the SV40 promoter/enhancer element) within the framework of this now clearly proven integration capability of the DNA fragments, two further main dangers arise.

1.4.4.2 Special hazards of the SV40 enhancer element : Why this SV40 enhancer element, which has long been used in vectors for gene therapies and plasmid vaccine trials, is present in the production plasmid for the spike gene-coding modRNA raises very big questions about the safety of genetic modRNA vaccines.

An insert for understanding: the 72 bp enhancer element of SV40

SV40 (Simian virus 40) is an oncogenic, i.e. cancer-causing, monkey virus from the polyomavirus family, which has very special properties. This virus can transport its DNA genome into the cell nucleus of the infected host cell (monkeys and humans) and integrate it into the genome of the host cells. Its genome is regulated by unusual control elements (promoters/enhancers), which allow the very rare reading of double-stranded DNA in both directions (i.e. strand and opposite strand). This is practically only observed in this SV40 virus; in other DNA viruses such as herpes viruses and also in eukaryotes up to humans, only the coding strand is read, but not the opposite strand. This bidirectional function allows the virus to code genes for different proteins on both strands of its DNA, which saves space and nucleic acid. Responsible for this special reading property is a region of 72 bases in length (72bp region) from the promoter, the so-called enhancer (Hertz GZ 1988). This 72bp region has also been shown to be the crucial gene sequence responsible for the nuclear transport of the viral genome into the cell nucleus, even in quiescent (non-dividing) cells with an intact nuclear membrane (Graessmann M, 1989; Dean DA 1999; Young JL 2003). This sequence is therefore also called "nuclear targeting sequence (NTS)". The nuclear transport property of this 72bp region from the SV40 enhancer has long been utilized in the field of vector design for gene therapy (Dean DA 1999, Curr Eyr res; Dean DA 1999) and also for plasmid vaccines (Li HS, 2007). Incorporation of these 72bp NTS ensures that the plasmid with the desired gene information enters the nuclei of the transfected cells to integrate into the genome (summarized in Dean DA). Even more effective in nuclear transport and integration of plasmids than the simple 72bp NTS is a "cassette" of two 72bp SV40 elements connected in series, which "incidentally" also have hypermutagenic properties, i.e. trigger accelerated gene changes with a high tumor risk, as was demonstrated in a recently published manuscript (Senigl F 2024).

Accordingly, the cassette used in the Comirnaty plasmid and detected by sequencing (Genbank sequence PP544445) with two 72bp nuclear targeting sequences of the enhancer is in principle, as explained above, suitable for the targeted transport of attached DNA from the Comirnaty production plasmid into the cell nucleus. This means that DNA fragments that are too large for diffusion can also enter the cell nucleus and the genome. If the SV40 72bp NTS element successfully integrates into the genome, its ability to initiate bidirectional reads (which are not intended in the human genome) can lead to completely unwanted RNA formation and thus trigger gene expression that is not naturally intended in the cell. There are also an increasing number of sequence analyses that show that in the Pfizer/BioNTech plasmid used, not only the actual strand with the gene sequence for antibiotic resistance and the actual spike protein is coded, but that there is also a very long reading frame for an unknown protein on the opposite strand, which can potentially be read due to the special property of the built-in SV40 enhancer element to work bidirectionally, should this area enter the cells undigested by the DNase.

With regard to the potential oncogenic properties of Comirnaty, it is important to note that the SV40 enhancer element that enters the vaccinated persons with the transferred plasmid or its degradation products itself serves as a mutation enhancer (SMH: somatic hypermutations) ("*Our results argue that the ability of the SV40 enhancer to target SHM to LT is a potential source of LT truncation events in various cell types that could contribute to carcinogenesis*." (Senigl F 2024).

This significantly increases the risk of unfavourable gene expression and even gene mutations in cells transfected with this short functional SV40 element as a by-product in Comirnaty.

1.4.4.3 Further dangers of DNA residues arise from the fact that after DNase digestion, DNA fragments (oligonucleotides) may be present as single strands which, like primers in PCR, bind with the RNA and also DNA and can, in principle, hinder or even prevent transcription and translation processes.

To understand: Primer

Oligonucleotides are short, 20-30 base long, single-stranded pieces of DNA that can bind precisely to opposing sections of nucleic acids. Oligonucleotides can therefore bind when DNA is present in single-stranded form, e.g. during cell division or in the context of RNA formation. This mechanism is utilized in PCR in that the DNA double strand is separated into the two single strands by heating and the selected special oligonucleotides can then bind as primers to the now single-stranded DNA. During PCR, the formation of the new double strands, the polymerase reaction, then starts on these bound primers. Oligonucleotides can also bind to the (single-stranded) normal mRNA and thereby block it. This mechanism is used in the so-called "siRNA" technique, in which individual cell functions are specifically switched off. Short oligonucleotides are used to specifically bind the mRNA and prevent it from being read. "si" means "small interfering" because small oligonucleotides are used to interfere with normal gene function. Thus, normal mechanisms on the DNA to RNA to protein pathway are disrupted by the bound oligonucleotides. This can lead to the termination of RNA formation (if DNA is blocked) or protein formation (if mRNA is blocked). In principle, the bound oligonucleotides can also serve as primers for a polymerase function and thus act as a starting point for gene amplification, a property that is utilized in the polymerase chain reaction (known from the genome detection of SARS-CoV-2 in patient samples).

1.4.4.4 Differently long RNA molecules and double-stranded RNA as a production-related admixture to the correct active substance RNA in Comirnaty was criticized by the EMA (EMA Assessment Report, (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf).

EMA Assessment Report p. 18:

"*According to the Applicant, the majority of fragments are expected to be comprised of truncated transcripts including the 5' region but lacking the 3' region and poly(A)tail. However, the results **indicating a substantial proportion of shorter/truncated mRNA** with both cap and poly(A)tail are not in agreement with this statement*."

S. 20:

"*In addition to **double stranded RNA**, there are **truncated RNA**, also referred to as fragmented species. Truncated RNA is reflected in the AS specification in terms of RNA integrity. However, the characterization of BNT162b2 AS is currently not found to be complete in relation to a specific parameter. This is especially important considering that the current AS and finished product acceptance criteria allow for a proportion of fragmented species*."

The EMA report (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf) refers in several places to the problem of RNA not being produced in a uniformly clean manner. In particular, the switch from process 1 to process 2, which was used for the vaccination campaign, appears to have caused increased defects in shape stability (integrity) (page 17): *"In comparability studies, a decrease in RNA integrity was observed for the initial Process 2 batches compared to Process 1 batches*."

These defects in the shape stability of the RNA are specified in more detail on various pages of the EMA report as truncated transcripts ("truncated RNA"), which lack the 3' region and the poly-A tail and are therefore unable to encode a complete protein. Furthermore, double-stranded RNA molecules (dsRNA) can also be produced instead of the desired single-stranded mRNA. In accordance with the natural function of dsRNA in cells, these dsRNA molecules can trigger massive interactions with intracellular regulatory mechanisms, as described in great detail in a recent review article (Chen YG 2022), and which may therefore be partly responsible for the pathogenesis of immune disorders, among other things.

"*From the available data, mRNA integrity, dsRNA and Poly(A) tail acceptance criteria are considered in relation with batches used in clinical studies and with the demonstrated manufacturing capability and need to be re-assessed and revised as appropriate as further data becomes available*" (page 21 under "Specification" in the EMA Assessment Report (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf)

Due to the extreme molecular similarity of these unwanted RNA products with the actually desired active ingredient, the intact, single-stranded RNA in the intended length, it can be assumed that these misproducts can hardly be separated technically from the active ingredient mixture - even less than the detected DNA, an aspect that the EMA (see above) also assesses critically. And similar to the unsatisfactorily separated DNA components from the manufacturing process, these faulty RNAs are also packed into the lipid nanoparticles and thus also transfected into the cells of the vaccinated person.

This means that the actual modRNA active ingredient product contains unwanted admixtures of various RNA and DNA components, which are also packed into the lipid particles due to their strong molecular similarity to the actual modRNA.

1.4.5 Packaging of RNA in lipid nanoparticles

Foreign "naked", i.e. single-stranded RNA, would be immediately identified as foreign by the body's safety mechanisms outside the cells and immediately degraded. Even if some molecules were to escape degradation, they would not be able to enter the cells through the cell membrane. The RNA must therefore be packaged in a "vehicle" that either specifically (vectors) or non-specifically (transfection using a lipid envelope) reliably protects the RNA outside the cells from degradation and at the same time can channel it through the cell membrane without also triggering foreign-recognizing alarm mechanisms. The principle is like the Trojan horse, in which the actual cargo (in this case the modRNA) passes undetected into the cell inside the camouflaging and protective envelope.

When promoting genetic RNA vaccines as part of the anti-Covid-19 vaccination campaign, it was emphasized to the public that these substances would explicitly remain in the muscle at the injection site and would then be degraded there in a timely manner. This function would be difficult to realize even if the appropriate LNP envelope components with a preference for muscle cells were selected, as it must always be assumed that they spread via blood and lymph vessels, which permeate the body's tissues everywhere. It was also already known from animal experiments in the approval studies from Japan that the lipid nanoparticles currently used in the genetic RNA vaccines spread very quickly throughout the entire organism and accumulate particularly in the tissues of the haematopoietic and immune system (spleen, lymph nodes and bone marrow) and the gonads (testicles, ovaries). The documents can only be viewed via the Wayback Machine. (https://web.archive.org/web/20210611193138/ https://www.pmda.go.jp/drugs/2021/P20210212001/672212000_30300AMX00231_I100_1.pdf)

This can also be found in a patent dispute between Northwestern University and Moderna, (Northwestern University v. Moderna, Inc. et al 1:2024cv01151 | US District Court for the District of Delaware | Justia) in which it is pointed out under point 113: "*When scientists studied the progress of LNPs following intramuscular administration of mRNA vaccines, they "detect[ed] the systemic trafficking of mRNA LNPs, which are rapidly and strongly expressed in the liver, at the same time as they are expressed in muscle and draining lymph nodes," which is achieved through "ApoE-mediated targeting*". Since Moderna's lipid nanoparticles follow the same principle as those of Pfizer/BioNTech, it can be assumed that the systemic distribution of LNPs after intramuscular injection in lymph nodes and liver, which is complained about in the patent dispute, also applies to Comirnaty.

Besides, it should be noted that the patent complaint contains the allegation (point 1) **that Moderna** *had already completed the first clinical batch of its* **Covid-19 vaccine more than a month before March 11, 2020** ("*Strikingly, Moderna had already completed the first clinical batch of its COVID-19 vaccine-more than a month before this declaration*"). This is very surprising in view of the fact that, according to C. Drosten (Corman VM 2020), the first available gene sequence of the new virus was available in the databases on January 10, 2020. This means that Moderna would have been able to research, preclinically test and manufacture its version of the genetic RNA vaccine as a clinically applicable product within a few days, many weeks before "Operation Warp Speed" was announced on May 15, 2020 for the rapid, unbureaucratic development of genetic RNA vaccines (Operation Warp Speed - Wikipedia). According to "Project lightspeed" (https://www.projektlightspeed.de), preclinical studies started in BioNTech's laboratories in February 2020.

In general, it should be noted that **the composition of the lipid envelope from the individual components essentially determines the behaviour in the organism with regard to tissue distribution and the target cells.** With regard to Comirnaty, according to several press releases from BioNTech, these lipid components were composed in such a way that the intended target cell type, the dendritic cells, especially in the lymph nodes, are actually preferentially targeted by the lipids. BioNTech CEO Sahin commented on this on September 2, 2020 in the newspaper "Die Presse" (https://www.diepresse.com/5861311/teil-des-covid-19-impfstoffes-konnte-aus-osterreich-kommen): "*For the Covid-19 candidate vaccine, we have chosen lipid nanoparticles* ***that favor migration from muscle cells to lymph nodes***. ***Dendritic*** *(antigen-presenting; note)* ***cells*** *then present the resulting S protein to the immune system*." Moreover, almost word for word on March 4, 2021 in

the German Medical Journal (https://www.aerzteblatt.de/nachrichten/121745/Biontech-Nanopartikel-sind-schwieriger-herzustellen-als-mRNA) "*The oncologist and vaccine researcher emphasized that the actual active ingredient of the vaccine, the mRNA, could be "produced within hours". The challenge lies rather in the production of the nanoparticles that coat the mRNA and transport it to its destination - primarily* ***dendritic cells in lymph nodes***."

The immunological problems resulting from the deliberate choice of dendritic cells as the target site of Comirnaty are discussed in more detail in section 3.1.

1.4.5.1 General problems of lipid packaging are listed in a review on the immunological and toxicological classification of various lipid materials for the packaging of therapeutic agents (Inglut CT 2020). Here, the lipid envelopes are referred to with the nomenclature "liposome", i.e. closed spherical lipid particles, whereas the lipid particles of genetic RNA vaccines are correctly referred to as lipid nanoparticles (LNP). In particular, intravenously injected liposomes or LNPs can interact with plasma proteins directly after injection, which leads to opsonization, i.e. the binding of lipid particles to antibodies and components of the complement system (various interacting proteins in the blood plasma, which are part of the innate immune system and play an important role in the defence against infections). On page 42 of the EMA's assessment report (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf), this is described as follows: "*ALC-0159 is included in the formulation to provide a steric barrier to: 1) facilitate the control of particle size and homogeneity during manufacturing and product storage, and 2)* ***regulate the association of plasma and proteins with the LNP surface****. The composition of the LNPs may also affect the distribution of injected BNT162b2. In addition, it cannot be excluded* ***the LNP composition contributes to the overall immunogenicity***." It is very likely that an initial non-specific immune activation takes place and healthy immune cells, especially monocytes, macrophages and dendritic cells, which come into contact with the opsonized lipids in the circulation, can be altered in their normal function. The binding of the components of the complement system can also be expected to disrupt blood clotting (increased tendency to thrombosis). Liposomal agents can generally stimulate or suppress the immune system depending on their physiochemical properties such as size, lipid composition and surface charge. Due to the pharmacokinetics of liposomes - which primarily target lymphoid organs - the active substances packaged in the lipid shells (here the modRNA including the unwanted additional nucleic acids) can be absorbed in organs of the mononuclear phagocyte system (part of the immune system, in which all cell types that are capable of phagocytosis, i.e. the absorption and storage of substances and particles, are grouped together). Classical phagocytes are macrophages and monocytes with all stages of maturation up to dendritic cells) are stored and thus impair the function of the liver and spleen. This description can be transferred 1:1 to the very small lipid nanoparticles (LNP) used in the genetic vaccines, as the envelope principle of the lipid membrane is identical and the - in comparison to liposomes - rather smaller lipid nanoparticles can be taken up even more easily by phagocytizing cells.

1.4.5.2 The size of the lipid particles is specified for Comirnaty as "nanoparticles". According to the Pharmazeutische Zeitung (Comirnaty from BioNTech/Pfizer | PZ - Pharmazeutische Zeitung (pharmazeutische-zeitung.de)), the lipid diameters of the nanoparticles should therefore be a maximum of 1 µm, because *"In pharmaceutical-medical applications, one generally speaks of*

nanotechnology when the structures are smaller than 1 micrometer (10^{-6} m)." In this article it is pointed out that the lipid nanoparticles of the genetic RNA vaccines are in the order of 100 nm (corresponding to 0.1 µm and thus by definition in the "nano" range).

However, our own light microscopic evaluations (Figure 12) of original Comirnaty showed that some of the lipid particles contained therein were considerably larger than the maximum size of 1 µm that would have allowed the term "nano" to be used. Whether the LNPs from Comirnaty, which were found to be up to 25 µm in diameter, were caused by problems in maintaining the planned LNP size during the manufacturing process or by very rapid fusion processes of many small LNPs to form larger structures during the thawing process cannot be assessed here. Several thawing and freezing cycles on the part of the manufacturer between LNP production (e.g. at Polymun near Vienna (https://www.diepresse.com/5861311/teil-des-covid-19-impfstoffes-konnte-aus-osterreich-kommen) and filling in Puurs (Belgium) are also conceivable disruptive factors that can cause the LNPs to fuse into larger lipid droplets. In any case, it is a fact that many very large lipid "drops" are found in the injection solutions, indicating a stability problem with the LNP mixtures. And the larger the injected lipid particles, the greater the risk of embolization (blockage) of blood vessels if these particles clog the small capillaries.

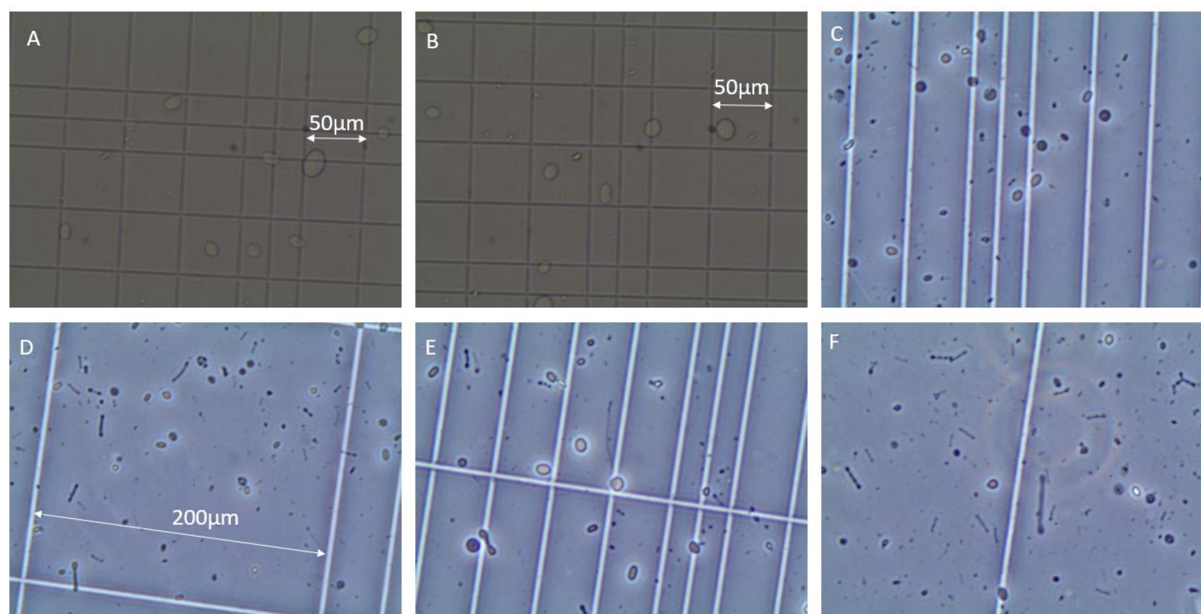


Figure 12: Typical lipid particles from Comirnaty original bottles in a calibrated counting chamber (Neubauer Improved) show large lipid droplets >10 µm in diameter, which clearly exceed the permitted maximum size of 100 nm (= 0.1 µm). (Q: Kämmerer U, own photos)

1.4.5.3 The individual components of the lipid envelopes of the genetic RNA vaccines are listed in Table 1 of (Comirnaty from BioNTech/Pfizer | PZ - Pharmazeutische Zeitung (pharmazeutische-zeitung.de).

