Covid-19 Vaccines: The Different Technologies Used

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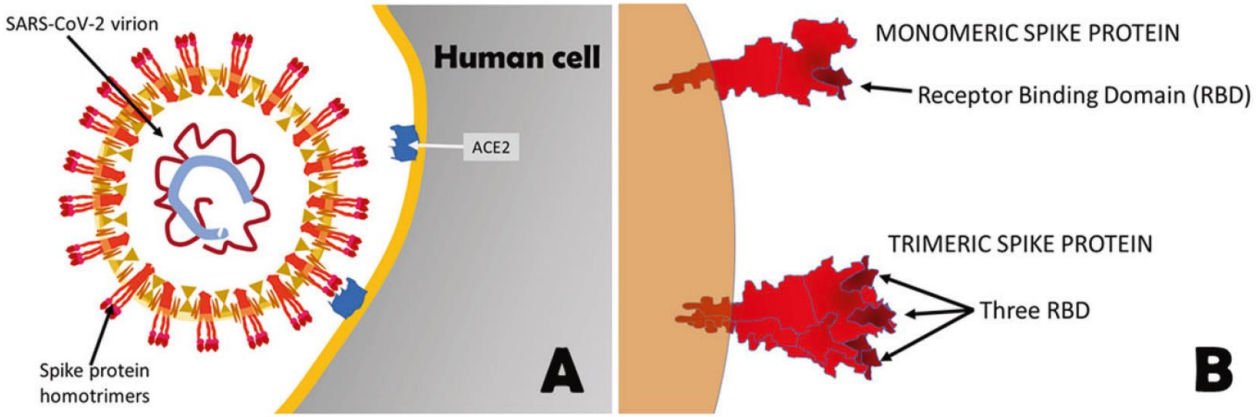
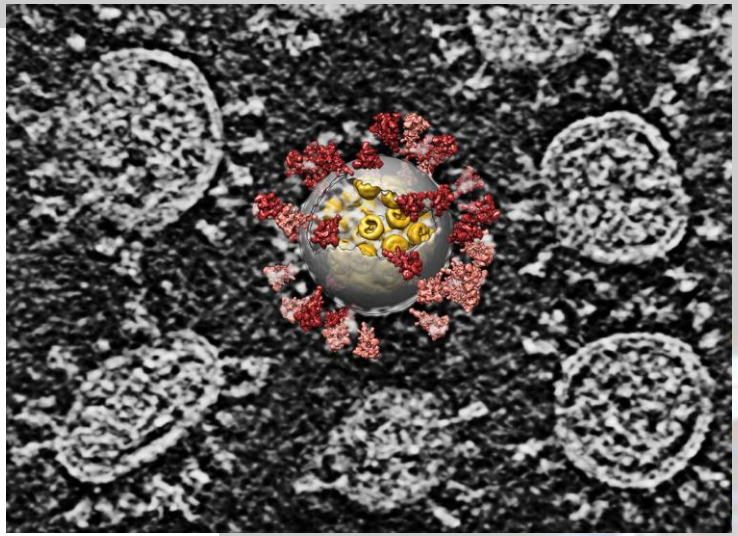
How the virus work:



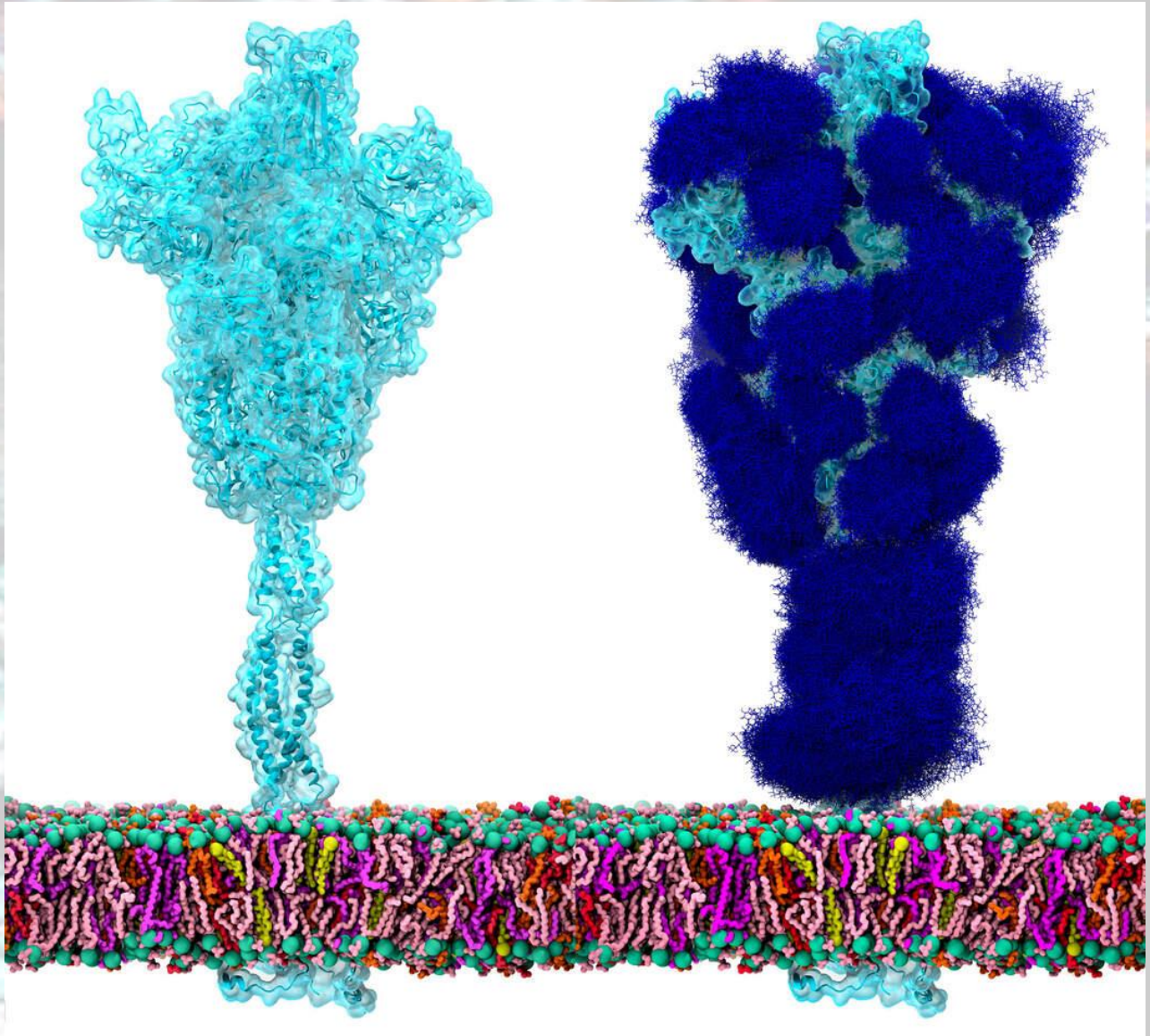


TEM image of covid-19

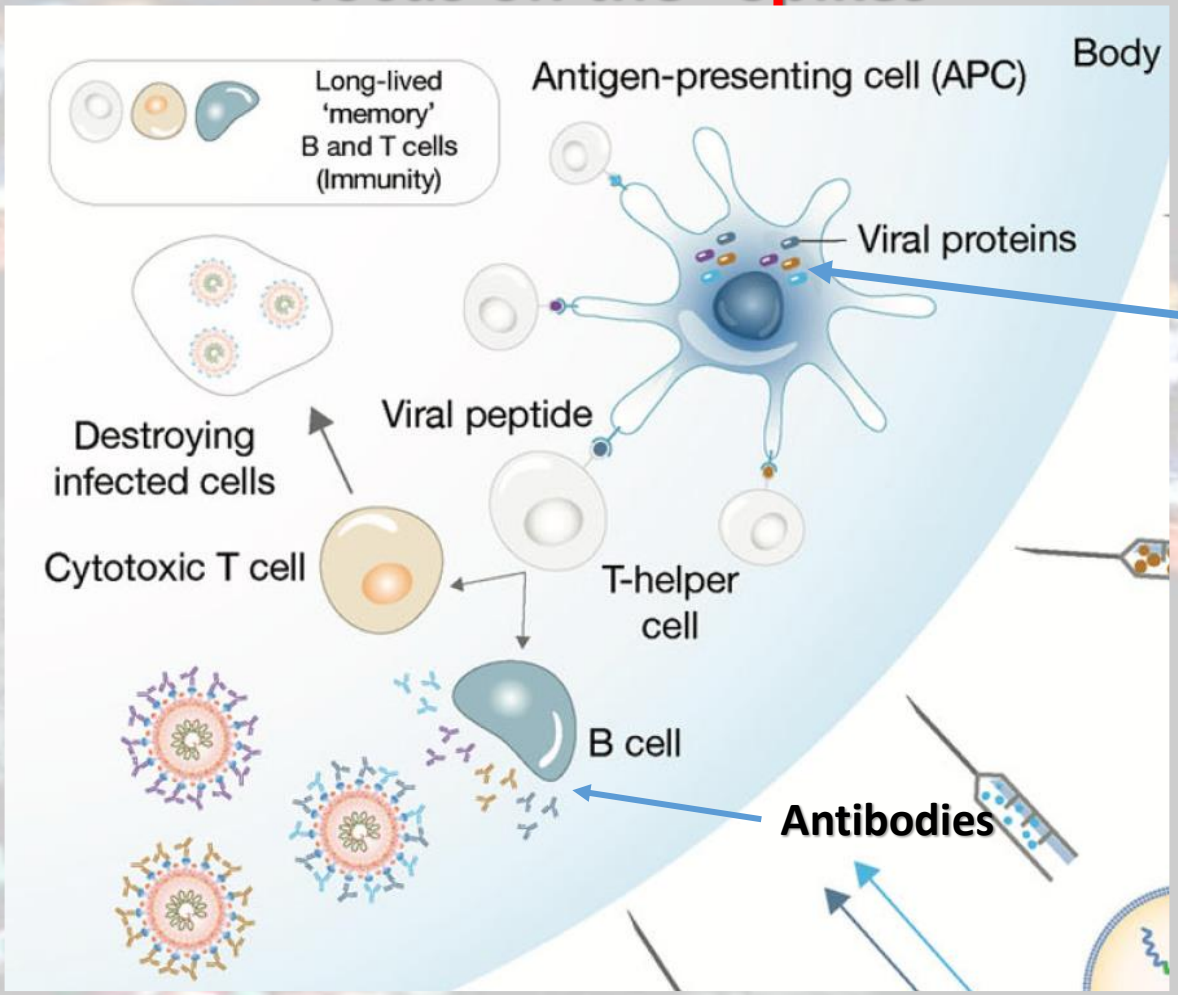
Fig. 2 Spike as a target for vaccine development. Within a few months after the identification of the new SARS-CoV-2, the freely available data made by numerous laboratories around the world provided a defined picture of the virus structure and of the steps of human cell infection. **A** SARS-CoV-2 is an oily spherical particle containing a single-stranded positive-sense RNA of about 30 kb wrapped and coiled by the Nucleocapsid protein. The virus outer shell consists of three other structural glycoproteins: Spike, Envelope, and Membrane, and a lipid coating. On the surface of SARS-CoV-2, three Spike glycoproteins aggregate protrudes outside the pericapsid and may interact at a high affinity with Angiotensin-Converting Enzyme 2 (ACE2), an exopeptidase normally present on the outer surface of a wide variety of human cells. **B** The Spike protein consists of two domains, S1 and S2. In the most external domain, a region known as Receptor-Binding Domain (RBD), allows the high-affinity binding of the SARS-CoV-2 to the N-terminal domain of the ACE2. The progressive elucidation of the critical role of this interaction provided the key insights that spurred several developers of innovative vaccine to target the Spike protein and its RBD [2, 66]. The recent reports on the protective efficacy of vaccines based on different platforms targeting the Spike protein [6–9] suggest that the freely available basic science data allowed to make a winning bet [10].

ACE2 receptors are mainly in human lungs

3D reconstruction of the spike



General Strategy of Vaccines: The immune responses induced by vaccines focus on the "Spikes"



Viral proteins: the "spikes"