Funktion der Hilfsstoffe	Comirnaty®	Covid-19 Vaccine Moderna
Lipidgemisch der LNP	ALC-0315: ((4-Hydroxybutyl)azandiyl)bis(hexan-6,1-diyl)bis(2-hexyldecanoat)	SM-102: Heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoat
Lipidgemisch der LNP	ALC-0159: 2-((Polyethylenglykol)-2000)-N,N-ditetradecylacetamid	PEG2000-DMG: 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylenglycol-2000
Lipidgemisch der LNP	DSPC (Colfoscerilstearat): 1,2-Distearoyl-sn-glycero-3 phosphocholin	DSPC (Colfoscerilstearat): 1,2-Distearoyl-sn-glycero-3 phosphocholin
Lipidgemisch der LNP	Cholesterol	Cholesterol
Isotonisierung	NaCl, KCl	
Pufferkomponente	KH_2PO_4	Tromethamin, Tromethamin HCl
Pufferkomponente	Na_2HPO_4 x 2 H_2O	Essigsäure, Natriumacetat
Kryoprotektor	Saccharose	Saccharose
Lösungsmittel	Wasser für Injektionszwecke	Wasser für Injektionszwecke

Tabelle 1: Hilfsstoffzusammensetzung der mRNA-Impfstoffe Comirnaty® und Covid-19 Vaccine Moderna

With regard to the lipids, it should be noted that cholesterol and DSPC were already used in pharmaceutical products prior to vaccine development, but the two cationic lipids ALC-0315 and -0159 were used exclusively in the technical field prior to their use in Comirnaty's LNPs and were not approved for use in animals or humans by the original manufacturer (Echelon) (package insert lipids ALC-0315 - Echelon Biosciences; ALC-0159 - Echelon Biosciences). Other manufacturers refer to the lipids ALC-0159 and ALC-0315 as "for research purposes only" (ALC-0315 | Lipid Nanoparticle Component | MedChemExpress page 1) and the EMA approval documents also criticize the lack of information and experience on the use and toxicology of the two "novel excipients" in the LNPs in several places. ("*Complete information is not provided for both the cationic lipid ALC-0315 and the PEGylated lipid ALC-0159*" page 34 in the EMA assessment report (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf).

Until fall 2024, these two lipids, which were used for the first time for medicines in humans, could not be found in the standard database of all chemicals approved in the EU (ECHA CHEM), neither under the product name nor under the molecular formula or CAS number (see ALC-0315 | Lipid Nanoparticle Component | MedChemExpress, pages 2+3), making it difficult to evaluate their properties.

The company Merck provides certificates of analysis that document a purity of at least 98% for a batch of ALC-0159, which still contains up to 2% impurities, and a purity of 99.81% for a batch of ALC-0315 (downloadable as a PDF from: ALC-0315 | Lipid Nanoparticle Component | MedChemExpress). In a recent publication, using a new gas chromatographic analysis method, detectable by-product contamination was found in ALC-0315 from 9 different producers, documenting purity as low as 82.3% and as high as 98.3% (Figure 3 in Birdsall RE 2024). Even two different batches from one producer show a high variability with regard to the integrity of the lipid, so that it must be assumed here that the lipids

integrated in the lipid nanoparticles are not really highly pure and therefore have very different properties. The deviations from the desired product mainly concern the hydrophobic properties and thus the possible interaction with other lipids or cell membranes and proteins within the body. According to Merck's data sheet (ALC-03125 Data sheet, ALC-0315 | Lipid Nanoparticle Component | MedChemExpress), ALC-0315 is the most common component in terms of quantity, accounting for 46% of the lipid shell, followed by cholesterol. (ALC-0315:DSPC:Cholesterol:ALC-0159 = 46.3:9.4:42.7:1.6). The problems that are becoming apparent here of being able to use the main component of the LNPs in a reproducibly high-purity and desired molecular structure give rise to many unfavourable and incalculable problems of the LNP properties.

A detailed evaluation of the lipids can be found in the article by Segalla G (2024) and will therefore not be further elaborated here.

The main problem with the use of technical foreign lipids without real knowledge of pharmacokinetics and toxicology is that the lipid envelopes inevitably fuse with the membrane of the cells during the transfection process and these foreign lipid molecules are then incorporated into the natural membranes.

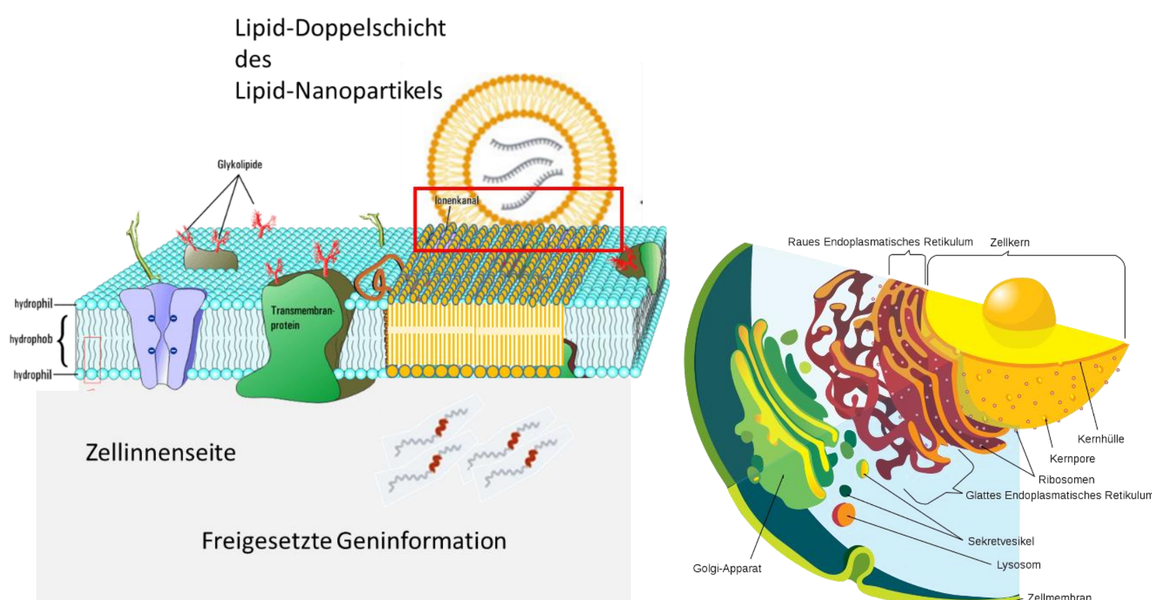


Figure 13 Fusion of lipid envelopes with cell membranes:

In order to transport the modRNA into the cytoplasm of the cell, the lipid envelope fuses with the cell membrane. During this process, the synthetic lipids of the LNP are incorporated into the biological membranes that form not only the cells but also all important organelles.

It should be noted that all membranes within a cell (cell wall, nuclear membrane, all organelles) are connected to each other (right part of the figure, source: https://de.wikipedia.org/wiki/Endomembransystem) and merge into each other. It is therefore to be expected that the foreign lipids introduced by transfection accumulate in all membrane structures of the cell - with unclear effects on e.g. the vital electron gradients and transport and signalling processes taking place at the membranes. Since membranes within tissues and organ systems are also exchanged

between cells via endo- and exosomes, the lipids from the transferred LNPs can also be distributed to cells that are not primarily affected. The extent to which these lipid layers interact with the various vital ion channels and transmembrane receptor complexes cannot yet be predicted due to the lack of previous experience with these molecules in cells or living organisms, but possible damage mechanisms are shown as examples in Figure 11 of Segalla G 2024. That the lipids were in principle selected by the developers to provide "(1) structural integrity, (2) mRNA loading and subsequent release in the cell and (3) stabilization in the body and durability of the product" and "biologically mimic the bilayer of the cell wall and are stealth-like so that they cannot be quickly removed from the body" (excerpts from the following quotes: "*LNPs are made from four lipids that provide (1) structural integrity, (2) drive mRNA loading, then release inside the cell, and (3) stabilization in the body, as well as for the product's shelf-life*" and "*The development of LNPs hails from research into nanoparticles that biologically mimic the bilayer of the cell wall and are stealth-like, avoiding rapid clearance from the body*") is explicitly presented in the publication by Pfizer employees (Thorn CR 2022).

1.4.5.4 Lipid quantity and distribution in the body

The lipid nanoparticles (LNPs) were explicitly designed to migrate from the injection site in the muscle into the lymph nodes in order to transfect the target cells, the dendritic cells. Furthermore, the very rapid and wide spread of LNPs into all organ systems was already known from preclinical data, so that the publicly repeatedly emphasized statement that Comirnaty would remain at the injection site in the muscle is definitely refuted. In this respect, it has been proven that LNPs with their cargo of modRNA can in principle transfect all cells in the body - and during this process the lipid layer necessarily fuses with the cell membrane. The LNPs act directly on cells in the organs throughout the body, into which they can reach via the blood and lymphatic system. This even applies to the brain, as the LNPs can migrate through the blood-brain barrier.

An important calculation to estimate the amount of LNPs injected per dose:

The amount of lipid nanoparticles in one dose of Comirnaty (0.3 ml, contains 30 µg of tozinameran) is anything but "small" at 1.32×10^{13} (13.2 trillion) molecules of RNA. For comparison, a 70 kg man is assumed to have approx. 36 trillion (= 3.6×10^{13}) cells and a 60 kg woman approx. 28 trillion (2.8×10^{13}) cells, so that in purely mathematical terms approx. 4 molecules of RNA are inoculated per body cell. Various models assume an average quantity of 10 RNA molecules per lipid nanoparticle, which would mean that 1.3 trillion LNPs are inoculated per dose and can therefore transfect a large number of body cells.

(Korosec CS 2022): "*The standard mRNA dose in BNT162b2 is 30 µg, and together with the known mRNA size of 4.3 kb[74] and average nucleotide molecular weight of 319 g/mol, there are an estimated 1.32×10^{13} of mRNA in each dose.*"

A recent study (Kent SJ 2024) investigated the distribution of lipid nanoparticles and modRNA in the blood after vaccination in subjects with the genetic vaccine Spikevax from Moderna; due to the very similar mode of action, the data can be transferred to Comirnaty.

The following average quantities were determined:

4 hours after injection into the muscle, 6.5-112 mRNA copies/µl of blood were found, which corresponds to 0.005-0.081 ng/ml), the amount of RNA in the blood reached its peak 1-2 (mean 1.3) days after vaccination (with peak values of up to 731 mRNA copies/µl, which corresponds to 0.529 ng/ml). With regard to lipids, a typical lipid of Moderna-LNP was analyzed here and found in the blood at a median of 3.22 ng/ml after 4 hours and on day 4 after vaccination the signals were still well above background at up to 1.16 ng/ml.

To calculate the actual amount of modRNA and lipids in circulation, an average blood volume of 70 ml/kg body weight is assumed. To simplify, 5 l per average adult. These 5 l correspond to 5 x 10^6 µl (i.e. 5 million µl). This means that after an average of 4 hours, the respectable amount of 5 x (6.5-112) x 10^6 = 32.5- 560 million RNA molecules enter the bloodstream per vaccination, reaching a maximum of 5 x 731 = 3.5 x 10^9 RNA molecules in the circulation after 2 days.

The amount of residual DNA fragments packed in the lipids and distributed in the body cannot be calculated because, assuming the maximum amount of 10 ng per dose approved by the EMA, it is not clear how many fragments are involved. On average, a specific molecular weight of 0.65 kilodaltons can be assumed for each base pair. Thus, for fragments of "only 100 bases", an average molecular weight of 65 kDa would have to be assumed; for fragments of 50 bases, twice the number of molecules would be assumed for the same specific molecular mass, etc. Since no exact data on the fragment size and their number is known, this is not validatable data that can be included in a calculation.

However, a very large number of DNA fragments were detected in Comirnaty samples (https://anandamide.substack.com/p/dna-fragments-detected-in-monovalent), which show a very large variability in fragment length. For technical reasons, the following graph does not include fragments less than 200 bp in length, which, thanks to DNase digestion, are to be expected in very large quantities in Comirnaty and can penetrate the genome "like shotgun pellets" and damage it (Min 8.55 in the lecture by vaccination and molecular expert Phillipp Buckhaults before the South Carolina Senate - USC Professor Dr. Phillip Buckhaults, SC Senate Hearing, September 12, 2023).

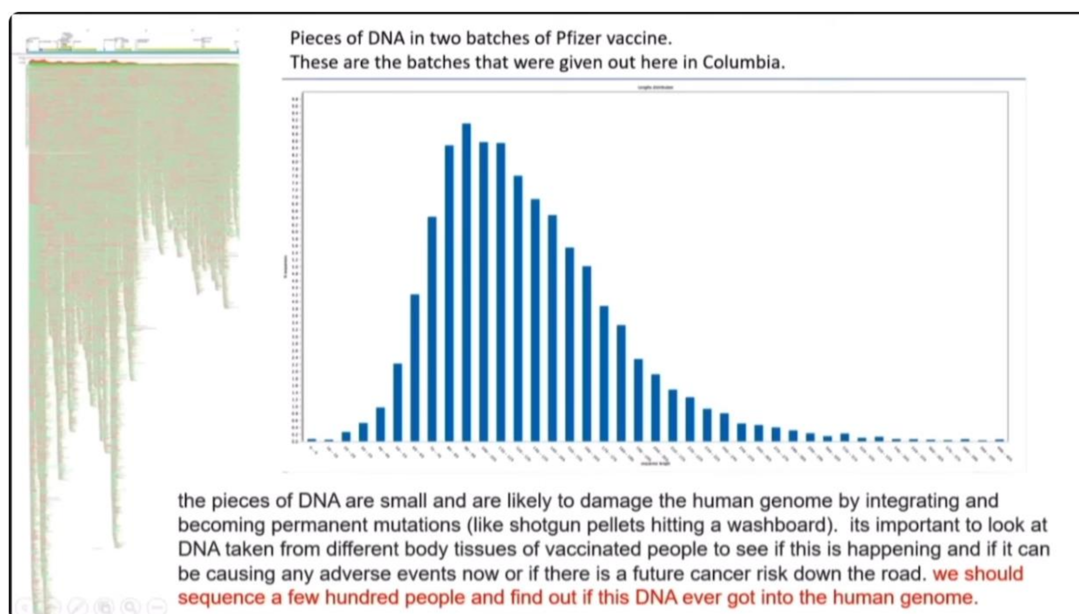


Figure 14: Size distribution of DNA fragments from Comirnaty. Slide from a Senate Hearing by Prof. P. Buckhaults (USC Professor Dr. Phillip Buckhaults, SC Senate Hearing, September 12, 2023)

With regard to lipids, the SM-102 from Moderna, for example, would have a clear quantity of 3.2 ng/ml x 5000ml = 16000 ng = 16 mg in the circulation

"*Vaccine mRNA was detected in the plasma samples of all 19 bivalent booster vaccine subjects at 4 h postvaccination (range 6.5-112 mRNA copies μL^{-1} , equivalent to 0.005-0.081 ng mL^{-1}), peaked at 1-2 (mean 1.3) days post vaccination (at peak levels of up to 731 mRNA copies μL^{-1} , equivalent to 0.529 ng mL^{-1}), and subsequently displayed log-linear decay kinetics*"

"*SM-102 levels peaked at 4 h to 2 days (mean 1.1 day) postvaccination (median 3.22 ng mL^{-1}) and subsequently showed log-linear decay kinetics. The SM-102 signals remained significantly above the background at day 4 postvaccination (up to 1.16 ng mL^{-1}) and approached background levels by day 7 postvaccination (up to 0.12 ng mL^{-1}).*"

Due to the enormous number of packaged nucleic acids (modRNA+DNA), the many LNPs and the proven rapid distribution of the lipid nanoparticles and the measured modRNA in the body, it can and must therefore be assumed that the LNPs loaded with modRNA and residual DNA reach all organs of the body in large quantities. A noticeable number of cells can be transfected here.

1.4.6 Final cleaning and filling

The various production steps up to the finished product, the deep-frozen ampoule with the genetic RNA vaccine doses, are briefly summarized in an illustration from the Wiener Zeitung (Figure 15). The still impure active substance (the modRNA including the discussed impurities from BioNTech in Mainz, point 1 in Figure 12A) is forwarded to other companies for processing. The critical step 2, the purification of the desired modRNA, probably takes place mainly at Rentschler in Laupheim. According to a press release (BIOPHARMA CLUSTER: BioRegionUlm: Detail), Rentschler describes this as follows:

"Purification to pure active ingredient
Rentschler will take over the downstream processing of the starting material and remove impurities from the previously synthesized mRNA that are present due to the manufacturing process in order to provide a high-purity active ingredient (drug substance).
This purification is important because it ensures the safety and tolerability of the vaccine for use in humans. At the same time, the yield of mRNA that can be obtained from the original manufacturing step can be maximized. Rentschler Biopharma will manufacture the high-purity active ingredient at its headquarters in Laupheim. [....].".

The packaging of the purified, concentrated RNA into the lipid nanoparticles (Figure 12 B from Comirnaty by BioNTech/Pfizer | PZ - Pharmazeutische Zeitung (pharmazeutische-zeitung.de) is then primarily carried out at the company Polymun (Klosterneuburg near Vienna), as described in a press release (Impfstoff-Hersteller Polymun ausweitet Produktion - Archiv | Wiener Zeitung) : *"[...] Polymun Scientific has specialized in lipid nanoparticles. These mini fat globules are used, for example, to coat the mRNA active ingredient from BioNTech/Pfizer. They stabilize the vaccine and protect it from degradation.*

Polymun has been working with BioNTech/Pfizer on the development of their vaccine since the beginning of last year. "We get the mRNA from BioNTech, the lipids from other manufacturers, and we have the know-how to mix them," says Katinger. [....]

Meanwhile, according to official documents (FOI 3659 document 1 (tga.gov.au), the LNPs are also produced at Pfizer in Puurs and Dermapharm in Grünwald and the RNA-LNP mixture is then transported from these LNP producers to the Pfizer plant in Puurs (Belgium) for final processing (sterile filtration, adjustment of the final concentration and filling into the injection vials). BioNTech and Pfizer are always mentioned together, so that the exact supply chain for the Comirnaty vaccinated in Germany cannot be traced with publicly accessible documents.

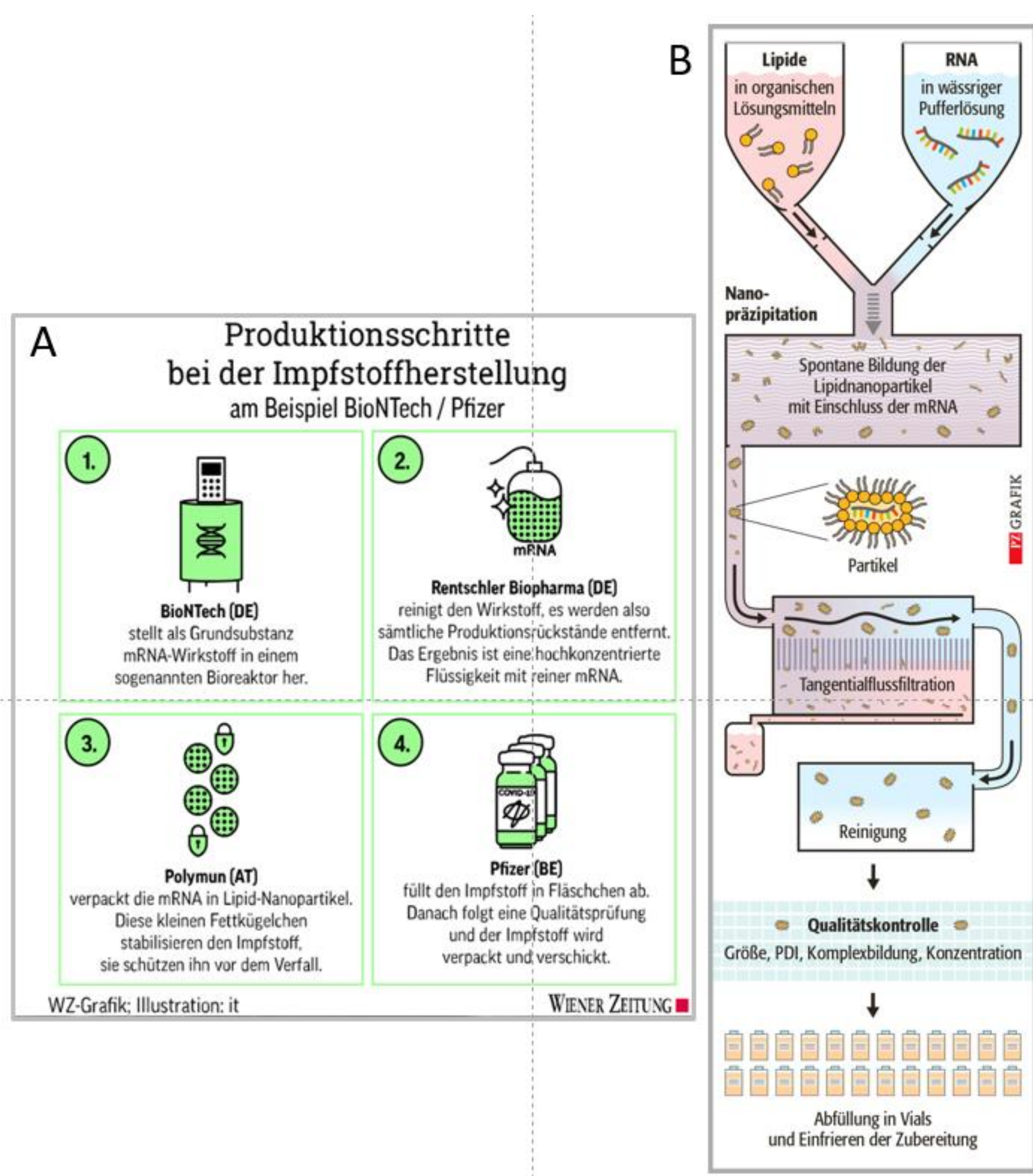


Figure 15 Production steps Comirnaty : In figure A with naming of the companies originally involved (Q: Wiener Zeitung) and in B more detailed (From Comirnaty by BiontechPfizer | PZ - Pharmazeutische Zeitung (pharmazeutische-zeitung.de))

2. The SARS-CoV-2 spike protein as a vaccine antigen

In order to understand the effect of Comirnaty, in addition to the general aspects of the new active ingredient platform (genetic modRNA/LNP vaccines, Point 1), which has been vaccinated millions of times worldwide for the first time, the special properties of the spike protein formed from it in the body are also of decisive importance.

2.1 Structure of the spike protein

2.1.1 General structure

The actual active ingredient of the genetic RNA vaccine Comirnaty, the modRNA, codes for the complete spike protein of the SARS-CoV-2 virus. This spike protein is a large (1273 amino acid) glycoprotein, i.e. a complex protein molecule with many glycosylations. The spike protein encoded in the original modRNA of Comirnaty, and ideally resulting from it, corresponds to the amino acid sequence of the so-called Wuhan-Hu 1 variant, the first published sequence of the virus first identified in Wuhan. (Note: adapted sequences are now being inoculated which contain sequences of the most common variants of the virus in addition to the original sequence). However, as part of Comirnaty's gene optimization, two of the original amino acids were exchanged for proline (2P). This exchange should help to ensure that the three-dimensional structure of the spike protein remains in a form that the virus protein has when it is newly formed within the cells (prefusion, Figure 13 A left) and does not change into a different spatial structure (postfusion, Figure 13 A right) when it is incorporated into the cell membrane (fusion). The two prolines are supposed to stabilize this prefusion form (Figure 13 B shows Comirnaty's principle) and deactivate the furin cleavage site (2.1.2.2.), but in reality this does not work reliably, so that the spike protein can still be at least partially cleaved into the two subunits S1 and S2 despite the amino acid modification (Amant F 2021).

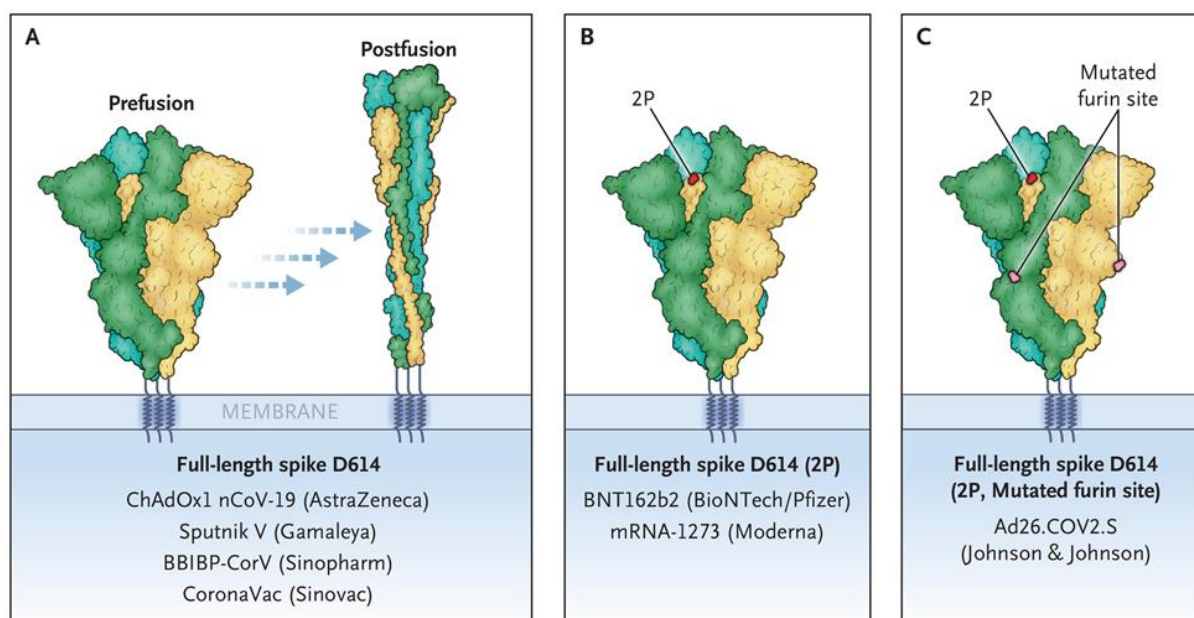


Figure 16 Spike protein and proline anchor from Comirnaty. Figure B shows the spike protein with the proline modifications (2P), which should keep it stabilized in the so-called prefusion form (Figure A left), even if it is expressed at the cell membrane, which in the case of the natural spike would result in conversion to the postfusion form (Figure A right). Figure modified from (Koenig P-A 2021)

2.1.2 Special features of the SARS-CoV-2 spike protein

2.1.2.1 Unusual structural elements: The basic 3D structure of the spike protein of SARS-CoV-2 (then still 2019-nCoV) was modelled very early on by a renowned Indian research group (Pradhan P 2020:) Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag | bioRxiv). The but a corresponding publication had to be withdrawn due to massive criticism but was available on a preprint server from 31.01.2020 (and is still available there). However, the data was subsequently confirmed by other groups, including the French HIV discoverer Luc Montagnier (Preprint: HIV MAN-MANIPULATED CORONAVIRUS GENOME EVOLUTION TRENDS - ScienceOpen) and his group. These sequence and amino acid sequence analyses suggest that the spike protein of this coronavirus SARS-CoV-2 contains important regions of the gp120 protein of the HI virus, which are arranged at prominent positions matching the receptor binding. In the modelled Figure 3 from Pradhan's publication (here Figure 17), the three areas coloured red, orange and yellow form a structural unit. In a database search of all known proteins, these three areas show a high degree of correspondence with components of the "button" of HIV, the gp120 protein. "*The first 3 inserts (insert 1, 2 and 3) aligned to short segments of amino acid residues in HIV-1 gp120*.").

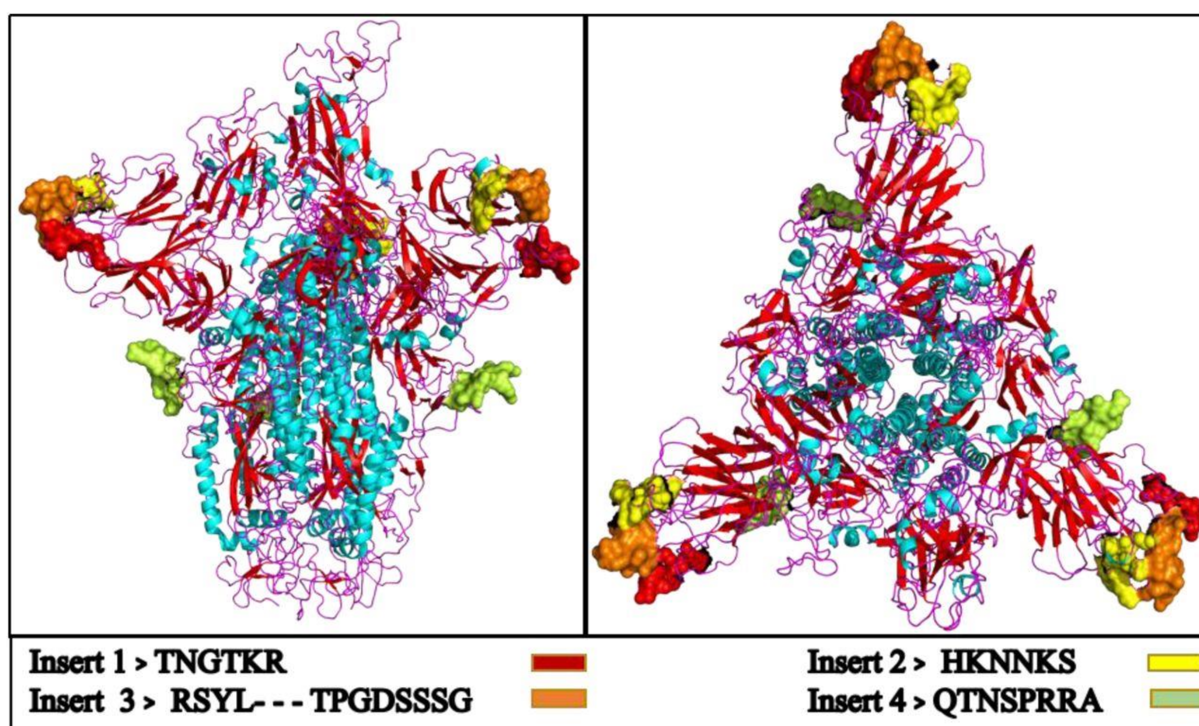


Figure 17 shows the HIV receptor binding sites in the spike protein as already described by Pradhan P in a preprint on January 31, 2020 (Q: Pradhan P Figure 3, Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag | bioRxiv)

While the properties of the first three HIV-like regions receive little attention when discussing the properties of the spike protein of SARS-CoV-2, the conspicuous furin cleavage site (in light green) has been confirmed many times. This corresponds to the amino acids **PRRAR** and was also already found in the publication by Pradhan P 2020 (Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag | bioRxiv). However, the other three areas are also exciting, as together they form the docking site on CD4 in the three-dimensional form of the spike protein, which in principle can be used to give the SARS-CoV-2 virus access to CD4-positive immune cells. Nevertheless, these binding sites have hardly been discussed so far, the focus is clearly on the so-called furin cleavage site.

2.1.2.2 The conspicuous furin cleavage site is a kind of "special feature" of the SARS-CoV-2 virus, as all closely related viruses of the Sarbeko group do not have this cleavage site (Furin cleavage motif makes SARS-CoV-2 more aggressive, scientists find (drugtargetreview.com)). The furin cleavage site serves a proteolytic (i.e. protein-cleaving) enzyme called furin to cleave the spike protein into two main components, the subunits S2 and S1. The furin cleavage site located in the spike of SARS-CoV-2 is extremely effective due to its very rare amino acid sequence with an arginine (R) in the third position, which means that a high percentage of spike molecules can be cleaved into the two subunits S1 and S2 using the cell's own enzymes such as furin (Segreto R 2021; Hoffmann M 2020).

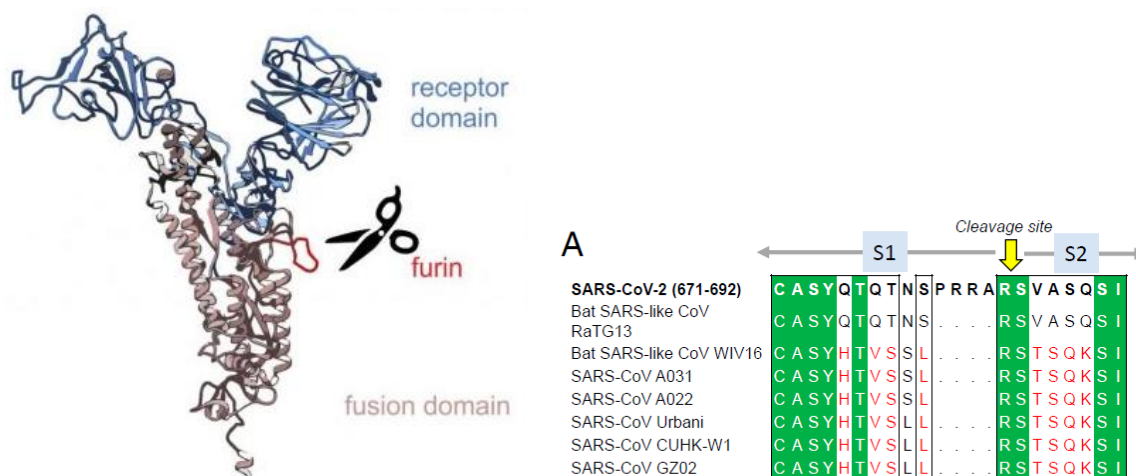


Figure 18 on the furin cleavage site: left the furin cleavage site shown as a red loop through which the SARS-CoV-2 spike protein is cleaved into the S1 and S2 subunits using the enzyme furin (shown as scissors). Figure from: Furin cleavage motif makes SARS-CoV-2 more aggressive, scientists find (drugtargetreview.com). right: Sequence comparison of various closely related coronaviruses shows the exclusivity of the inserted furin cleavage site (amino acids PRRA) in SARS-CoV-2. Figure from: Cheng MH 2020.

The cleavage of the spike protein with the ubiquitous cellular enzyme furin produces the S1 subunits with the docking sites on cellular receptors (receptor domain), and the "cut free" S2 subunit with the so-called "fusion domain" which can lead to the fusion of membranes.

2.1.2.3 Possible neurotoxic properties: Sequence comparisons at RNA and protein level have revealed further unusual properties in the SARS-CoV-2 spike protein, which may be responsible for the aggressiveness and toxicity of the protein. Many properties can be found as so-called "superantigens" in the immediate vicinity of the furin cleavage site, which, in addition to the details described below, can also unusually bind the spike protein to receptors on T cells and CD28 and thus trigger overactivation of T cells and a so-called cytokine storm (Cheng MH 2020). This T-cell activation combined with the neurotoxic details described below can potentially trigger a neurotoxic immune response.

The potentially neurotoxic segments of the SARS-CoV-2 spike protein within the superantigen region in the immediate vicinity of the furin cleavage site are named in detail as:

A sequence with

1. high similarity to the neurotoxin of the Indian cobra (*Naja naja*) and monocle cobra (*Naja kaouthia*),
2. and the neurotoxin of the *multicolored* krait (*Bungarus multicinctus*), a Chinese venomous snake.
3. Furthermore, a protein with high sequence similarity to the G protein of the rabies virus (Rabies) with which this virus enters neurons.

In addition to the potentially neurotoxic motifs, two binding sites were identified in this superantigen region that show a clear similarity or even complete agreement with

1. a binding site for the neuropilin receptors NRP1 and NRP2 (important docking sites for the virus in addition to the actual receptor ACE2),
2. a binding site for heparin with a very high affinity for heparan sulfate

2.1.2.4 A master pattern of prions was also identified outside the superantigen region at the interface between the S1 and S2 region of the SARS-CoV-2 spike protein in the original Wuhan version of the spike protein (which corresponds to the protein encoded by the genetic RNA vaccines but also by the vector vaccines). According to a preprint publication by Luc Montagnier's group (Perez JC 2022 HIV MAN-MANIPULATED CORONAVIRUS GENOME EVOLUTION TRENDS - ScienceOpen), a longer amino acid sequence from the S1 subunit of the SARS-CoV-2 spike protein from the original Wuhan 1 variant shows extreme similarity to prions. Prions are protein structures that are practically non-degradable in the body (as they are misfolded) and are associated with severe to fatal neurological diseases such as Creutzfeld-Jakob or BSE (mad cow disease). In the preprint of Perez JC 2022, reference is made to 16 cases of acute and extremely rapidly fatal Creutzfeldt-Jakob disease, each of which occurred after genetic vaccinations (RNA or vector) against SARS-CoV-2. Sequence analyses identified a region of 38 amino acids that determines the prion property and which occurs in the Wuhan-1 variant (and thus in all genetic vaccines based on this sequence) and the delta variant and was no longer active in the Omicron variant, for example, due to several mutations. It is not yet possible to conclusively assess how active this region actually is in humans, but this amino acid pattern definitely represents a potential source of danger.

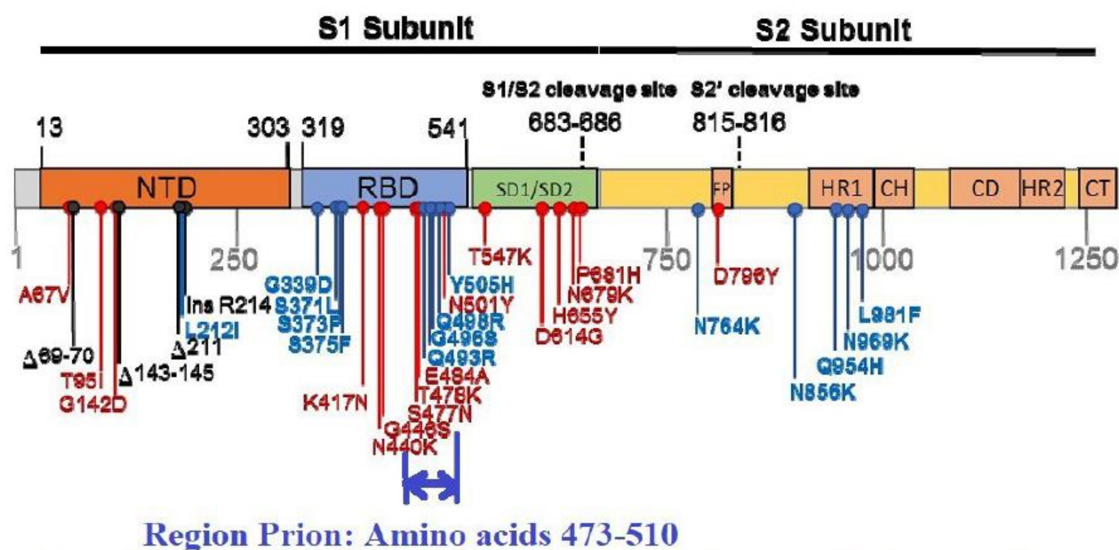


Figure 19 shows the prion sequence found within the spike protein within the blue bars (double arrow) at the bottom: the region within the sequence of the spike protein in which the amino acid sequence matches the "master motif" of prions. (Q: Figure 19 from Preprint: HIV MAN-MANIPULATED CORONAVIRUS GENOME EVOLUTION TRENDS - ScienceOpen

2.1.2.5 Receptor binding domains in the spike molecule. Receptors on cell surfaces play a decisive role in the tropism and pathogenicity of viruses. The more different receptors a virus can use to dock and penetrate the cell, the more tissue types (and organs) the virus can infect and replicate in. The spike protein of the corona viruses is practically the key on which the binding partners ("receptor binding domains" RBD) to various receptors on human cells (the "lock") are encoded.

For the sake of understanding, this could be compared to a security key on which small "buttons" or indentations allow the locking function to be wasted.