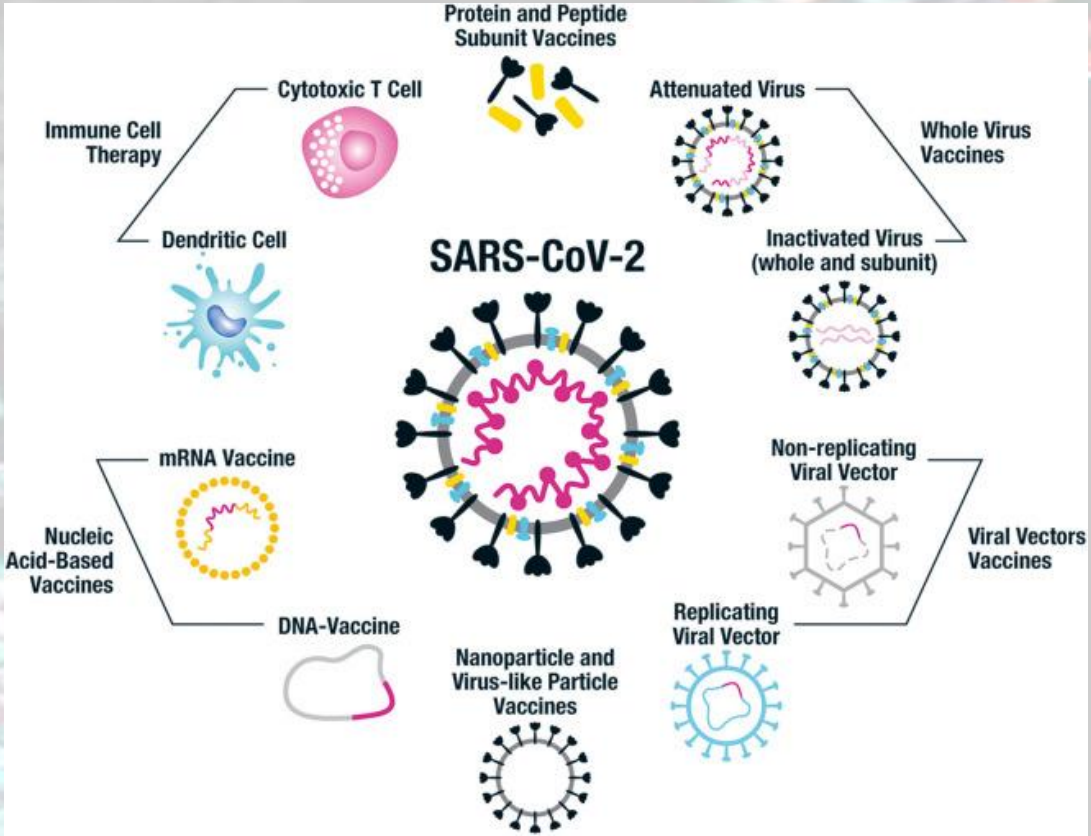
The immune responses induced by vaccines. Antigen-presenting cells (APCs) can process vaccine antigen and present it to CD8+ T cells and CD4+ T cells. The activated B cells can produce NAbs.

A WAY HAD TO BE FOUND TO DELIVER THE PROTEINS THAT CONSIST THE SPIKES WITHOUT THE VIRUS

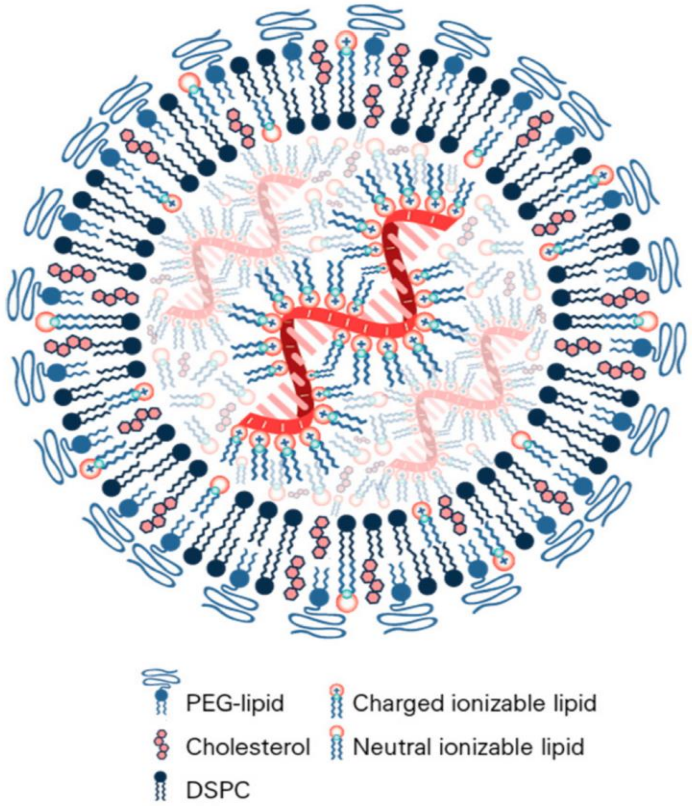
Nikolaos C. Kyriakidis, Andrés López-Cortés, Eduardo Vásconez González, Alejandra Barreto Grimaldos and Esteban Ortiz Prado, npj Vaccines (2021) 28

Technologies used for current vaccines:

1. **Nucleic acid vaccines (mRNA),**
2. **Non-replicating Adenovirus-based vector vaccines**
3. **Inactivated and attenuated virus vaccines,**
4. **Recombinant subunits vaccines**