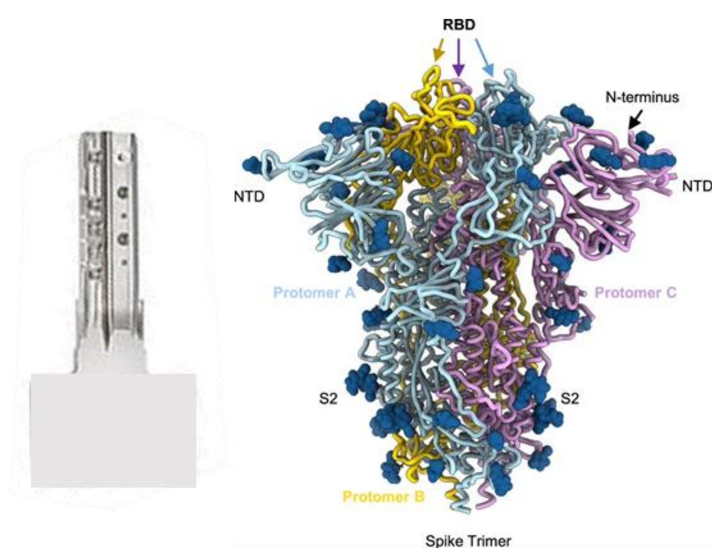


Figure 20 on the function of the receptor binding domains in comparison with a security key. The entire protein (key) has the amino acid patterns (receptor binding domains; RBD) on its surface with which it can bind to individual receptors.

In the spike protein of SARS-CoV-2, in addition to the well-known main binding domain (docking site) to the ACE2 receptor, further binding possibilities were found (Gu Y 2021), which occur exclusively only in SARS-CoV-2 and not, for example, in SARS and MERS (Figure 21).

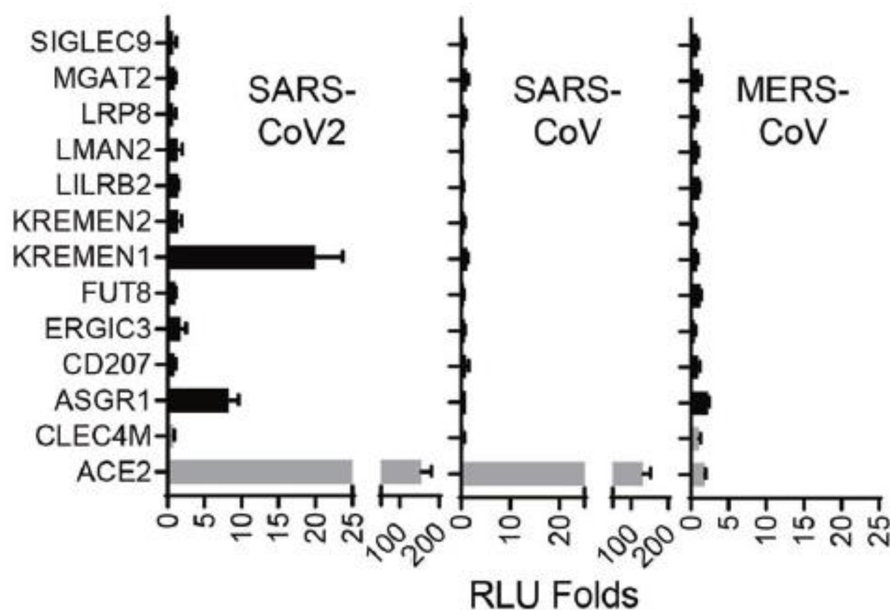


Figure 21 of the unusual receptor binding sites, Figure 2a from Gu Y 2021: Possible receptors for the spike protein of SARS-CoV-2 compared to SARS and MERS. The larger the bar, the better the spike protein can bind to this receptor. The main receptor is ACE2, but the two receptors KREMEN1 and ASGR1 are also bound very effectively.

The two receptors KREMEN1 and ASGR1 are particularly effective in binding to the spike protein. KREMEN1 is a receptor that is involved in the signalling cascade of controlled cell death (apoptosis) when activated. This receptor is used as the main receptor by another group of viruses, the enteroviruses. KREMEN-1 is found as a normal receptor in many tissues of the body, especially on endocrine glands and in the intestine, but also on muscle cells, in the brain and in the bone marrow.

ASGR1 is used by the hepatitis C virus to enter liver cells and is found almost exclusively in the liver. By utilizing ASGR1, the spike can bind very effectively to liver cells.

By using these two additional receptors, SARS-CoV-2 can infect a much wider range of cell types via spike binding than if it only used the ACE2 receptor like SARS. The ACE2 receptor is mainly found on cells of the blood vessels (endothelia) and on epithelia of the respiratory tract and gastrointestinal tract. However, ACE2 is also found in the kidneys and pancreas as well as in the testicles and ovaries.

With the help of the three receptors, the spike protein of SARS-CoV-2 can bind to nearly all organs in the body and thus also give the virus access there or influence the cells as a vaccine spike via the receptors (see 2.2. Special effect of the spike in the body).

Further binding partners for the spike protein are described in a review paper (Suprewicz L 2023). This article deals with damage to the brain (see also point 2.2.2.3.) and the focus is on binding partners at the blood-brain barrier and on nerve cells. It should also be noted here that the damage mechanisms of the spike (and the S1 subunit separated by furin cleavage) are discussed on the basis of the viral

infection with SARS-coV-2. Since the spike proteins formed over a large area in the body due to genetic vaccination are sequence-identical to the viral spike proteins (including the furin cleavage site, which is active in the body despite the two proline mutations), it is to be expected that they trigger the same mechanisms.

Meaning: The spike protein of SARS-CoV-2 (and identically the one from the genetic vaccinations) has many cellular and extracellular binding partners beyond the ACE2 receptor. These binding partners are primarily associated with vascular damage, increased blood clotting (thrombosis formation), access to the brain via the blood-brain barrier and severe inflammation (also in the brain).

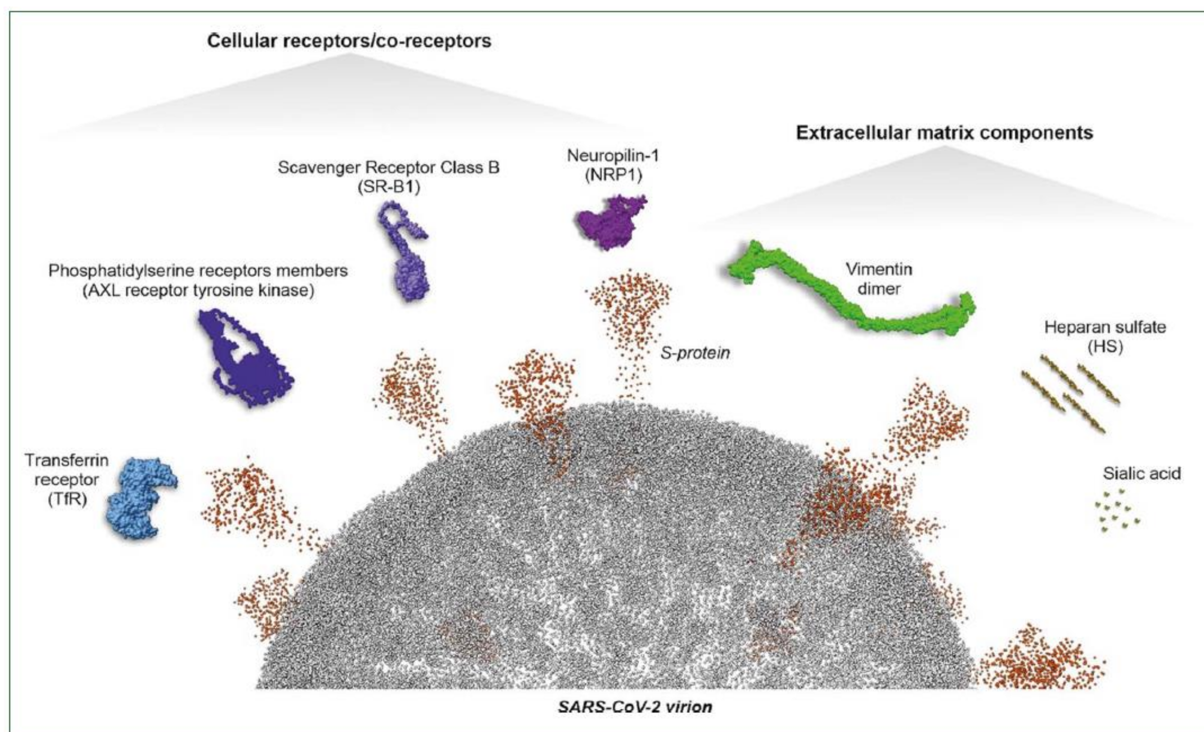


Figure 22 shows binding partners at the blood-brain barrier for the spike protein with the help of which the virus (but also the vaccine spike) can enter the brain and trigger inflammation in the nerve cells. (Q: Figure 2 from Suprewicz L, 2023)

2.1.2.6 Summarizing the structure of the spike protein of SARS-CoV-2 (original Wuhan variant, corresponding to the spike encoded in the genetic vaccines), it should be noted with regard to the effect:

This molecule contains a conspicuous furin cleavage site, which means that, unlike SARS-1 and MERS, for example, this spike can be cleaved by the cell's own enzymes into a soluble (and thus spread in the body) S1 subunit and a membrane-bound S2 subunit. The S1 subunit is a receptor molecule, which, in addition to the known ACE2 receptor, can utilize a variety of different docking sites on cells and has a heparin binding site, which can interfere with blood clotting. In several places, the amino acid structure and the spatial arrangement of the amino acids on the surface show strong similarities with neurotoxic peptides (short pieces of protein) and a prion property. The S2 subunit remaining on the cell surface after cleavage has fusing properties and can therefore fuse neighbouring cell membranes and thus destroy the individual integrity of individual cells.

2.2 Special effects of the spike protein in the body

From Wikipedia:

The spike glycoprotein contributes to the pathogenesis of Covid-19 disease through various mechanisms:

- Binding to the ACE2 receptor can lead to deregulation of the functions of this enzyme in several organs, including the lungs, blood vessels, heart, kidneys, intestines and brain. Many symptoms and consequences of Covid-19 can be understood through this deregulation.[39]
- Among other mechanisms that may lead to the deregulation of blood coagulation mechanisms in the vessels by SARS-CoV-2 is the direct or indirect influence of the S1 region of the spike glycoprotein on receptors and other structures of the blood vessels.[40][41]
- The spike glycoprotein can also bind to other receptors and thus lead to the deregulation of other functions.[42]

Note: ACE2 is an enzyme called angiotensin-converting enzyme 2.

2.2.1 The S2 subunit triggers cell fusions

By separating the S1 subunit of the spike protein at the very effective furin cleavage site, the S2 subunit is activated to connect (fuse) cell membranes. In the event of a viral infection, the SARS-CoV-2 viruses are taken up into the cells via this mechanism and passed on between cells. Regardless of the virus, the spike alone can trigger a fusion of cells, resulting in the formation of so-called syncytia (Figure 23). Syncytia are several fused cells without partitions with multiple cell nuclei, which usually become dysfunctional as a result. Physiologically, syncytia in humans only occur in the placenta and in striated skeletal muscle, pathologically in various viral infections and in SARS-CoV-2. The spike protein of SARS-Cov-2 in particular, with its S2 subunit, is extremely fusiogenic ("*SARS-CoV-2 S protein showed a remarkable fusogenic activity*" (Theuerkauf SA, 2021). To demonstrate this very effective fusogenic property of the spike protein, cell culture experiments were carried out by the Paul-Ehrlich-Institut working group, in which the spike gene was introduced into cells by transfection of a spike-encoding plasmid. This procedure is comparable to the basic mechanism of genetic engineering vaccines. The cells transfected in this way then formed the spike protein. Extreme syncytia formation was observed in the spike-forming cell cultures. This matches the particular pathology in SARS-Cov-2-infected lungs, where syncytia were also observed. This unusual syncytia formation is a unique feature of the SARS-CoV-2 virus. ("*Syncytia formation has recently been described as main and unique lung pathology in patients affected with COVID-19 in an occurrence not seen in other lung infections* before." Theuerkauf SA, 2021). Regardless of the viral infection, the SARS-CoV-2 spike protein produced in cells alone is sufficient to trigger cell fusions, a significant potential danger of genetic vaccines. According to the publication from the PAI (Theuerkauf SA 2021), these fusion properties can only be prevented with difficulty by specific neutralizing antibodies ("*This suggests that cell fusion is not only proceeding with minimal amounts of S protein but also difficult to access for neutralizing antibodies*."). Based on the findings in this publication from the PAI, it is very likely that the spike, which is formed in the body due

to the genetic vaccinations, also has a considerable syncytia formation property analogous to the cell cultures and that this cannot be prevented even with high antibody formation against the spike.

Note: this publication originates from the Paul-Ehrlich Institute (PAI) with the then director Klaus Cichutek as one of the authors, i.e. the PAI knew very early on about this problem of syncytia formation even through "*low levels of S protein*".

The problem of syncytia formation by the spike protein is summarized very well here: [Coronavirus makes our cells merge - Spike protein of SARS-CoV-2 triggers cell fusions even in non-infected cells - scinexx.de]() .

The fact that syncytia formation can contribute to immunodeficiency not only by disrupting the function of the affected cells but also by fusing and killing lymphocytes with the syncytia (Figure 20) was discussed early on in a paper from China (Zhang Z 2021).

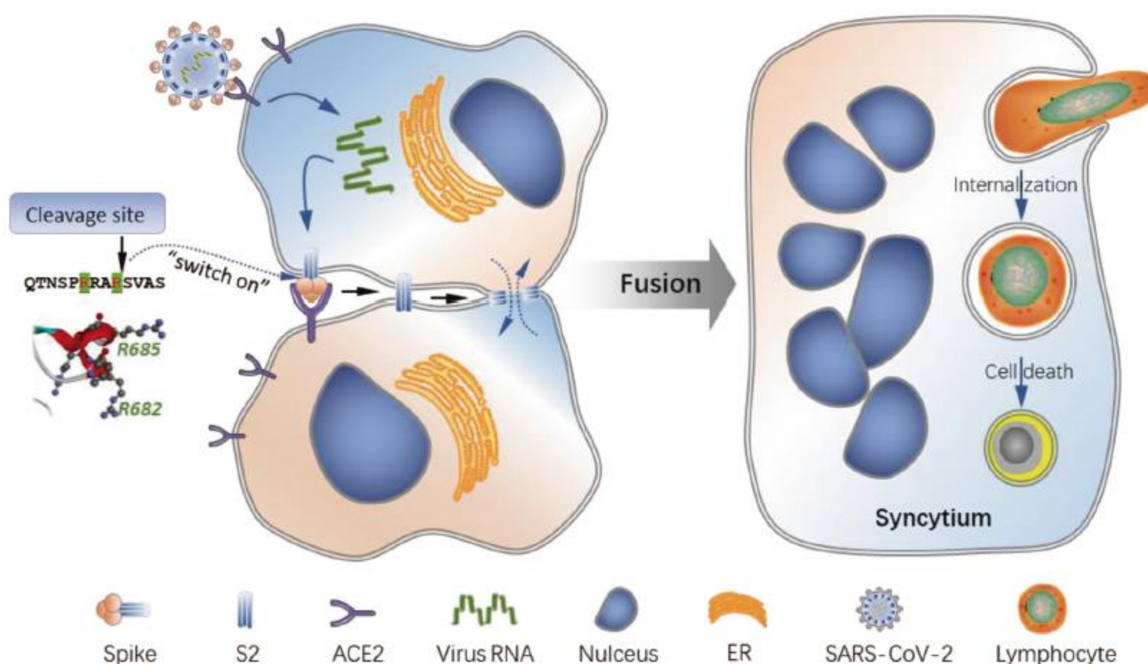


Figure 23 shows the formation of syncytia by the S2 subunit of the spike protein (left) and the mechanism by which the syncytia can internalize and then kill lymphocytes (right). In this way, the spike protein can force a reduction of lymphocytes (lymphocytopenia) and thus a general immunodeficiency. (Q: Figure 5 from Zhang Z 2021)

2.2.2 The S1 subunit can spread throughout the body and persist for a long time

Due to the furin cleavage already described several times, the S1 subunit of the spike protein of SARS-CoV-2 and thus also the spike, which is formed in the body by the genetic vaccinations, can be detached from the transfected cells and spread in the body. The S1 subunit can bind to the wall cells of the blood vessels (endothelia) in all organs including the brain (S1 is even able to cross the blood-brain barrier) via its ACE2 receptor binding site. Accordingly, the S1 of the spike is regularly detected in organ damage

both after severe Covid-19 diseases ("LongCovid") and after genetic vaccinations ("PostVac") using various methods, especially in the area of the capillaries (Figure 21 B-D). Our own staining shows that even small blood vessels in placentas of newly vaccinated pregnant women (2nd or 3rd trimester) in individual cases clearly stain for the spike protein (see Figure 21 A). In a study (publication in preparation), of 92 placentas of vaccinated women examined, 28 were found to have clear spike detection (without nucleocapsid detection, i.e. excluding infection with the virus). This indicates that the genetic vaccination or at least the spike protein can also reach the unborn child via the placental barrier. However, the S1 subunit can also dock onto other cells with the help of the various receptor binding sites and can often be taken up into them. For example, in a study from the USA (Patterson BK 2022), the S1 subunit was detected in macrophages from patients up to 15 months after a viral infection, and without persistent virus (*"It is important to note that the S1 protein detected in these patients appears to be retained from prior infection or phagocytosis of infected cells undergoing apoptosis and is not the result of persistent viral replication."*). This astonishingly long period of time suggests either that the spike protein appears to be surprisingly long-lived or that it is replicated in the body. Nothing was known in the publication about the vaccination status of the people concerned.

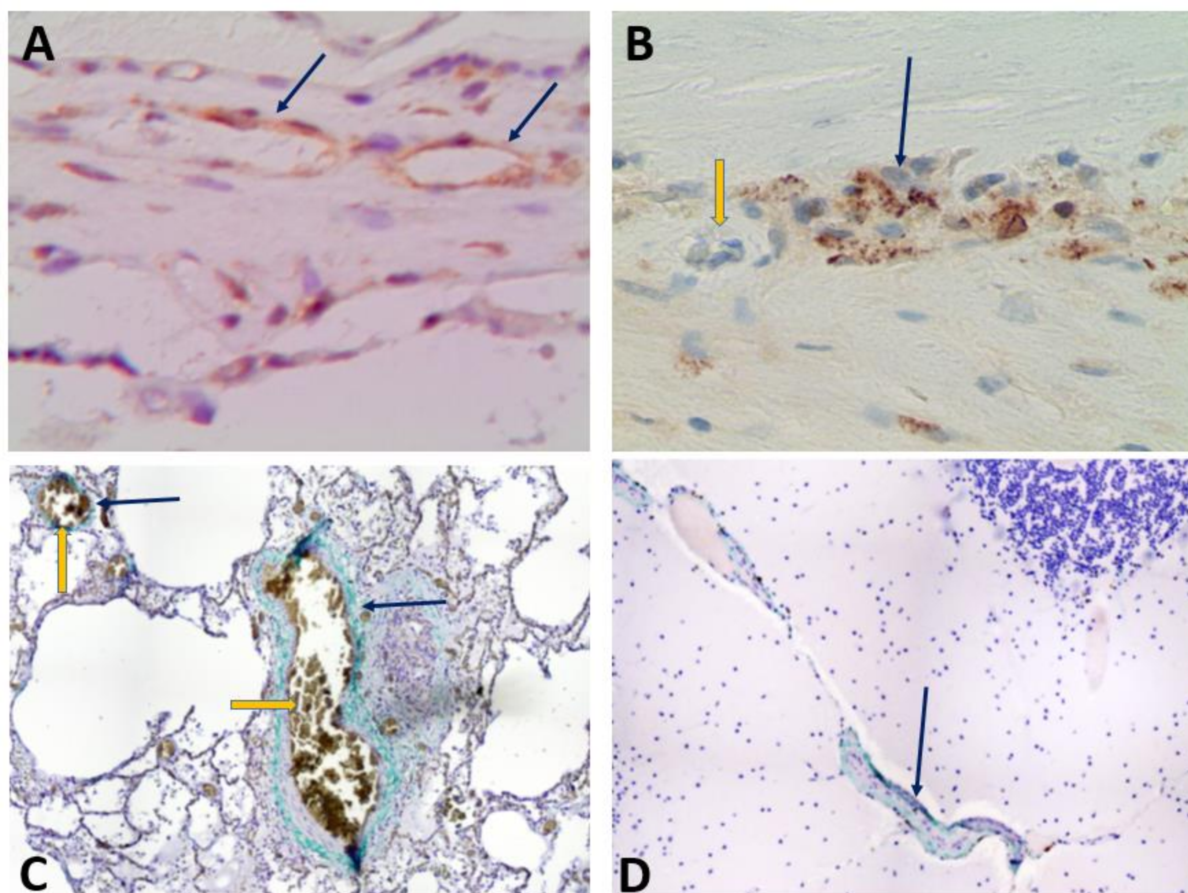


Figure 24: Spike staining on blood vessels of A) placenta of a woman vaccinated with Comirnaty (3rd dose) in week 37 of pregnancy. The staining was performed after the birth of the child in the 40th week of pregnancy. There is a clear positivity for the spike protein of the vessel walls (endothelia, arrows, stained brown) in a mature placental villus (on the fetal side of the placenta). B) Vascular wall of a large capillary artery (coronary artery) of a patient who died acutely of cardiac arrest. Here, the areas around the small blood vessels (vasa vasorum externae) that supply the muscular wall of the right coronary artery (arteria coronaria dextra) are clearly spike-positive. (Positive are endothelial cells and macrophages in brown). The yellow arrow points to the lumen of a small vessel with a microthrombus.

C) Spike detection (here green) in the adventitia of a small pulmonary artery (arrow) with remnants of a thrombus (brown in the lumen, yellow arrow); D) Spike detection in the wall of a blood vessel in the cerebellum. In all cases, counterstaining with antibodies against the nucleocapsid protein (as evidence of a viral infection) was negative. Sources: A) U. Kämmerer, Women's Hospital Würzburg; B) M. Mörz, Institute of Pathology "Georg Schmorl" Dresden Friedrichstadt. C+D) V. Schmidt-Krüger, Labor Inmodia GmbH (via MWGFD)

2.2.2.1 The S1 subunit triggers inflammation in macrophages

Using cell cultures of mouse and human macrophages, it was shown that a recombinant (i.e. artificially produced) S1 subunit of the SARS-CoV-2 spike protein stimulates macrophages to produce typical pro-inflammatory messenger substances (cytokines) in a dose-dependent manner (Shirato K 2021; Chiok K, 2023). The interaction of the S1 subunit with a specific pro-inflammatory receptor, TLR4, appears to be a key factor in this process. (Note: there are many different TLRs, in the context of the discussion about the deimmunizing modRNA - point 1.4.3 - an inhibitory interaction with TLRs 7 and 8 plays a role, this is to be distinguished from TLR4).

For understanding: Due to the basically identical mechanisms of spike formation in cells both after an infection with SARS-CoV-2 in the context of its multiplication within an infected cell and after transfection with the genetic information for the spike protein by means of genetic vaccines, the findings on the effect of the viral spike (infection) can be directly transferred to the effects of the vaccine spike (transfection).

As outlined in Figure 21 (from Chiok K 2023): it makes no difference whether the pro-inflammatory S1 subunit circulates in the body via the virus as part of an infection or as part of the vaccination spike. These practically identical mechanisms often make it difficult to differentiate between long-Covid and post-Vac.

However, the fact that the viral infection (typical respiratory virus) usually only occurs on superficial mucosal cells and therefore only a small amount of soluble spike enters the circulation, an enormous amount of formed spike is detectable during genetic vaccination with subsequent transfection and significantly more pronounced effects can be expected.

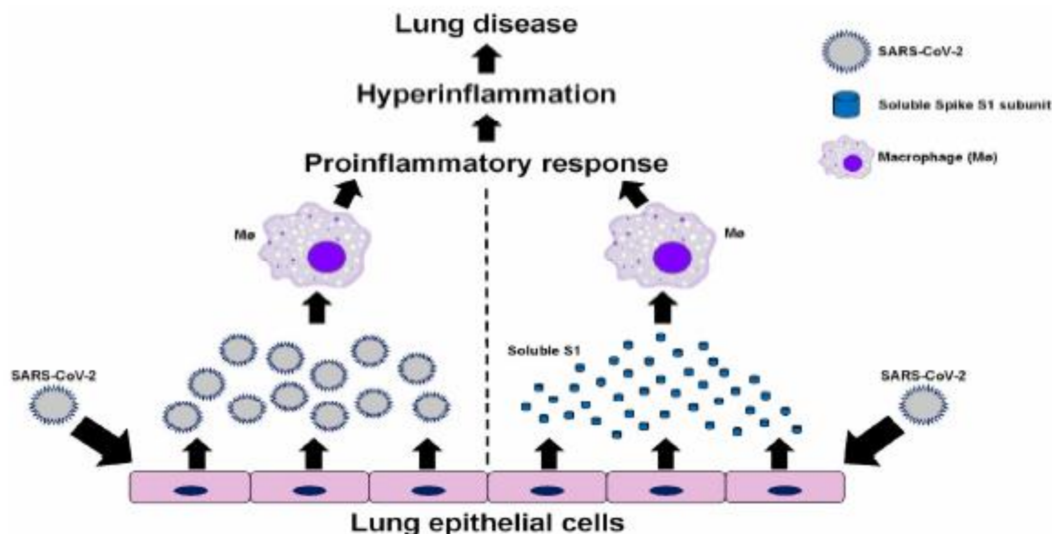


Figure 25: Sketch of the connection between spike and inflammation shows that both the viral infection and the genetic vaccination stimulate macrophages to an inflammatory reaction via the S1 of the spike protein, which can then lead to pneumonia. (Q: Figure 4 from Chiok K 2023)

If macrophages are activated by the S1 subunit to promote inflammation, this can lead to inflammation in all tissues and organs, as macrophages are found everywhere in the body. The two tissue structures most frequently affected by the S1 subunit as a result of genetic vaccination, with effects on the frequent symptoms of vasculitis/thrombosis and neurological damage, are the cells of the blood vessel walls (endothelia) and the nerve cells (neurons) in the body. The endothelia are not only directly affected by the spike, but apparently macrophages activated by the S1 subunit increase the sensitivity of the vessel walls to respond to the spike S1 protein with thrombosis-promoting inflammation (" [...] *our findings highlight the relevance of innate immune cells in the spike-dependent induction of a pro-coagulative phenotype in the endothelium and provide a pathogenetic model for endothelial dysfunction in COVID-19 based on a crosstalk between immune and endothelial cells, both targets of the spike S1 protein*." (Rotoli BM 2021).

The spike protein and especially the S1 subunit can also trigger pro-inflammatory damage in all other cell types. In the following, however, only endothelial damage and the effect on the nervous system will be discussed, as these are at the forefront of the spike-induced damage that causes patients to suffer from post-vac problems.

2.2.2.2 S1 as a cause of vascular inflammation and damage

For understanding: Most of the published work on the topic of Sars-CoV-2 spike protein and vascular damage is discussed as an explanation for the observed vascular damage and thrombosis as a result of the actual virus infection. However, the underlying experiments were carried out with recombinant (i.e. genetically engineered) spike protein or its subunits (but not with the virus itself). Since the recombinant spike protein and its separated S1 subunit are also produced in the human body after

genetic vaccination, the experimental data can be transferred accordingly to the effect of the "vaccine-induced" spike protein.

In a mouse model, mechanisms were investigated that can explain the vascular damage that is also observed in humans as a result of severe COVID-19 disease or vaccination damage. The authors conclude from their results that the S1 subunit of the spike protein of SARS-CoV-2 alone, without the infectious virus, is capable of triggering inflammatory mechanisms:

(Nuovo GJ 2020*) "In sum, the data presented indicates that the full-length S1 subunit of the spike protein of SARS-CoV-2 alone is capable, without the infectious virus, of inducing systemic microendothelial cell damage in mice with a cognate pattern of complement activation and increased cytokine expression and the concomitant thromboses/hypercoagulable state. This disease pattern strongly parallels the extra-pulmonary manifestations of severe human COVID-19 and suggests that the latter may not represent systemic infectious virus. "*

The binding of the S1 subunit to endothelial cells can occur not only via the ACE2 receptor, but also via another factor, the so-called P-selectin. This P-selectin is also found on blood platelets (thrombocytes) and in a study from China (Wang C 2024). This explains the underlying mechanism of platelet activation and vascular cell inflammation by the S1 subunit of the SARS-CoV-2 spike protein (Figure 22). Activated platelets in combination with inflammation (and thus dysfunction) of the vessel walls (endothelial cells) lead to thrombosis. ("*Platelet activation and endothelial dysfunction are the critical events in the pathogenesis of vascular thrombosis*"). Many small thromboses deplete the blood platelets and are no longer available to stop the bleeding as a result of the thrombocytopenia triggered by this. This thrombocytopenia as a result of the thromboses can lead to extensive bleeding in non-occluded vessels, even with minor injuries (corresponding to the tendency of patients taking blood thinners to develop very large haematomas).

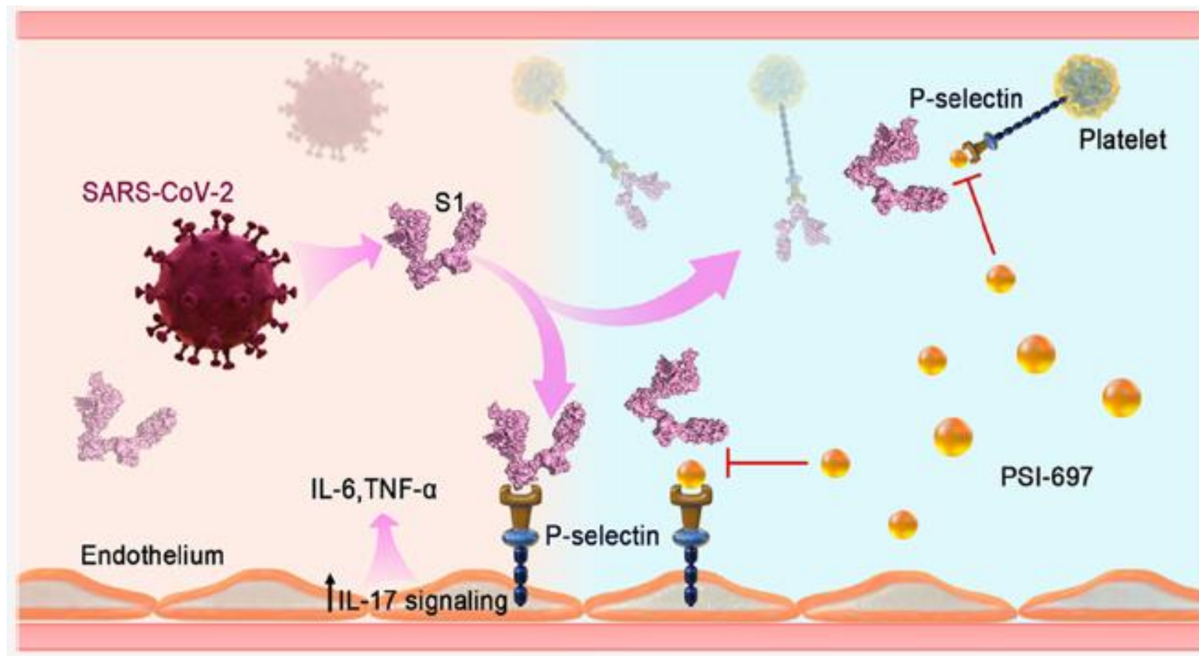


Figure 26: Thrombosis induction via the S1 subunit is achieved by binding to P-selectin. Here in the figure (abstract from Wang C 2024), a substance (PSI-697) is discussed that inhibits the binding of P-selectin and could therefore possibly be used as an anti-thrombosis agent for spike-induced thrombosis.

2.2.2.3 Summary of spike and thrombosis/embolism due to vascular damage

As the cleaved S1 fragment of the spike protein formed can activate platelets (causing them to clump more quickly), bind heparin (reducing its anti-thrombotic effect) and cause inflammatory changes to vessel walls (thrombi then accumulate at the sites of inflammation), it contributes significantly (now scientifically recognized) to the formation of thrombi in capillaries. This can also be detected microscopically - see photos in Figure 21. It should also be noted here that it is not just a matter of "deposits" of the spike protein, because in addition to the separated S1 protein subunit distributed in the bloodstream, the lipid nanoparticles are also distributed throughout the body after vaccination (see point 1.4.5.) and can therefore transfect cells everywhere. The first cells to be affected by these LNPs are the endothelial cells of the vascular walls. As a result of the transfection of the endothelial cells, the spike protein is also formed "locally", i.e. directly in the capillaries. This local spike formation means that the S2 subunit in the walls of the endothelial cells can contribute to syncytia formation through furin cleavage (see points 2.1.1.; 2.1.2.2.; 2.2.1). Furthermore, the endothelia, like all cell types that express the spike on their surface, can be attacked and killed by cytotoxic T cells (Figure 30). This results in damaged areas in the blood vessels, which in turn promote the formation of thrombosis. All of this together causes an extremely high risk of thrombi, as seen both in severe Covid-19 diseases and especially as a result of genetic vaccinations, which are now also listed as side effects in the package inserts.

2.2.2.4 S1 subunit is neuroinflammatory and can trigger ME/CSF in the brain

Since the S1 subunit is able to cross the blood-brain barrier (Frank MG 2021+2024), it can also reach the nerve cells directly and not "only" affect the endothelia of the capillaries in the brain (see Figure 20D). In the meantime, experiments have shown that the S1 subunit (independent of the virus) is extremely inflammatory and can thus trigger systemic inflammation (encephalitis) in the brain, as described in a publication from Dresden (Mörz M, 2022). In principle, this can lead to the clinical picture of ME/CSF, a frequent diagnosis in the context of long Covid and/or post-vac damage.

These mechanisms are discussed in three recent papers. The review by Suprewicz L 2023 focuses on the mechanisms by which the spike protein or its S1 subunit can bind and cross the blood-brain barrier and thus, in the case of viral infection, enable this virus (unusual for coronaviruses) to access the brain, where it leads to symptoms such as "*fatigue, dizziness, headache, sleep disorders, malaise, disturbances of memory and mood*" via massive inflammatory reactions and vascular damage. Fatigue, dizziness, headache, sleep disorders, malaise, disturbances of memory and mood are frequently described by Long-Covid and PostVac patients, and are also observed in ME/CSF of other causes. But in addition, the expression of spike in the brain can also contribute to more serious complications such as strokes and encephalopathies ("*Damage to the brain vessels mediated by the coronavirus spike protein (S-protein) and overactive immune responses have been identified as leading causes of this condition*"). Another review (Klein RS 2022) confirms these mechanisms and also emphasizes the role of the spike protein with its inflammation- and thrombosis-promoting properties as a central element of neurological damage, whereas true viral infections in the brain are described as rare. ("*While the development of neurologic diseases during acute COVID-19 is rarely associated with evidence of viral neuroinvasion, new evidence suggests SARS-CoV-2 Spike (S) protein exhibits direct inflammatory and*

pro-coagulation effects. This, in conjunction with immune dysregulation resulting in cytokine release syndrome (CRS) may result in acute cerebrovascular or neuroinflammatory diseases."

An earlier review (Theoharides TC 2022) expresses the suspicion that the typical symptoms of Long Covid (at that time there was no post-vac) are linked to "antigen persitence", i.e. the long-lasting presence of the spike antigen. The author describes the sum of the resulting neuro-inflammation, damaged blood vessels and brain cells as "autoimmunity of the brain" and concludes the article with a call to limit or prevent the harmful effects associated with spikes, especially on the brain and their possible contribution to the development of long-term COVID.

3. Disruption of the immune system at several central sites

3.1 Link between genetic vaccinations and infections

There are increasing observations that people have an increased susceptibility to typical diseases after genetic vaccination, which can be interpreted as a sign of general immunosuppression. This includes a reactivation of herpes viruses, especially the varicella-zoster virus (chickenpox virus), which manifests itself in the form of shingles. But also an increased susceptibility to long-lasting viral and bacterial infections or even protozoa such as mycoplasma. There is currently an unusual increase in pneumonia caused by mycoplasma, which indicates that the immune system of those affected is poor, as only then can this intracellular parasite spread easily in the population.

In a letter to the editor of the "Virology Journal" (Yamamoto K 2022), the author summarizes the aspects of possible immunosuppression by genetic RNA vaccines already known in 2022 as Comirnaty as follows (with citation of the literature, omitted here in the translation - see original):

"Some studies suggest a link between COVID-19 vaccines and reactivation of the virus that causes shingles. This condition is sometimes referred to as vaccine-acquired immunodeficiency syndrome [....] ***The decrease in immunity is caused by several factors.*** *First, N1-methylpseudouridine is used as a replacement for uracil in the genetic code. The altered protein can trigger the activation of regulatory T cells, leading to reduced cellular immunity. The spike proteins do not disintegrate immediately after the administration of mRNA vaccines. The spike proteins present on exosomes circulate in the body for more than 4 months. In addition, in vivo studies have shown that lipid nanoparticles (LNP) accumulate in the liver, spleen, adrenal glands and ovaries. LNP-encapsulated mRNA is highly inflammatory. Newly formed spike protein antibodies damage the cells and tissues that are designed to produce spike proteins, and vascular endothelial cells are damaged by spike proteins in the bloodstream; this can damage immune system organs such as the adrenal gland. In addition, antibody-dependent enhancement may occur, in which infection-enhancing antibodies weaken the effect of neutralizing antibodies in preventing infections"*

3.1.1 The more genetic vaccinations, the more Covid-19 diseases. In a large study of over 50,000 employees of the Cleveland Clinic (Shrestha NK 2023), the relationship between genetic vaccinations (87% Comirnaty from Pfizer, 13% Spikevax from Moderna) against Covid-19 and the risk of disease was investigated. The sobering conclusion was that the risk of contracting Covid-19

increased with the number of vaccinations ("***The risk of COVID-19 also increased** with time since the most recent prior COVID-19 episode and **with the number of vaccine doses previously received**.*") The correlation can be seen very clearly in Figure 2 of the paper, in which the cumulative risk of contracting Covid-19 clearly increases with each vaccine dose. The increase is strongest between employees who have not been vaccinated against SARS-CoV-2 and those who have been vaccinated, so that from the very first injection there is an increased susceptibility to the disease against which the vaccination was actually intended to protect (exactly the opposite of what would be expected with a vaccination).