1. Nucleic acid vaccines (mRNA)

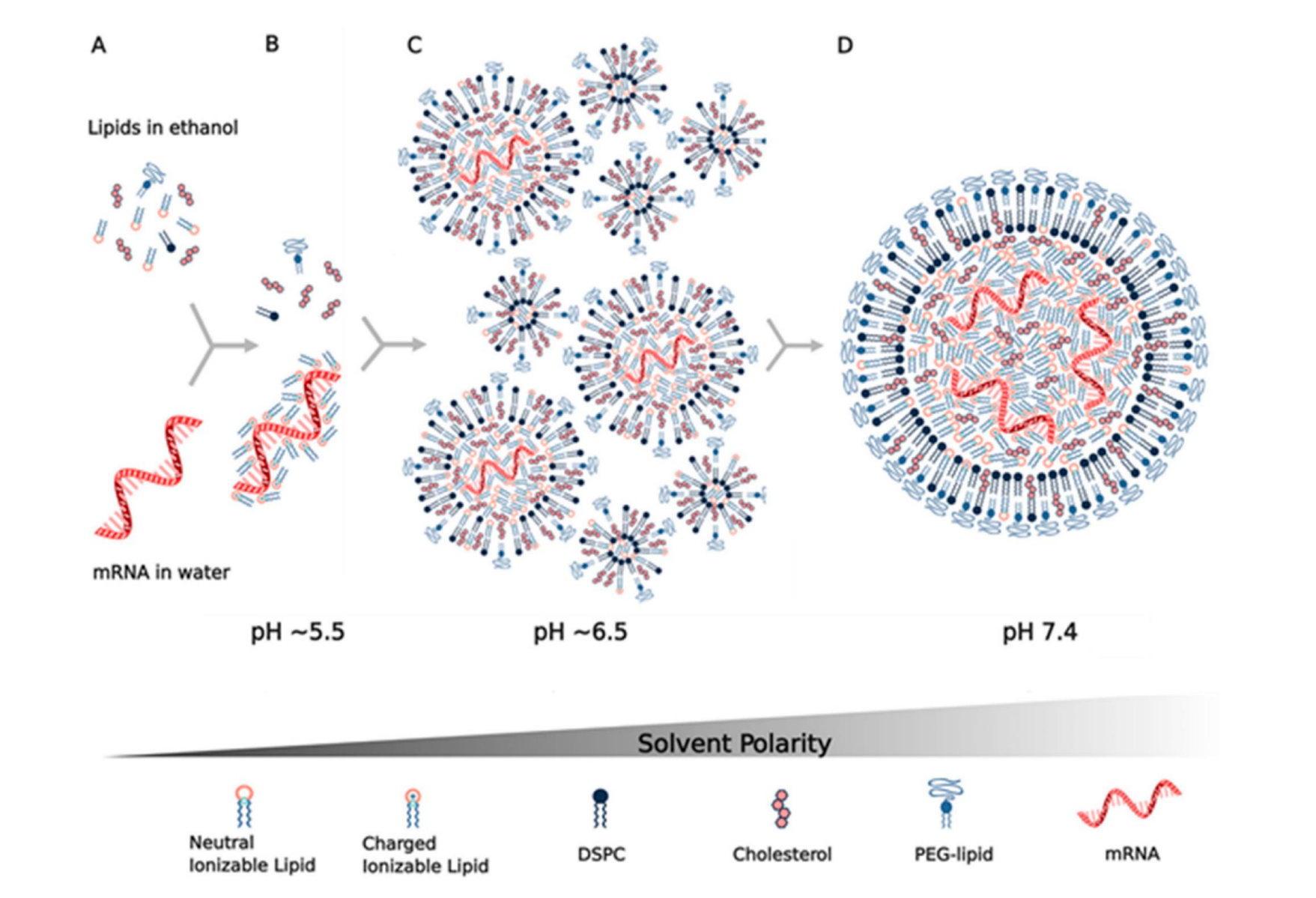


Electrostatic interactions arises between positively charged lipids and negatively charged mRNA from the phosphoric groups

Figure 1. mRNA lipid nanoparticle structure. Recent studies using cryoelectron microscopy [96], small-angle neutron scattering and small-angle X-ray scattering [89] have shown that the mRNA Lipid nanoparticle includes low copy numbers of mRNA (1–10) and that the mRNA is bound by the ionizable lipid that occupies the central core of the LNP. The polyethylene glycol (PEG) lipid forms the surface of the lipid nanoparticle (LNP), along with DSPC, which is bilayer forming. Cholesterol and the ionizable lipid in charged and uncharged forms can be distributed throughout the LNP. Structural schematics of other delivery systems are available in a recent review [14].

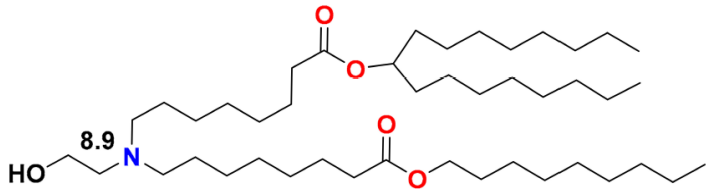
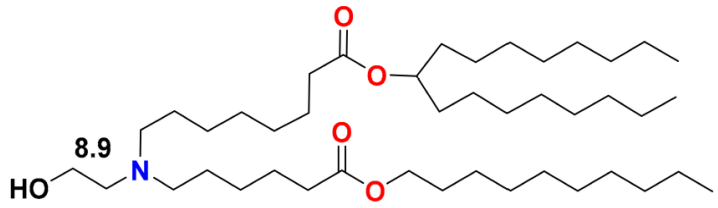
Buschmann, M.D.; Carrasco, M.J.; Alishetty, S.; Paige, M.; Alameh, M.G.;Weissman, D. Nanomaterial Delivery Systems for mRNA Vaccines. Vaccines 2021, 9, 65. https://doi.org/10.3390/vaccines 9010065

Formation of the final NP at neutral pH



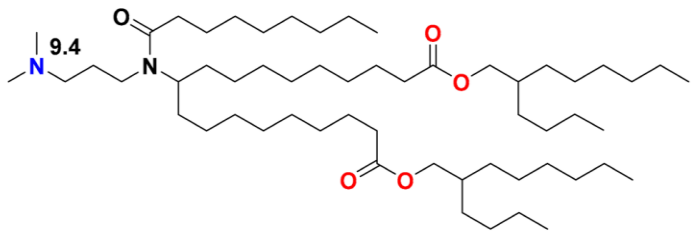
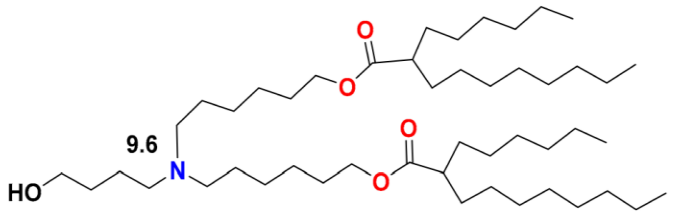
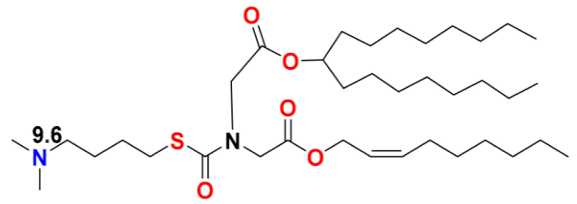
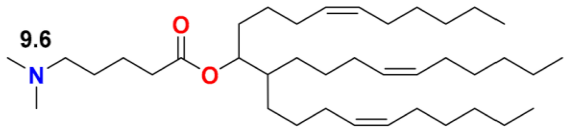
Buschmann, M.D.; Carrasco, M.J.; Alishetty, S.; Paige, M.; Alameh, M.G.;Weissman, D. Nanomaterial Delivery Systems for mRNA Vaccines. Vaccines 2021, 9, 65. https://doi.org/10.3390/vaccines 9010065

Ionizable lipids of Moderna:

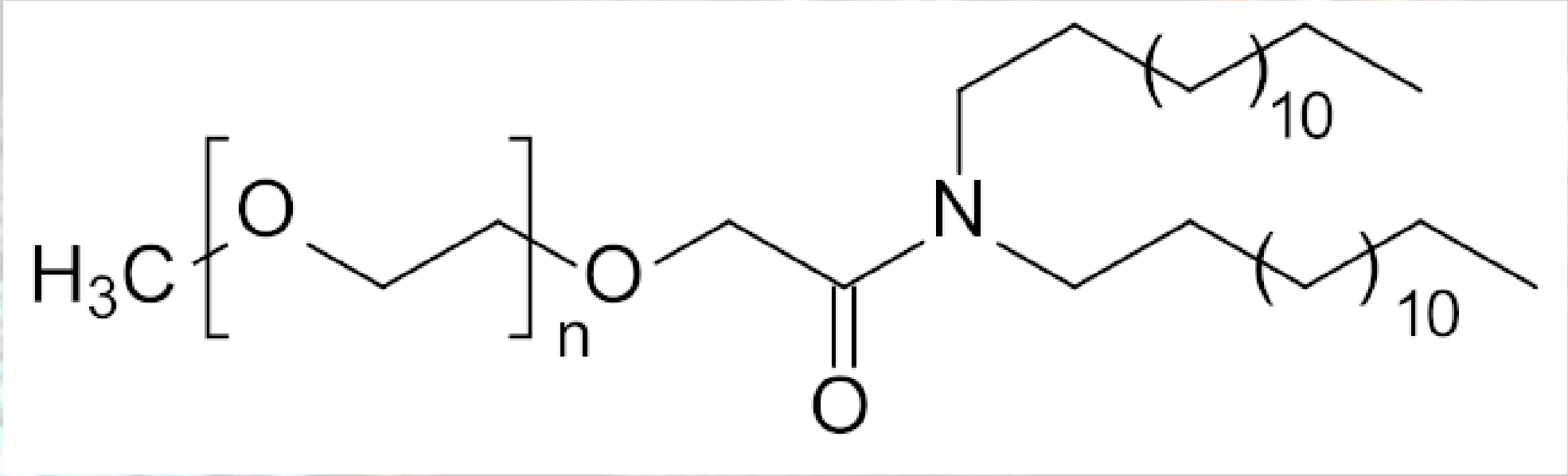
Moderna Lipid 5 [101]		TNS pKa	6.56
Moderna Lipid H, SM-102 [42]			6.75