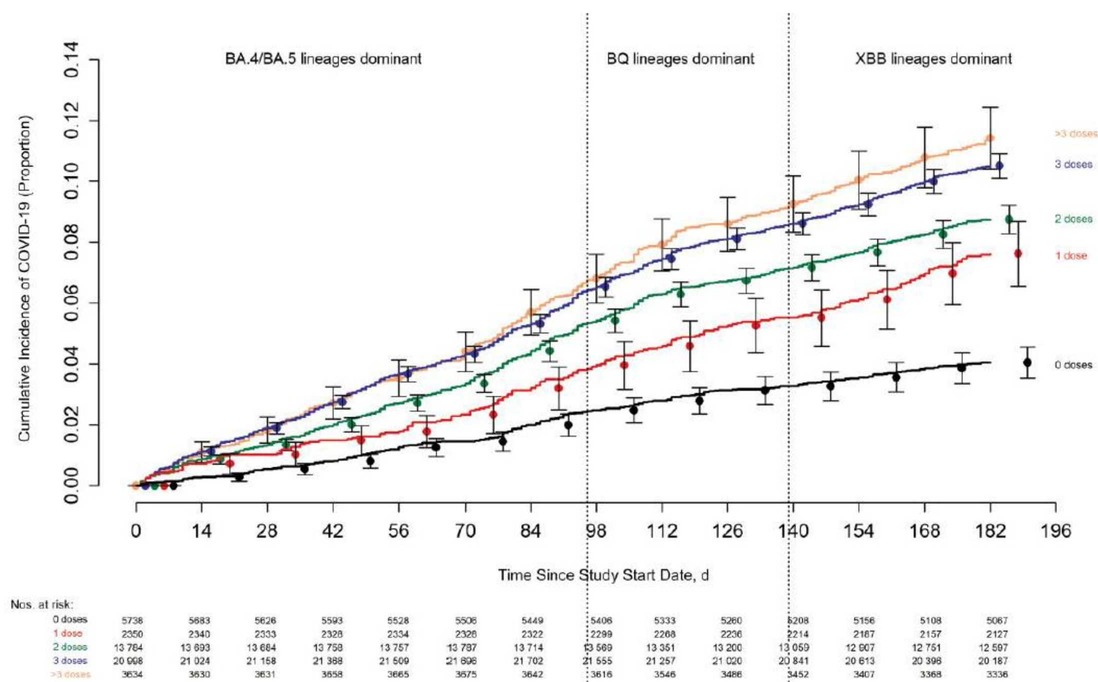


Figure 2. Cumulative incidence of coronavirus disease 2019 (COVID-19) for study participants stratified by the number of COVID-19 vaccine doses previously received. Day 0 was 12 September 2022, the date the bivalent vaccine was first offered to employees. Point estimates and 95% confidence intervals are jittered along the x-axis to improve visibility.

Figure 27 on the increasing risk of Covid-19 disease after vaccination. Data from the large Cleveland Clinic study show a clear link between genetic vaccinations and the cumulative susceptibility to contracting Covid-29. The more vaccinations, the greater the risk of contracting Covid-19 (Q: Shresta NK 2023, Figure 2).

These data from the USA with Comirnaty from Pfizer fit in with the observation of increasing "vaccination breakthroughs" with the (identical) Comirnaty from BioNTech available in Germany and suggest a negative effect on the immune system due to the genetic RNA vaccinations.

The "Supplemental material" of a study from the Netherlands (https://www.sciencedirect.com/science/article/pii/S1933021924000278?via%3Dihub#s0115) illustrates very well the correlation between the number of vaccinations (in the Netherlands mainly Comirnaty) and the rate of subsequent SARS-CoV-2 infections:

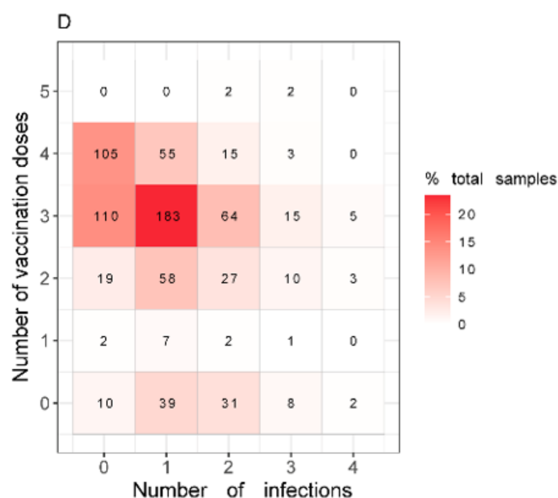


Figure 28 on the accumulation of Covid-19 in three genetic vaccinations (Q: https://www.sciencedirect.com/science/article/pii/S1933021924000278?via%3Dihub#s0115)

Some important basics for understanding the immunosuppressive effect are presented below. The point of the immunosuppressive effect of modRNA with the base Ψ has already been dealt with under point 1.4.3.

3.2 Dendritic cells, not muscle cells, are the target of transfection

According to BioNTech and many publications by U. Sahin, Comirnaty's declared target cells are the so-called **dendritic cells** (Figure 26). A BioNTech press release on Kathalin Kariko's Nobel Prize (Statement on the award of the 2023 Nobel Prize in Physiology or Medicine to Katalin Karikó and Drew Weissman | BioNTech) reads: "*The Sahin-Türeci researchers developed strategies for the targeted uptake of mRNA into **dendritic cells**, which increased the potency of mRNA by a factor of 1.000-fold*" and in an interview with the Ärzteblatt (https://www.aerzteblatt.de/nachrichten/121745/Biontech-Nanopartikel-sind-schwieriger-herzustellen-als-mRNA) U. Sahin is quoted as saying: "*The challenge lies rather in the production of the nanoparticles that envelop the mRNA and transport it to its destination - primarily **dendritic cells** in lymph nodes.*" (BioNTech: Nanoparticles are more difficult to produce than mRNA)

According to BioNTech, this clear preference of Comirnaty's for dendritic cells is contrary to the public statement (also made by the director of the PAI, Klaus Cichutek, among others) that the genetic vaccine would remain almost completely in the muscle near the injection site. "*Furthermore," Cichutek explains, "the majority of the mRNA remains in the muscle after vaccination. Although the smallest amounts can also enter the blood or organs, they are* harmless *there*" (Faktencheck MDR Wissen from 15.06.2021 No organ damage from spike protein after mRNA vaccination | MDR.DE). "*Of course, the vaccine does not remain 100 percent in the upper arm muscle, but is also distributed somewhat in the body." But what is distributed in the body are irrelevant small amounts, says Watzl*." (Correktiv

fact check with Carsten Watzel: No evidence that spike protein produced during mRNA vaccination has a "toxic" effect)

On the BioNTech website (Figure 29), the principle of action of Comirnaty on human cells is illustrated using a transfected "APC" (antigen-presenting cell, to which dendritic cells belong). Incidentally, muscle cells do not appear in any of the diagrams on the mode of action of Comirnaty; the typical starfish-like dendritic cells are always shown, which actually corresponds to the aim of transferring the dendritic cells from the muscle.

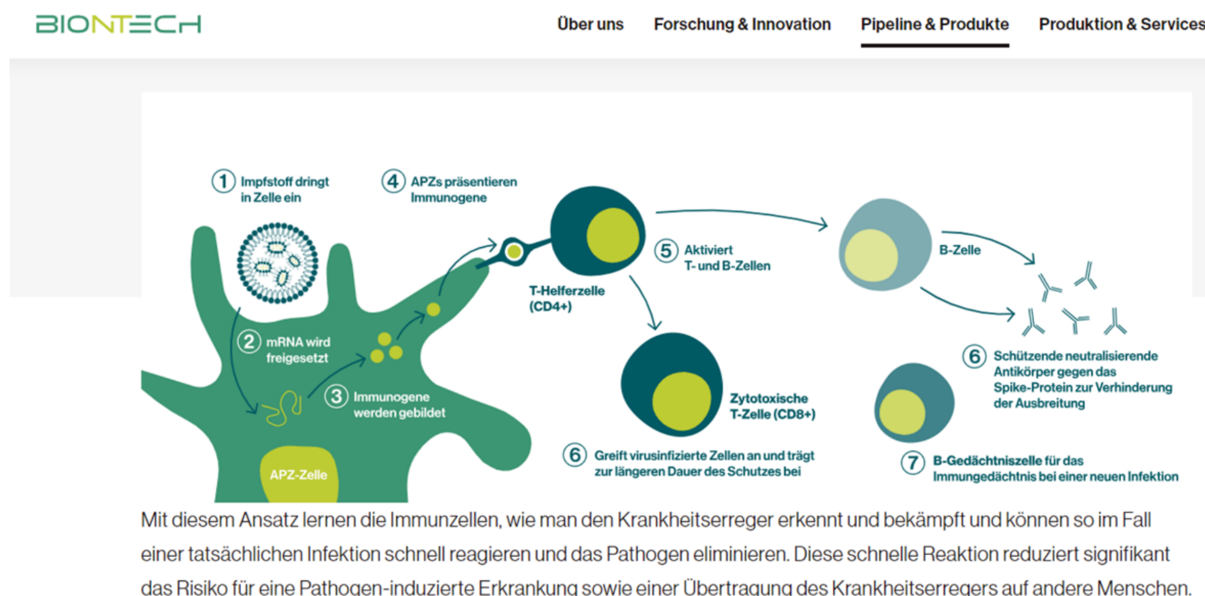


Figure 29 shows Comirnaty's target cell: Dendritic cell (here: APC for antigen-presenting cell (Q: BioNTech webpage)

Insert for understanding: dendritic cells

Dendritic cells (DC) are so called because they have many extensions (dendrites) that protrude from their surface and are clearly visible under the microscope. DC are often referred to as "key cells of immunity" because they orchestrate immune responses via a variety of mechanisms. A key property of DC is their ability to present antigens specifically to other immune cells, which is why they, like macrophages, are classified as antigen-presenting cells (APC), of which they are the most important representative. During antigen presentation, the DC can use additional cofactors to control whether the presented antigen should be tolerated by the immune system or recognized as dangerous and fought against. The normal function of dendritic cells is controlled in a complex interplay with the environment. Immature dendritic cells are found throughout the body in a function as guard cells. As soon as they notice a foreign antigen (e.g. components of a virus), they absorb it. The DC migrate towards the nearest lymph nodes with the absorbed foreign antigen. During their migration, they mature and break down the antigen into small components, the peptides (protein fragments). These peptides are bound to special receptors, the MHC molecules, and transported to the surface of the DC. Once in the lymph nodes, these MHC molecules with the bound peptides are presented to the inactive T cells present there. (You can imagine this as in US crime novels, where a number of possible

perpetrators are presented to a witness). If a T cell is found that recognizes this peptide, it can be activated by the presenting DC, provided it is a foreign antigen, and begins to divide. In the case of infections, this is often noticeable by swelling lymph nodes. These activated T cells then swarm from the lymph node into the body and search for the peptides (and thus the associated antigens). If these T cells then encounter cells that have this antigen on their surface, e.g. because they are infected with a virus, the cells are attacked and killed. Under the guidance of DCs, T cells learn to recognize the body's own antigens or, for example, food components as "own" and taboo. The appropriate T cells become tolerant and suppress a rejection reaction. In the case of autoimmune problems, the wrong T cells are activated by a DC error and instead of tolerance, they become attack ("cytotoxic") T cells, which can now attack the body's own structures. In this respect, dendritic cells play a key role in the coordination of the immune system and the correct activation or deactivation of cytotoxic T cells in particular; DC as target cells for transfection with foreign genes can therefore lead to massive disruptions in DC function and thus in the finely tuned immune network.

3.2 Spike expression on dendritic cells other than physiological

However, in the very simplistic representation of the effect of Comirnaty on dendritic cells as outlined in Figure 29 by BioNTech or Figure 30 (Deutsche Apothekerzeitung), it is assumed that the APCs present the vaccine antigen on their surface in such a way that it serves to activate T cells via normal presentation mechanisms of APCs (note: means only small fragments (peptides) of the spike protein coupled to special receptors, the MHC molecules). However, a large review paper (Pardi N 2018) led by D. Weissman, who received the Nobel Prize together with Ms. Kariko for the modRNA vaccine development, shows the more correct effect of the transfected modRNA on spike formation and presentation in dendritic cells (Figure 1). Since the lipid nanoparticles are used to introduce the complete gene in the form of modRNA for the spike protein into the cells, this complete spike is in principle also formed in the cells and not immediately broken down into peptides again. It is precisely the modifications of the RNA with the incorporation of Ψ that serve to immunologically paralyse these cells (see point 1.4.3) and ensure that they recognize the spike protein to be formed as their own and do not break it down. This leads to the dendritic cells incorporating the complete spike protein (here in the figure "native AG expression") on their surface and only partially presenting it correctly to the T cells as peptides. If the peptides of the spike proteins have actually been successfully presented by some DC for the activity of cytotoxic T cells, there is now an immense risk that these activated T cells will turn against their own dendritic cells if these are identified as supposedly virus-infected by the incorporation of the spike protein into their surface and are consequently destroyed.

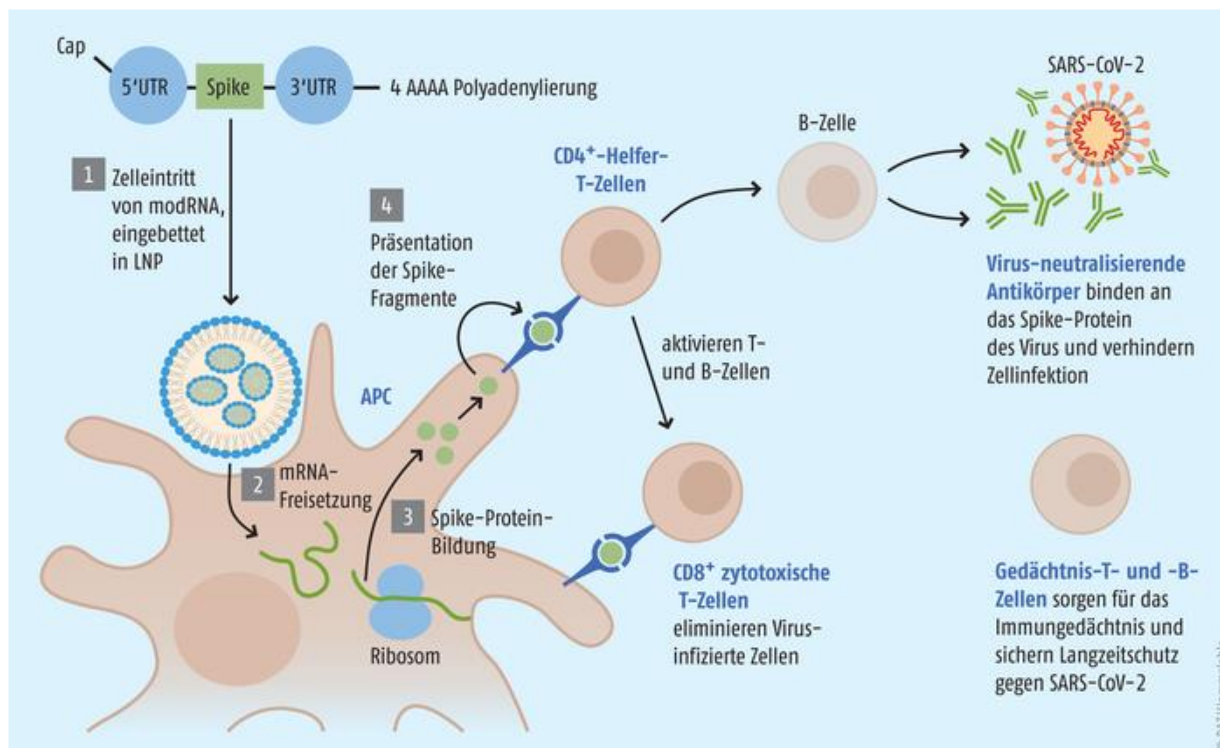


Figure 30: Theoretical effect of Comirnaty on dendritic cells . In contrast to the simplified sketch from the BioNTech website (Figure 26), an important aspect is shown here: The T cells activated by the spike formation recognize cells that express the spike protein on their surface as "virus-infected" (bottom center of the graphic) and "eliminate (i.e. destroy) them. This is an important mechanism that can promptly destroy infected cells in the event of a real infection and thus stop virus replication. With repeated genetic vaccinations, however, the cells that now form the spike protein thanks to the genetic information and show it on their surface to the immune system are also regarded as supposedly infected and "eliminated" (classic autoimmunity). And since the dendritic cells are the main targets of the LNPs, there is a considerable risk that these key cells of immunity will then be attacked and killed by their own cytotoxic T cells. (Q: Deutsche Apothekerzeitung https://www.deutsche-apotheker-zeitung.de/daz-az/2020/daz-50-2020/was-ueber-den-biontech-impfstoff-bekannt-ist)

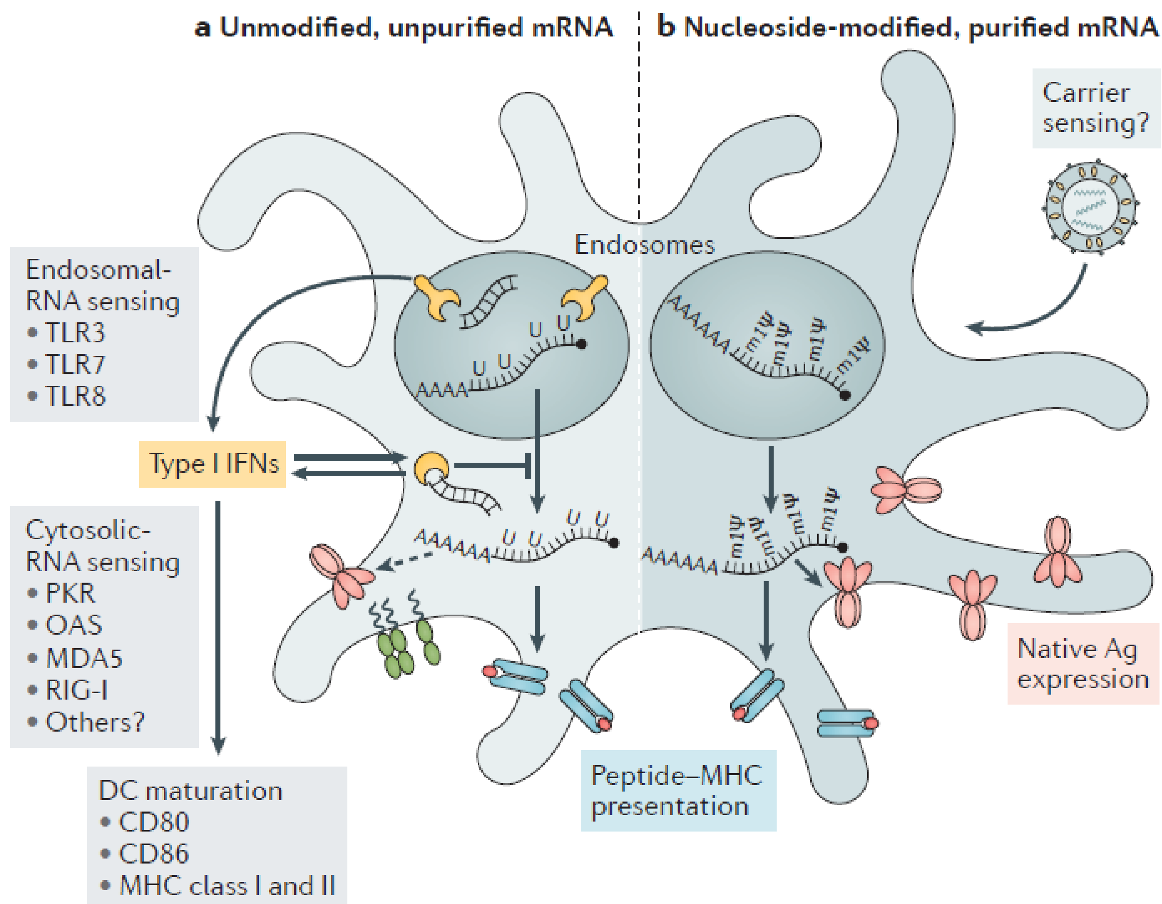


Figure 1 | **Innate immune sensing of mRNA vaccines.** Innate immune sensing of two types of mRNA vaccine by a dendritic cell (DC), with RNA sensors shown in yellow, antigen in red, DC maturation factors in green, and peptide–major histocompatibility complex (MHC) complexes in light blue and red; an example lipid nanoparticle carrier is shown at the top right. A non-exhaustive list of the major known RNA sensors that

Figure 31 of the effect of modRNA on dendritic cells: from the working group of D. Weissman (Pardi N 2018). The right-hand side (b) shows the mechanism when the RNA modified byΨ is transfected into DC. The normal mechanisms of activation, maturation and cofactor formation by the DC (left in part a) are suppressed, the important control centre cell of immunity is transformed into a passive pure antigen production site, which also presents the full (native) antigen on its surface and thus makes itself a target cell of specific cytotoxic T cells. This point, the elimination of the cell, has been added to the original figure from BioNTech (Figure 26 above) by the German Pharmacist's Journal (https://www.deutsche-apotheker-zeitung.de/daz-az/2020/daz-50-2020/was-ueber-den-biontech-impfstoff-bekannt-ist), namely that the presenting cell can also be eliminated as a supposedly virus-infected cell by the activated cytotoxic T cells (bottom centre in Figure 27).

3.3 Antibody class change as a sign of an immune problem: IgG4

Interestingly, genetic vaccination with Comirnaty appears to have a strong effect not only on the cellular immune response, but also on the antibody response. In a study by the University of Erlangen (Irrgang P 2022), it was shown that in healthy healthcare workers, several weeks to months after the second dose of Comirnaty, there was a so-called class switch in the antibodies formed from the desired, immune defence-associated classes IgG1 and 3 to an increasingly higher proportion of undesired class IgG4 immunoglobulins (in the case of vaccination). ("*In summary, our study demonstrates an mRNAvaccine-induced antiviral IgG4 antibody response appearing late after secondary immunization*."). An IgG4 antibody class is associated with a reduced inflammatory and immune response. ("*Importantly, this class switch was associated with a reduced capacity of the spike-specific antibodies to mediate antibody-dependent cellular phagocytosis and complement deposition*").

Insert for understanding: Antibodies and Comirnaty

There are different types of antibodies, each of which is produced by specialized B cells. In respiratory infections such as SARS-CoV-2, two antibody groups in particular play a role: the so-called IgA (immunoglobulin A) antibodies, which are located on the cells of the mucous membrane and can become active on "first contact" with the virus. Provided that they bind the components of the virus. This is either because you have already had an infection with this virus (e.g. the infection with the "Alpha" variant protects against "Omicron"), or with a closely related virus - such as normal cold coronaviruses. The majority of respiratory infections (i.e. all "cold and flu viruses, including coronaviruses) are already prevented on the mucous membrane if sufficient neutralizing IgA antibodies (and corresponding immune cells) are present. (Note: Since the genetic vaccination is injected into the muscle and does not act directly on the mucous membrane of the respiratory tract like the viruses, no IgA is formed on the mucous membrane cells during vaccination). Should the virus nevertheless manage to penetrate mucosal cells and multiply and even enter the bloodstream, the so-called IgG antibodies take effect in addition to the cellular immune response, which is also important here. Within the group of so-called IgG antibodies, which are predominantly soluble in the blood and represent the immune response, there are four main classes: IgG2 and IgG3 are characteristic for an active, combating immune response, IgG2 and IgG4 for a tolerogenic immune response and a reducing immunity respectively. IgG4 antibodies are mainly formed when an antigen comes into contact with the immune system permanently (such as cellular structures) or very frequently (such as from common food) in order to prevent autoimmunity or allergies. The development of class IgG4 antibodies is therefore the desired goal of anti-allergy therapies. The aim here is to trigger tolerance to the allergen through targeted repeated vaccinations with the allergy antigen, e.g. pollen from a plant ("hyposensitization"), so that an excessive inflammatory immune response no longer occurs.("*High levels of antigen-specific IgG4 have been reported to correlate with successful allergen-specific immunotherapy by blocking IgE-mediated effect*")

IgG4-based immune recognition is therefore accompanied by tolerance, which is desirable in the treatment of allergies, but is counterproductive in the case of a vaccination against a potentially dangerous pathogen such as SARS-CoV-2 and presumably increases susceptibility to the pathogen (and close relatives, such as SARS-CoV-2).SARS-CoV-2 and beta-coronaviruses) against which the IgG4

response was triggered. ("*Since Fc-mediated effector function could be critical for viral clearance, an increase in IgG4 subclasses might result in longer viral persistence in case of infection*."). Parasites such as worms or some tumours can also actively evade an immune response by promoting the formation of IgG4 antibodies in order to persist and grow in the host without triggering its immune response. This and other functions of IgG4 antibodies are summarized in a review paper (Rispens T 2023) as follows: *"IgG4 is largely unable to activate antiboldy-dependent immune effector responses [....] These properties of IgG4 have a blocking effect, either on the immune response or on the target protein of IgG4.*" This underlines the unfavourable effect of IgG4-based immunity with regard to effectively combating a pathogen such as the SARS-CoV-2 virus, i.e. an extremely counterproductive effect of Comirnaty on the desired immune response to an infection with SARS-CoV-2: although the virus is recognized, the immune system does not fight it, but due to a lack of immune activation, the virus can persist and multiply in the body for a particularly long time. The properties of IgG4 antibodies are summarized in Figure 32.

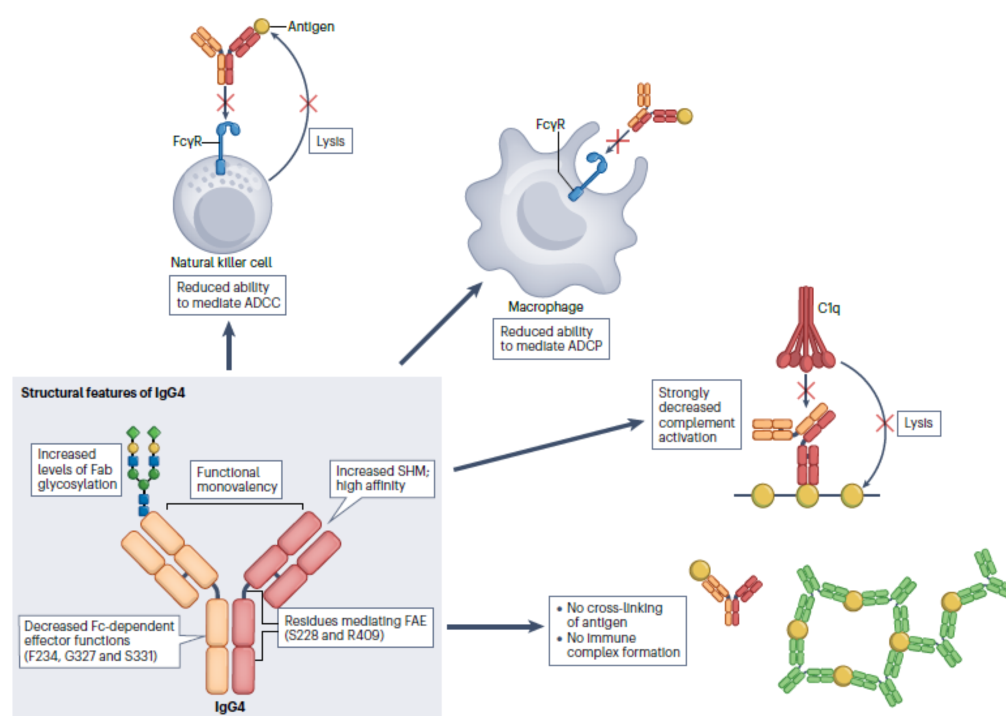


Fig. 2 | Structural and functional characteristics of IgG4. IgG4 has several unique structural features compared with other IgG subclasses, including specific biases in the IgG4 response repertoire (high affinity and increased levels of Fab (fragment antigen binding) glycosylation), functional monovalency (owing to Fab-arm exchange (FAE)) and a reduced ability to induce effector functions mediated by interactions in the Fc (fragment crystallizable) region. Important residues mediating Fab-arm exchange of IgG4 are serine at position 228 (S228) and arginine at position 409 (R409); C1q and Fc receptor binding are particularly reduced by phenylalanine at position 234 (F234), glycine at position 327 (G327) and serine at position 331 (S331) of IgG4, although residues at other positions may also contribute to the altered binding patterns of IgG4. The functional consequences of these structural features include reduced ability to mediate the Fc-dependent effector functions of antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), a poor ability to activate complement through the Fc domain and interference with immune complex formation through the inability to cross-link antigen. SHM, somatic hypermutation.

Figure 32 shows the class change of the antibodies : This class change towards anti-spike IgG4 was again significantly increased by the "boosting" with the third genetic vaccination or also by an infection with SARS-CoV-2 after a triple Comirnaty vaccination, so that in some test subjects predominantly antibodies of class IgG4 against the spike protein were found after a longer period of time. This IgG4 dominance was accompanied by a corresponding population of B cells which increasingly matured in the direction of the tolerogenic IgG4-producing B cells over the course of the study period. (*... that the switch to IgG4 is a consequence of ongoing GC maturation and that it takes several months until IgG4-switched memory B cells appear*.") (Q: Figure 2 from Rispens T 2023).

In a study from Hamburg (Kobbe R 2024), the development of an "*unusual*" IgG4 immune response against the S1 subunit of the vaccine spike protein was detected in 5-11 year old children one year after vaccination with Comirnaty. The authors explain that the effect of this antibody formation on the long-term immune status of the children is unclear ("*remains unclear how the specific subclass kinetics with delayed IgG2 and IgG4 induction by mRNA vaccination, here first described in children, affects long-term immunity*."). The authors also appear to have safety concerns regarding the genetic RNA-LNP vaccinations that can trigger this unexpected IgG4 class switch, as they emphasize that it is important to clarify the underlying mechanisms in order to assess the risk of future vaccinations with substances such as Comirnaty. ("*Understanding the role and interplay of these regulatory factors will be crucial to design safe and effective vaccines for all age groups in the future*")

In addition to this class change of the IgG antibody types to the non-protective IgG4 anti-spike antibodies, the genetic vaccinations with Comirnaty cause another unfavorable effect on antibodies: in an exciting scientific publication (Sheikh-Mohamed S 2022) it was shown that the mucosal antibodies of the IgA group are also negatively affected. There is no class change here as with IgG, but antibody formation is massively reduced after Comirnaty vaccinations.

The findings are shown in the graphic abstract.

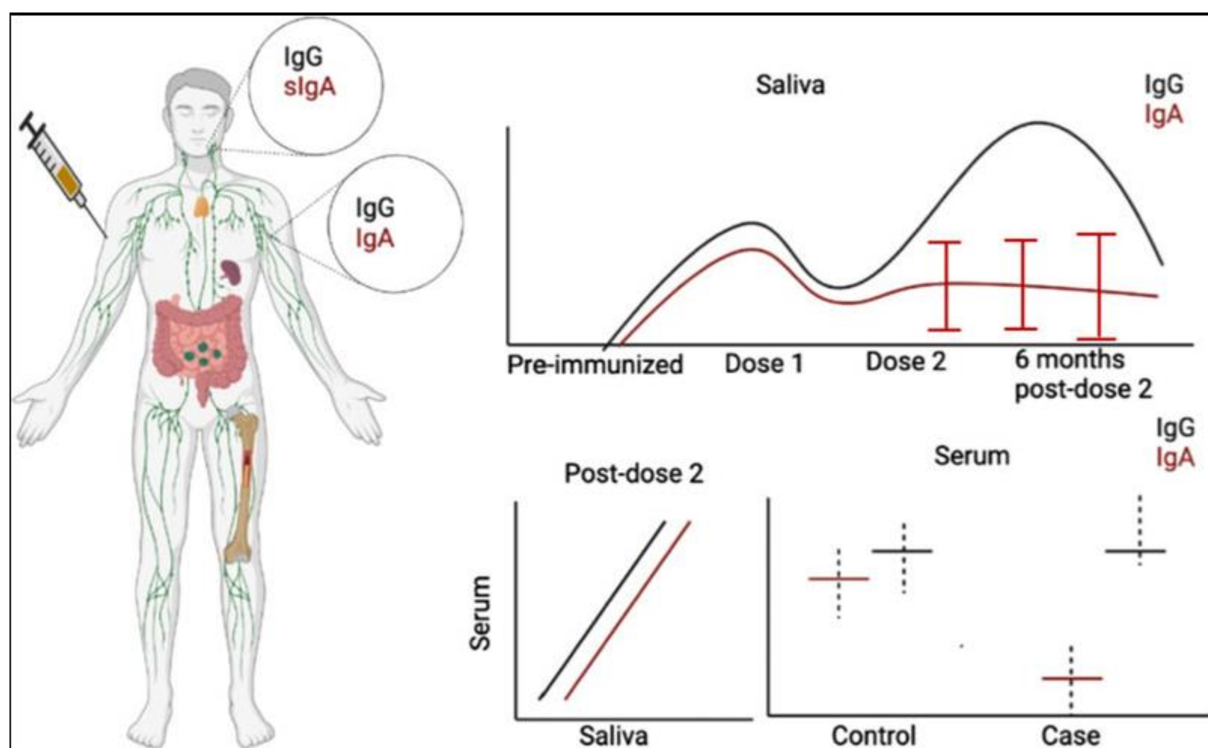


Figure 33 on the lack of mucosal immunity after vaccination. After the second dose of Comirnaty, the IgG antibodies in the blood increase (but here without specifying the class), but what is striking is the significant drop in IgA antibodies, which are supposed to intercept the pathogen on the mucous membrane and which even fall below the values of the unvaccinated control group after a single infection (recovered) due to the administration of modRNA vaccinations (Comirnaty or Spikevax) (Q: Sheikh-Mohamed S 2022)

In a Dutch study (Verheul MK 2024), in which 98% of subjects aged 18 and over were vaccinated (1-4 times), it was confirmed that protective mucosal immunity could be achieved with an infection, but not with a vaccination. ("*However, the commonly used SARS-CoV-2 vaccines in the population are administered intramuscularly and likely induce a reduced mucosal response compared with intranasal immunization or natural infection*").

4. Conclusion from points 1-3

The "ideal scheme" of Comirnaty's vaccination effect, as shown in the sketch from the Deutsche Apothekerzeitung (Figure 34) and in Verbeke R 2021, does not correspond to reality in that the various immune components are massively disrupted by the components and mechanisms of action of this genetic modRNA-LNP vaccination.

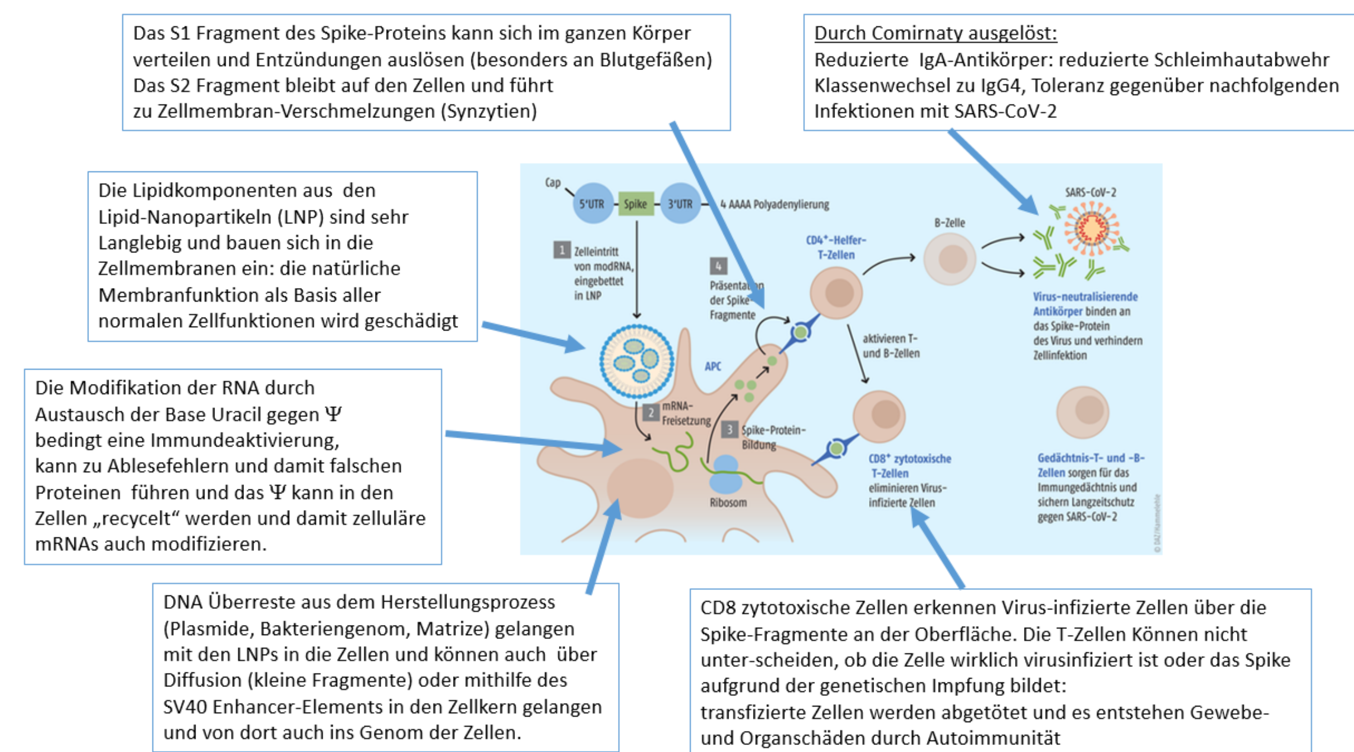


Figure 34 summarizes the most important points of the Comirnaty effect from the preliminary remarks.

Figure from the "deutsche Apothekerzeitung" supplemented with summarizing remarks

5. Bibliography

Aida V, Pliasas VC, Neasham PJ, North JF, McWhorter KL, Glover SR, Kyriakis CS. Novel Vaccine Technologies in Veterinary Medicine: A Herald to Human Medicine Vaccines. Front Vet Sci. 2021 Apr 15;8:654289. doi: 10.3389/fvets.2021.654289. PMID: 33937377; PMCID: PMC8083957.

Amanat F, Strohmeier S, Rathnasinghe R, Schotsaert M, Coughlan L, García-Sastre A, Krammer F. 2021. Introduction of two prolines and removal of the polybasic cleavage site lead to higher efficacy of a recombinant spike-based SARS-CoV-2 vaccine in the mouse model. mBio 12:e02648-20. https://doi.org/10.1128/mBio.02648-20.

Birdsall RE, Han D, DeLaney K, Kowalczyk A, Cojocaru R, Lauber M, Huray JL. Monitoring stability indicating impurities and aldehyde content in lipid nanoparticle raw material and formulated drugs. J Chromatogr B Analyt Technol Biomed Life Sci. 2024 Feb 15;1234:124005. doi: 10.1016/j.jchromb.2024.124005. Epub 2024 Jan 18. PMID: 38246008.

Chen YG, Hur S. Cellular origins of dsRNA, their recognition and consequences. Nat Rev Mol Cell Biol. 2022 Apr;23(4):286-301. doi: 10.1038/s41580-021-00430-1. Epub 2021 Nov 23. PMID: 34815573; PMCID: PMC8969093.

Cheng MH, Zhang S, Porritt RA, Noval Rivas M, Paschold L, Willscher E, Binder M, Arditi M, Bahar I. Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation. Proc Natl Acad Sci U S A. 2020 Oct 13;117(41):25254-25262. doi: 10.1073/pnas.2010722117. Epub 2020 Sep 28. PMID: 32989130; PMCID: PMC7568239.