A key feature of the ionizable lipids used in lipid nanoparticles is that the pKa of the ionizable lipid in the LNP, as measured by the TNS dye-binding assay, should be in the range of 6–7. The theoretically calculated pKa of most of the ionizable groups is in the range of 8–9.5, as shown below on the nitrogen atoms, using commercial software that theoretically estimates these values in aqueous media. The 2–3 point drop in pKa from the theoretical value to the TNS value is due to the much higher energy of solvation of protons in the lipid phase, creating a pH increase of 2–3 points in the lipid compared to the aqueous phase, where pH is measured during the TNS assay.

Ionizable lipids of Pfizer-BioNTech

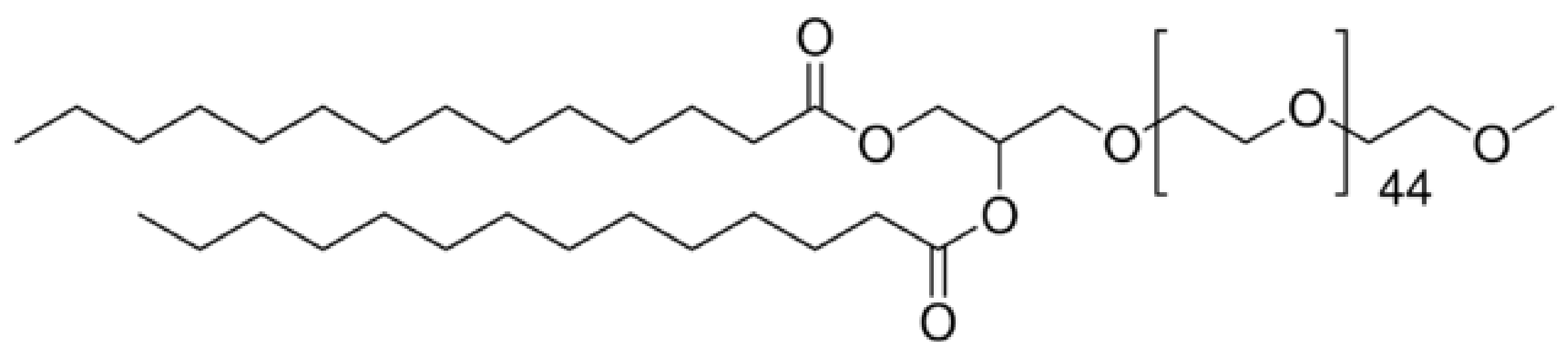
Name	Ionizable Lipid Structure and Theoretical pKas	TNS pKa
Acuitas A9 [59]		6.27
Acuitas ALC-0315 [47]		6.09
Arcturus Lipid 2,2 (8,8) 4C CH3 [57]		6.69
Genevant CL1[61]		NA

PEG Lipid of Pfizer-BioNTech



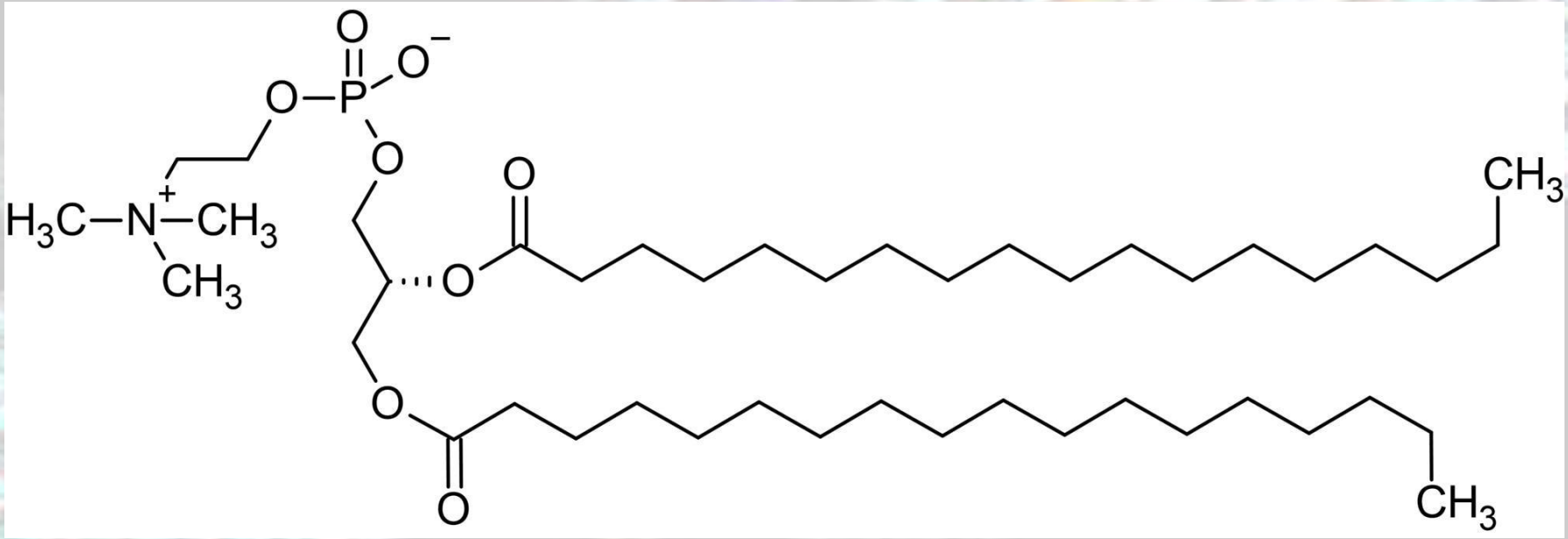
2[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide

PEG Lipid of Moderna



1 monomethoxypolyethyleneglycol-2,3-dimyristylglycerol

DSPC



In conclusion, mRNA lipid nanoparticle assembly is achieved by:

(A) rapid mixing in a microfluidic or T-junction mixer of four lipids (ionizable lipid, DSPC, cholesterol, PEG–lipid) in ethanol with mRNA in an aqueous buffer near pH=4.

(B) When the ionizable lipid meets the aqueous phase, it becomes protonated at a pH ~5.5, which is intermediate between the pKa of the buffer and that of the ionizable lipid.

(C) The ionizable lipid then electrostatically binds the anionic phosphate backbone of the mRNA while it experiences hydrophobicity in the aqueous phase, driving vesicle formation and mRNA encapsulation.

(D) After initial vesicle formation, the pH is raised by dilution, dialysis or filtration, which results in the neutralization of the ionizable lipid, rendering it more hydrophobic and thereby driving vesicles to fuse and causing the further sequestration of the ionizable lipid with mRNA into the interior of the solid lipid nanoparticles. The PEG–lipid content stops the fusion process by providing the LNP with a hydrophilic exterior, determining its thermodynamically stable size, and the bilayer forming DSPC is present just underneath this PEG–lipid layer.

Ingredients of the mRNA vaccines:

The Pfizer-BioNTech Vaccine exact composition: Each 0.3 mL dose of the Pfizer-BioNTech COVID-19 Vaccine contains 30 mcg of a nucleosidemodified messenger RNA (modRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2. Each dose of the Pfizer-BioNTech COVID-19 Vaccine also includes the following ingredients: lipids (0.43 mg (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 0.05 mg 2[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 0.09 mg 1,2-distearoyl-sn-glycero-3- phosphocholine, and 0.2 mg cholesterol), 0.01 mg potassium chloride, 0.01 mg monobasic potassium phosphate, 0.36 mg sodium chloride, 0.07 mg dibasic sodium phosphate dihydrate, and 6 mg sucrose. The diluent (0.9% Sodium Chloride Injection) contributes an additional 2.16 mg sodium chloride per dose. The dosing regimen is two doses of 0.3 mL each, 3 weeks apart.

The Moderna lipid nanoparticle exact composition Vaccine: The mRNA-1273 IP is an lipid nanoparticle (LNP) dispersion of an mRNA encoding the prefusion stabilized S protein of SARS-CoV-2 formulated in LNPs composed of 4 lipids (1 proprietary and 3 commercially available): the proprietary ionizable lipid SM-102; cholesterol; 1,2-distearoyl-snglycero-3 phosphocholine (DSPC); and 1 monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000- DMG). The mRNA-1273 is provided as a sterile liquid for injection and is a white to off- white dispersion in appearance, at a concentration of 0.2 mg/mL in 20 mM Tris buffer containing 87 mg/mL sucrose and 10.7 mM sodium acetate at pH 7.5.

How it works:

- **The vaccine is based on an mRNA molecule that contains the information for the synthesis of the stabilized prefusion form of the SARS-CoV-2 Spike (S) protein encapsulated in a lipid nanoparticle (LNP) vector that enhances uptake by host immune cells.**

- **The administered mRNA uses the host cell transcription and translation machinery (ribosomes) to produce the viral antigen that is afterward presented in T lymphocytes and is also directly recognized by B lymphocytes of the host, thereby initiating an adaptive immune response directed against the S protein of the virus.**

Limitations:

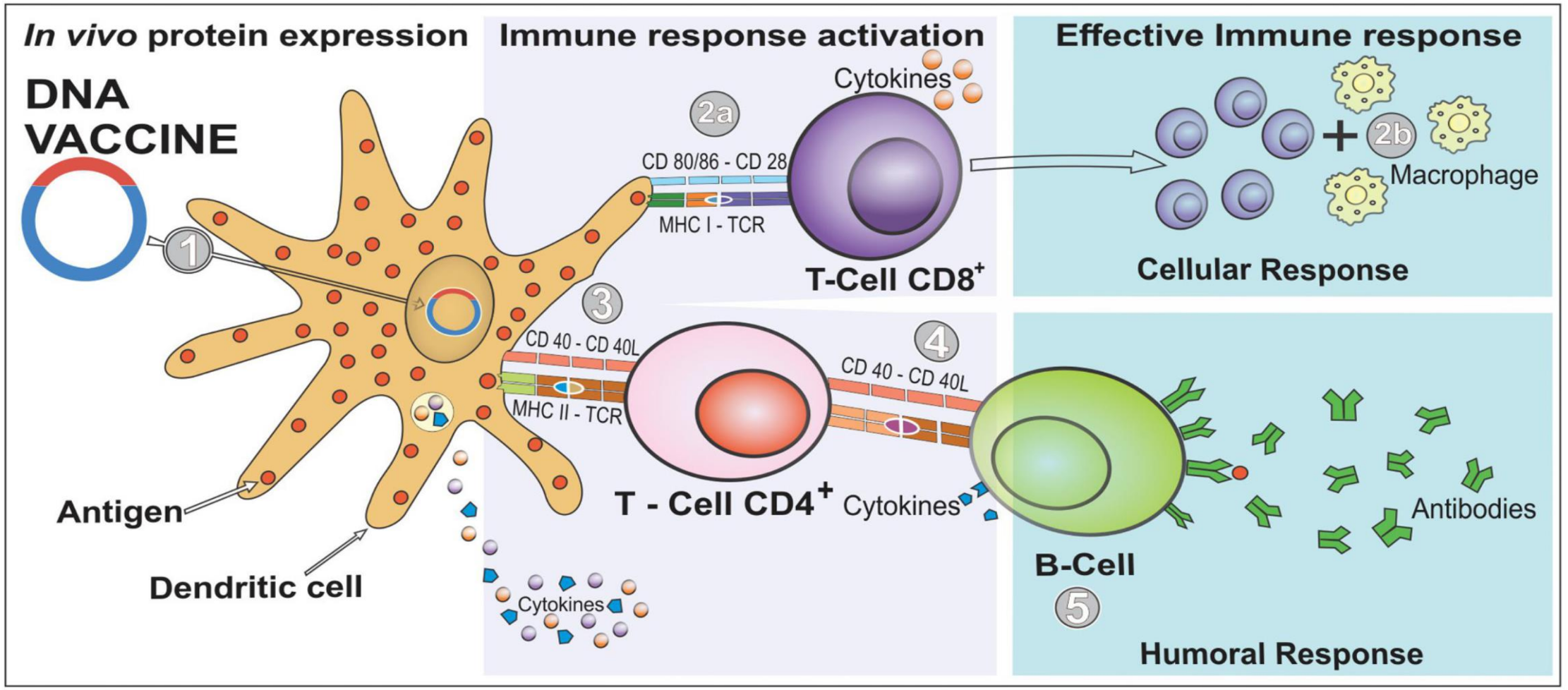
- **The mRNA molecules are significantly more unstable than DNA. Hence, mRNA vaccines commonly require temperatures between −70 °C and −20 °C for long-term storage that complicate the distribution logistics of these kind of vaccines.**

- **The Moderna COVID-19 vaccine needs to be stored from -25 ºC to -15 ºC, but is also stable between 2 ºC and 8 ºC for up to 30 days and between 8 C and 25 C for up to 12 h. The Pfizer/BioNTech COVID-19 vaccine needs to be stored from -80 ºC to -60 ºC and then thawed and stored from 2 ºC to 8 ºC for up to 5 days prior to dilution with saline before injection.**

- **The dry ice temperatures required for the Pfizer vaccine are more difficult to achieve during distribution and storage than the regular freezer temperature required by the Moderna vaccine.**

- **The Moderna mRNA LNPs are frozen in two buffers, Tris and acetate, while the Pfizer/BioNTech vaccine only uses a phosphate buffer. Phosphate buffers are known to be suboptimal for freezing due to their propensity to precipitate and cause abrupt pH changes upon the onset of ice crystallization.**

2. Adenovirus-based vector vaccines



Adenoviruses do not have enzymes that induce fusion of the incorporated genes to the genome of the organism.