Chiok K, Hutchison K, Miller LG, Bose S, Miura TA. Proinflammatory Responses in SARS-CoV-2 and Soluble Spike Glycoprotein S1 Subunit Activated Human Macrophages. Viruses. 2023 Mar 15;15(3):754. doi: 10.3390/v15030754. PMID: 36992463; PMCID: PMC10052676.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3):2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045. Erratum in: Euro Surveill. 2020 Apr;25(14). doi: 10.2807/1560-7917.ES.2020.25.14.20200409c. Erratum in: Euro Surveill. 2020 Jul;25(30). doi: 10.2807/1560-7917.ES.2020.25.30.2007303. Erratum in: Euro Surveill. 2021 Feb;26(5). doi: 10.2807/1560-7917.ES.2021.26.5.210204e. PMID: 31992387; PMCID: PMC6988269.

Dean DA, Strong DD, Zimmer WE. Nuclear entry of nonviral vectors. Gene Ther. 2005 Jun;12(11):881-90. doi: 10.1038/sj.gt.3302534. PMID: 15908994; PMCID: PMC4403635.

Dean DA, Dean BS, Muller S, Smith LC. Sequence requirements for plasmid nuclear import. Exp Cell Res. 1999 Dec 15;253(2):713-22. doi: 10.1006/excr.1999.4716. PMID: 10585295; PMCID: PMC4152905.

Dean DA, Byrd JN Jr, Dean BS. Nuclear targeting of plasmid DNA in human corneal cells. Curr Eye Res. 1999 Jul;19(1):66-75. doi: 10.1076/ceyr.19.1.66.5344. PMID: 10415459.

Dell'Aversana Orabona G, Abbate V, De Fazio GR, Calvanese C, Vaira L, Bonavolontà P, Romano A, Improta G, Piombino P, Califano L. Effects of serial swabs on the nasopharyngeal mucosa: our experience in SARS-CoV2 screening. J Infect Dev Ctries. 2024 Jul 29;18(7):987-992. doi: 10.3855/jidc.17957. PMID: 39078778.

Frank MG, Ball JB, Hopkins S, Kelley T, Kuzma AJ, Thompson RS, Fleshner M, Maier SF. SARS-CoV-2 S1 subunit produces a protracted priming of the neuroinflammatory, physiological, and behavioral responses to a remote immune challenge: A role for corticosteroids. Brain Behav Immun. 2024 Oct;121:87-103. doi: 10.1016/j.bbi.2024.07.034. Epub 2024 Jul 21. PMID: 39043345.

Frank MG, Nguyen KH, Ball JB, Hopkins S, Kelley T, Baratta MV, Fleshner M, Maier SF. SARS-CoV-2 spike S1 subunit induces neuroinflammatory, microglial and behavioral sickness responses: Evidence of PAMP-like properties. Brain Behav Immun. 2022 Feb;100:267-277. doi: 10.1016/j.bbi.2021.12.007. Epub 2021 Dec 13. PMID: 34915155; PMCID: PMC8667429.

Graessmann M, Menne J, Liebler M, Graeber I, Graessmann A. Helper activity for gene expression, a novel function of the SV40 enhancer. Nucleic Acids Res. 1989 Aug 25;17(16):6603-12. doi: 10.1093/nar/17.16.6603. PMID: 2550897; PMCID: PMC318353.

Gu Y, Cao J, Zhang X, Gao H, Wang Y, Wang J, He J, Jiang X, Zhang J, Shen G, Yang J, Zheng X, Hu G, Zhu Y, Du S, Zhu Y, Zhang R, Xu J, Lan F, Qu D, Xu G, Zhao Y, Gao D, Xie Y, Luo M, Lu Z. Receptome profiling identifies KREMEN1 and ASGR1 as alternative functional receptors of SARS-CoV-2. Cell Res. 2022 Jan;32(1):24-37. doi: 10.1038/s41422-021-00595-6. Epub 2021 Nov 26. Erratum in: Cell Res. 2022 Jun;32(6):600. doi: 10.1038/s41422-022-00654-6. PMID: 34837059; PMCID: PMC8617373.

Guimaraes LC, Costa PAC, Scalzo Júnior SRA, Ferreira HAS, Braga ACS, de Oliveira LC, Figueiredo MM, Shepherd S, Hamilton A, Queiroz-Junior CM, da Silva WN, da Silva NJA, Rodrigues Alves MT, Santos AK, de Faria KKS, Marim FM, Fukumasu H, Birbrair A, Teixeira-Carvalho A, de Aguiar RS, Mitchell MJ, Teixeira MM, Vasconcelos Costa V, Frezard F, Guimaraes PPG. Nanoparticle-based DNA vaccine protects against SARS-CoV-2 variants in female preclinical models. Nat Commun. 2024 Jan 18;15(1):590. doi: 10.1038/s41467-024-44830-1. PMID: 38238326; PMCID: PMC10796936.

Hertz GZ, Mertz JE. The enhancer elements and GGGCGG boxes of SV40 provide similar functions in bidirectionally promoting transcription. Virology. 1988 Apr;163(2):579-90. doi: 10.1016/0042-6822(88)90299-1. PMID: 2833024.

Hoffmann M, Kleine-Weber H, Pöhlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell. 2020 May 21;78(4):779-784.e5. doi: 10.1016/j.molcel.2020.04.022. Epub 2020 May 1. PMID: 32362314; PMCID: PMC7194065.

Inglut CT, Sorrin AJ, Kuruppu T, Vig S, Cicalo J, Ahmad H, Huang HC. Immunological and Toxicological Considerations for the Design of Liposomes. Nanomaterials (Basel). 2020 Jan 22;10(2):190. doi: 10.3390/nano10020190. PMID: 31978968; PMCID: PMC7074910.

Irrgang P, Gerling J, Kocher K, Lapuente D, Steininger P, Habenicht K, Wytopil M, Beileke S, Schäfer S, Zhong J, Ssebyatika G, Krey T, Falcone V, Schülein C, Peter AS, Nganou-Makamdop K, Hengel H, Held J, Bogdan C, Überla K, Schober K, Winkler TH, Tenbusch M. Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination. Sci Immunol. 2023 Jan 27;8(79):eade2798. doi: 10.1126/sciimmunol.ade2798. Epub 2023 Jan 27. PMID: 36548397; PMCID: PMC9847566.

Kämmerer U, Schulz V, Steger K. BioNTech RNA-Based COVID-19 Injections Contain Large Amounts Of Residual DNA Including A SV40 Promoter/Enhancer Sequence. Science, Public Health Policy and the Law. 2024 Dec 02; v5.2019-2024

Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005 Aug;23(2):165-75. doi: 10.1016/j.immuni.2005.06.008. PMID: 16111635.

Kent SJ, Li S, Amarasena TH, Reynaldi A, Lee WS, Leeming MG, O'Connor DH, Nguyen J, Kent HE, Caruso F, Juno JA, Wheatley AK, Davenport MP, Ju Y. Blood Distribution of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans. ACS Nano. 2024 Oct 1;18(39):27077-27089. doi: 10.1021/acsnano.4c11652. Epub 2024 Sep 19. PMID: 39298422; PMCID: PMC11447892.

Klein RS. Mechanisms of coronavirus infectious disease 2019-related neurologic diseases. Curr Opin Neurol. 2022 Jun 1;35(3):392-398. doi: 10.1097/WCO.0000000000001049. Epub 2022 Mar 11. PMID: 35283461; PMCID: PMC9186403.

Kobbe R, Rau C, Schulze-Sturm U, Stahl F, Fonseca-Brito L, Diemert A, Lütgehetmann M, Addo MM, Arck P, Weskamm LM. Delayed Induction of Noninflammatory SARS-CoV-2 Spike-Specific IgG4 Antibodies Detected 1 Year After BNT162b2 Vaccination in Children. Pediatr Infect Dis J. 2024 Jul 30;43(12):1200-3. doi: 10.1097/INF.0000000000004488. Epub ahead of print. PMID: 39078156; PMCID: PMC11542969.

König B, Kirchner JO. Methodological Considerations Regarding the Quantification of DNA Impurities in the COVID-19 mRNA Vaccine Comirnaty® . Methods Protoc. 2024 May 8;7(3):41. doi: 10.3390/mps7030041. PMID: 38804335; PMCID: PMC11130870.

Koenig PA, Schmidt FI. Spike D614G - A Candidate Vaccine Antigen Against Covid-19. N Engl J Med. 2021 Jun 17;384(24):2349-2351. doi: 10.1056/NEJMcibr2106054. PMID: 34133867.

Korosec CS, Farhang-Sardroodi S, Dick DW, Gholami S, Ghaemi MS, Moyles IR, Craig M, Ooi HK, Heffernan JM. Long-term durability of immune responses to the BNT162b2 and mRNA-1273 vaccines based on dosage, age and sex. Sci Rep. 2022 Dec 8;12(1):21232. doi: 10.1038/s41598-022-25134-0. PMID: 36481777; PMCID: PMC9732004.

Lewis LM, Badkar AV, Cirelli D, Combs R, Lerch TF. The Race to Develop the Pfizer-BioNTech COVID-19 Vaccine: From the Pharmaceutical Scientists' Perspective. J Pharm Sci. 2023 Mar;112(3):640-647. doi: 10.1016/j.xphs.2022.09.014. Epub 2022 Sep 18. PMID: 36130677; PMCID: PMC9482796.

Li HS, Liu Y, Li DF, Zhang RR, Tang HL, Zhang YW, Huang W, Liu Y, Peng H, Xu JQ, Hong KX, Shao YM. Enhancement of DNA vaccine-induced immune responses by a 72-bp element from SV40 enhancer. Chin Med J (Engl). 2007 Mar 20;120(6):496-502. PMID: **17439744.**

Mörz M. A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 mRNA Vaccination against COVID-19. Vaccines (Basel). 2022 Oct 1;10(10):1651. doi: 10.3390/vaccines10101651. PMID: 36298516; PMCID: PMC9611676.

Norman M, Magnus MC, Söderling J, Juliusson PB, Navér L, Örtqvist AK, Håberg S, Stephansson O. Neonatal Outcomes After COVID-19 Vaccination in Pregnancy. JAMA. 2024 Feb 6;331(5):396-407. doi: 10.1001/jama.2023.26945. PMID: 38319332; PMCID: PMC10848052.

Nuovo GJ, Magro C, Shaffer T, Awad H, Suster D, Mikhail S, He B, Michaille JJ, Liechty B, Tili E. Endothelial cell damage is the central part of COVID-19 and a mouse model induced by injection of the S1 subunit of the spike protein. Ann Diagn Pathol. 2021 Apr;51:151682. doi: 10.1016/j.anndiagpath.2020.151682. Epub 2020 Dec 24. PMID: 33360731; PMCID: PMC7758180.

Oldfield PR, Gutchi LM, McCullough PA, Speicher DJ. Pfizer/BioNTech's COVID-19 modRNA Vaccines: Dangerous genetic mechanism of action released before sufficient preclinical testing. J. Amercian Physicians and Surgeons 2024 Winter 29(4):118-126

Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018 Apr;17(4):261-279. doi: 10.1038/nrd.2017.243. Epub 2018 Jan 12. PMID: 29326426; PMCID: PMC5906799.

Patterson BK, Francisco EB, Yogendra R, Long E, Pise A, Rodrigues H, Hall E, Herrera M, Parikh P, Guevara-Coto J, Triche TJ, Scott P, Hekmati S, Maglinte D, Chang X, Mora-Rodríguez RA, Mora J. Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection. Front Immunol. 2022 Jan 10;12:746021. doi: 10.3389/fimmu.2021.746021. PMID: 35082777; PMCID: PMC8784688.

Rispens T, Huijbers MG. The unique properties of IgG4 and its roles in health and disease. Nat Rev Immunol. 2023 Nov;23(11):763-778. doi: 10.1038/s41577-023-00871-z. Epub 2023 Apr 24. PMID: 37095254; PMCID: PMC10123589.

Rosa SS, Prazeres DMF, Azevedo AM, Marques MPC. mRNA vaccines manufacturing: Challenges and bottlenecks. Vaccine. 2021 Apr 15;39(16):2190-2200. doi: 10.1016/j.vaccine.2021.03.038. Epub 2021 Mar 24. PMID: 33771389; PMCID: PMC7987532.

Rotoli BM, Barilli A, Visigalli R, Ferrari F, Dall'Asta V. Endothelial Cell Activation by SARS-CoV-2 Spike S1 Protein: A Crosstalk between Endothelium and Innate Immune Cells. Biomedicines. 2021 Sep 14;9(9):1220. doi: 10.3390/biomedicines9091220. PMID: 34572407; PMCID: PMC8470710.

Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics--developing a new class of drugs. Nat Rev Drug Discov. 2014 Oct;13(10):759-80. doi: 10.1038/nrd4278. Epub 2014 Sep 19. PMID: 25233993.

Segalla G Chemical-physical criticality and toxicological potential of lipid nanomaterials contained in a COVID-19 mRNA vaccine. (2023). International Journal of Vaccine Theory, Practice, and Research , 3(1), 787-817. https://doi.org/10.56098/ijvtpr.v3i1.68

Segreto R, Deigin Y, McCairn K, Sousa A, Sirotkin D, Sirotkin K, Couey JJ, Jones A, Zhang D. Should we discount the laboratory origin of COVID-19? Environ Chem Lett. 2021;19(4):2743-2757. doi: 10.1007/s10311-021-01211-0. Epub 2021 Mar 25. PMID: 33786037; PMCID: PMC7993900.

Šenigl F, Soikkeli A, Prost S, Schatz DG, Slavková M, Hejnar J, Alinikula J. The SV40 virus enhancer functions as a somatic hypermutation-targeting element with potential tumorigenic activity. Tumor Virus Res. 2024 Oct 25;18:200293. doi: 10.1016/j.tvr.2024.200293. Epub ahead of print. PMID: 39490533; PMCID: PMC11564006.

Sheikh-Mohamed S, Isho B, Chao GYC, Zuo M, Cohen C, Lustig Y, Nahass GR, Salomon-Shulman RE, Blacker G, Fazel-Zarandi M, Rathod B, Colwill K, Jamal A, Li Z, de Launay KQ, Takaoka A, Garnham-Takaoka J, Patel A, Fahim C, Paterson A, Li AX, Haq N, Barati S, Gilbert L, Green K, Mozafarihashjin M, Samaan P, Budylowski P, Siqueira WL, Mubareka S, Ostrowski M, Rini JM, Rojas OL, Weissman IL, Tal MC, McGeer A, Regev-Yochay G, Straus S, Gingras AC, Gommerman JL. Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. Mucosal Immunol. 2022 May;15(5):799-808. doi: 10.1038/s41385-022-00511-0. Epub 2022 Apr 25. PMID: 35468942; PMCID: PMC9037584.

Shirato K, Kizaki T. SARS-CoV-2 spike protein S1 subunit induces pro-inflammatory responses via toll-like receptor 4 signaling in murine and human macrophages. Heliyon. 2021 Feb 2;7(2):e06187. doi: 10.1016/j.heliyon.2021.e06187. PMID: 33644468; PMCID: PMC7887388.

Shrestha NK, Burke PC, Nowacki AS, Simon JF, Hagen A, Gordon SM. Effectiveness of the Coronavirus Disease 2019 Bivalent Vaccine. Open Forum Infect Dis. 2023 Apr 19;10(6):ofad209. doi: 10.1093/ofid/ofad209. PMID: 37274183; PMCID: PMC10234376.

Speicher DJ, Preprint (OSF Preprints | DNA fragments detected in monovalent and bivalent Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines from Ontario, Canada: Exploratory dose response relationship with serious adverse events).

Suprewicz Ł, Fiedoruk K, Czarnowska A, Sadowski M, Strzelecka A, Galie PA, Janmey PA, Kułakowska A, Bucki R. Blood-brain barrier function in response to SARS-CoV-2 and its spike protein. Neurol Neurochir Pol. 2023;57(1):14-25. doi: 10.5603/PJNNS.a2023.0014. Epub 2023 Feb 22. PMID: 36810757.

Theoharides TC. Could SARS-CoV-2 Spike Protein Be Responsible for Long-COVID Syndrome? Mol Neurobiol. 2022 Mar;59(3):1850-1861. doi: 10.1007/s12035-021-02696-0. Epub 2022 Jan 13. PMID: 35028901; PMCID: PMC8757925.

Theuerkauf SA, Michels A, Riechert V, Maier TJ, Flory E, Cichutek K, Buchholz CJ. Quantitative assays reveal cell fusion at minimal levels of SARS-CoV-2 spike protein and fusion from without. iScience. 2021 Mar 19;24(3):102170. doi: 10.1016/j.isci.2021.102170. Epub 2021 Feb 9. PMID: 33585805; PMCID: PMC7871100.

Thorn CR, Sharma D, Combs R, Bhujbal S, Romine J, Zheng X, Sunasara K, Badkar A. The journey of a lifetime - development of Pfizer's COVID-19 vaccine. Curr Opin Biotechnol. 2022 Dec;78:102803. doi: 10.1016/j.copbio.2022.102803. Epub 2022 Sep 1. PMID: 36162187; PMCID: PMC9433349.

Verbeke R, Lentacker I, De Smedt SC, Dewitte H. The dawn of mRNA vaccines: The COVID-19 case. J Control Release. 2021 May 10;333:511-520. doi: 10.1016/j.jconrel.2021.03.043. Epub 2021 Mar 30. PMID: 33798667; PMCID: PMC8008785.

Verheul MK, Kaczorowska J, Hofstee MI, Schepp RM, Smits GP, Wessels Beljaars D, Kuijer M, Schuin W, Middelhof I, Wong D, van Hagen CCE, Vos ERA, Nicolaie MA, de Melker HE, van Binnendijk RS, van der Klis FRM, den Hartog G. Protective mucosal SARS-CoV-2 antibodies in the majority of the general population in the Netherlands. Mucosal Immunol. 2024 Aug;17(4):554-564. doi: 10.1016/j.mucimm.2024.03.008. Epub 2024 Mar 27. PMID: 38553008.

Wang C, Wang S, Ma X, Yao X, Zhan K, Wang Z, He D, Zuo W, Han S, Zhao G, Cao B, Zhao J, Bian X, Wang J. P-selectin Facilitates SARS-CoV-2 Spike 1 Subunit Attachment to Vesicular Endothelium and Platelets. ACS Infect Dis. 2024 Aug 9;10(8):2656-2667. doi: 10.1021/acsinfecdis.3c00728. Epub 2024 Jun 24. PMID: 38912949.

Yamamoto K. Adverse effects of COVID-19 vaccines and measures to prevent them. Virol J. 2022 Jun 5;19(1):100. doi: 10.1186/s12985-022-01831-0. PMID: 35659687; PMCID: PMC9167431.

Young JL, Benoit JN, Dean DA. Effect of a DNA nuclear targeting sequence on gene transfer and expression of plasmids in the intact vasculature. Gene Ther. 2003 Aug;10(17):1465-70. doi: 10.1038/sj.gt.3302021. PMID: 12900761; PMCID: PMC4150867.

Xia X. Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines. Vaccines (Basel). 2021 Jul 3;9(7):734. doi: 10.3390/vaccines9070734. PMID: 34358150; PMCID: PMC8310186.

Zhang Z, Zheng Y, Niu Z, Zhang B, Wang C, Yao X, Peng H, Franca DN, Wang Y, Zhu Y, Su Y, Tang M, Jiang X, Ren H, He M, Wang Y, Gao L, Zhao P, Shi H, Chen Z, Wang X, Piacentini M, Bian X, Melino G, Liu L, Huang H, Sun Q. SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination. Cell Death Differ. 2021 Sep;28(9):2765-2777. doi: 10.1038/s41418-021-00782-3. Epub 2021 Apr 20. PMID: 33879858; PMCID: PMC8056997.