Marcelle Moura Silveira, Gustavo Marçal Schmidt Garcia Moreira, Marcelo Mendonça, Life Sciences 267 (2021) 118919

- *The AstraZeneca vaccine uses a chimpanzee adenovirus vaccine vector. This is a harmless, weakened adenovirus that usually causes the common cold in chimpanzees. It has been genetically changed so that it is impossible for it to grow in humans. The chimpanzee adeno (ChAd)-vectored vaccine platform encode a codon-optimized full-length SARS-CoV-2 S protein (ChAdOx1 nCoV-19).*
- *The Johnson & Johnson COVID-19 (and Sputnic) vaccine is a viral vector vaccine based on a human adenovirus that has been modified to contain the gene for making the spike protein of the SARS-CoV-2 virus that causes COVID-19. The body's immune system responds to this spike protein to produce antibodies. The vaccine requires only one dose and does not need to be stored frozen.*
- *Human adenoviruses can be prematurely deactivated due to their recognition by human immune system.*

3. Inactivated and attenuated vaccines

- ✓ Inactivated pathogen vaccines use a dead form of the pathogen, thus ensuring a better safety profile than live attenuated vaccines.
- ✓ However, chemically, irradiated or heat-inactivated pathogens, sometimes lose their immunogenicity rendering this strategy less efficacious than live attenuated pathogen immunization.
- ✓ Inactivated pathogen vaccines often fail to induce cellular adaptive responses unless and thus require the addition of adjuvants, specific compounds that act as stimulants of immune cells and amplifiers of immune responses, is required.

- ✓ **The traditional vaccine strategy of attenuated pathogen administration was first developed on bacteria by Pasteur in 1880 and led to the production of some of the vaccines against *measles, mumps, rubella, and poliomyelitis*. The success of this strategy mainly lies in the fact that in administering a live version of the pathogen it mimics almost precisely the natural infection without causing disease.**

- ✓ **Normally, to achieve attenuated strains of a pathogen, exhaustively long cell or animal cultures are required. By replicating in a foreign host, the wild-type virus needs to accumulate mutations that adapt it to the new host and potentially impair its virulence in the human host.**

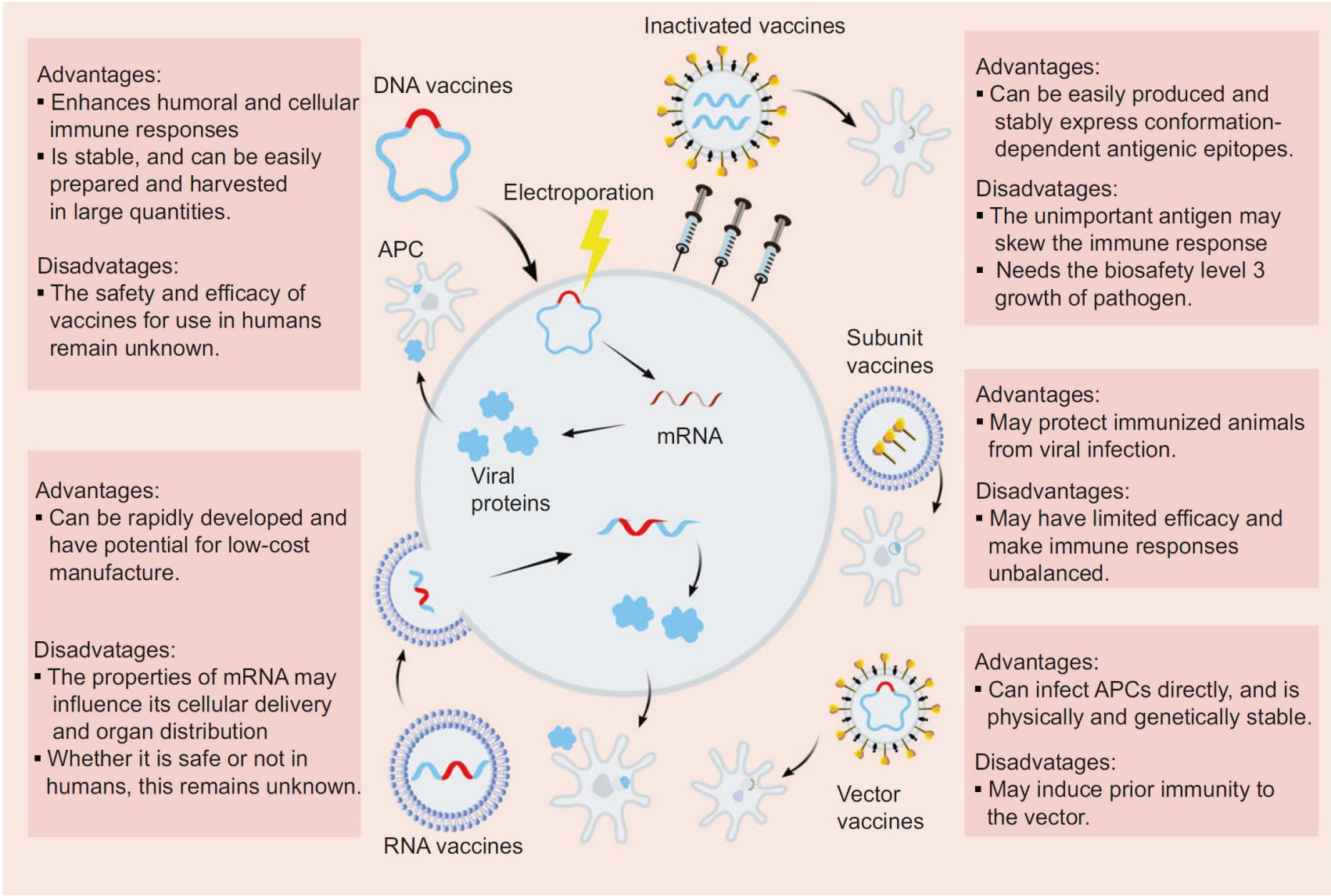
- ✓ **In this regard, coronaviruses are known to frequently recombine in nature, further complicating the development of an attenuated live vaccine against SARS-CoV-2, as the attenuated strain could recombine with other wild coronaviruses resulting in a fully virulent strain.**

4. Recombinant subunits vaccines

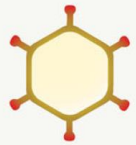
- ✓ **Protein subunit vaccines are generated through recombinant synthesis of protein antigens or protein isolation and purification methods after cultivating large amounts of the pathogen. This strategy eliminates the possibility of severe adverse effects, but frequently raises the necessity to increase booster doses to achieve stronger and more durable immunization.**
- ✓ **As in the case of other vaccine technologies, the administered antigen is uptaken by adjuvant activated antigen-presenting cells (APCs) and presented to adaptive immune cells.**

- ✓ **A plethora of protein subunit candidates against SARS-CoV-2 is currently in human clinical trials. Each one of these candidates is using different forms of the entire Spike protein or its receptor binding domain (RBD), the region of the S protein that mediates viral binding to the ACE2 receptor of target host cells.**

Advantages and disadvantages of the strategies



Yetian Dong, Tong Dai, Yujun Wei, Long Zhang , Min Zheng and Fangfang Zhou, Signal Transduction and Targeted Therapy (2020) 5:237

	mRNA vaccine		Replication-defective viral vector vaccine				Inactivated pathogen vaccine				Protein subunit vaccine		Virus-like particle
													
Candidate vaccine name	mRNA-1273	BNT162b2 / Comirnaty	Ad5-nCoV	AZD1222	Gam-COVID-Vac / Sputnik V	JNJ-78436735 / Ad26.COV2.S	CoronaVac	Undisclosed	BBIBP-CorV	BBV152/Covaxin	NVX-CoV2373	ZF2001	CoVLP
Manufacturer(s)	Moderna/NIAID	BioNTech/Pfizer/ Fosun Pharma	CanSino Biological / Beijing Institute of Biotechnology / Academy of Military Medical Sciences	AstraZeneca / Oxford University	Gamaleya Research Institute / Health Ministry of the Russian Federation / Acellena Contract Drug Research and Development	Janssen Pharmaceutical Companies of Johnson & Johnson / Beth Israel Deaconess Medical Center	Sinovac Research and Development Co.	Wuhan Institute of Biological Products / China National Pharmaceutical Group-Sinopharm	Beijing Institute of Biotechnology / China National Pharmaceutical Group-Sinopharm	Bharat Biotech	Novavax	Longcom / Chinese Academy of Medicine	Medicago / Glaxo Smith Kline
Phase 3 trials starting date	July 27th, 2020	July 27th, 2020	September, 2020	August, 2020	September 7th, 2020	September 23rd, 2020	July 21st, 2020	July 18th, 2020	July 16th, 2020	November 16th, 2020	September 23rd, 2020	November 18th, 2020	November 19th, 2020
Number of participants	30,000	44,000	40,000	30,000 🟢	40,000	90,000 🔴	8,870	21,000	63,000	26,000	45,000	29,000	30,612
Cold chain required for storage	Remains stable at: -20°C for up to 6 months 2°C - 8°C for 30 days Room temperature for up to 12 hours	Remains stable at: -70°C for up to 6 months 2°C - 8°C for 5 days 🟡	2–8°C	2–8°C	Two presentations: 1. Frozen (-18°C) 2. Lyophilised (2-8°C)	2–8°C	2–8°C	2–8°C	2–8°C	2–8°C	2–8°C	2–8°C	2–8°C
Immunization regimen	100 µg (2 doses - 4 weeks apart)	30 µg (2 doses - 3 weeks apart)	0.5x10 11 vp (1 dose)	0.5x10 11 vp (2 doses - 4 weeks apart)	0.5 ml per dose (2 doses - 3 weeks apart) 🔵	0.5x10 11 vp (1 dose)	3 µg (2 doses - 2 weeks apart)	Unknown quantity of dosage (2 doses - 3 weeks apart)	4 µg (2 doses - 3 weeks apart)	3 µg per dose (2 doses - 4 weeks apart) 🔵	5 µg SARS-CoV-2 rS + 50 µg Matrix-M1 adjuvant (2 doses - 3 weeks apart)	25 µg / 0.5 mL per dose (2-3 doses - 4 weeks apart)	3.75 µg of CoVLP + 0.5 mL of AS03 adjuvant per dose (2 doses - 3 weeks apart)
Immunity: Antibody response rate	Neutralizing antibodies produced in all participants (Phase 1)	Neutralizing antibodies produced in all participants (Phase 1)	Neutralizing antibodies produced in 97% of the participants (Phase 2)	Neutralizing antibodies produced in all participants that received the prime-boost regime (Phase 1/2)	Neutralizing antibodies produced in all participants (Phase 1/2)	Neutralizing antibodies in 92% of the participants (Phase 1/2a)	Neutralizing antibodies in 92.4% and 97.4% of the participants that received two doses of 3 ug of vaccine 2 or 4 weeks apart, respectively (Phase 2)	High titers of neutralizing antibodies in all participants that received the higher dose (Phase 1/2)	High titers of neutralizing antibodies in all participants (Phase 1/2)	Neutralizing seroconversion rate: 93.4% in the 3 ug per dose group (Phase 1)	High titers of neutralizing antibodies in all participants (Phase 1/2)	Not reported	Not reported
Immunity: T cell response rate	CD4+ T cell activation reported (Th1 skewed phenotype) / Low CD8+ T cell responses only induced with 100 ug dose	Virus specific Th1 and CD8+ T cell responses reported	T cell responses observed in 88% of the participants	T cell responses observed in all participants	CD4+ and CD8+ T cell responses observed in all participants	CD4+ T cell responses in 80% of the participants (Th1 skewed phenotype) / Robust CD8+ T cell responses	Not reported	Not reported	Not reported	Virus-specific CD4+ and CD8+ T cell responses reported. Th1 skewed.	CD4+ T cell activation in all tested participants (Th1 skewed phenotype)	Not reported	Not reported
Country / Region Emergency use approval	US* on Dec 18th, Canada on Dec 23th, Israel on Jan 4th (2021), EMA* on Jan 6th (2021)	UK* on Dec 2nd, Canada on Dec 9th, US on Dec 11th, EMA* on Dec 21st, and other countries 🟣	Limited use for 1 year in Chinese military personnel / June 25, 2020	UK on Dec 30th, Argentina on Dec 30th, India on Jan 3rd (2021)	Belarus and Argentina. Limited use in Russia for voluntary application in small population groups / August 12, 2020	No	Limited use in China / July, 2020	Limited use in China and UAE* / Sep 2020	UAE on Dec 9nd, Bahrein on Dec 13th China on Dec 31th, Egypt on Jan 3rd (2021)	India on Jan 3rd (2021)	No	No	No
Clinical trial registry number	NCT04470427	NCT04368728	NCT04526990	NCT04516746 NCT04540393 CTRI/2020/08/027170 ISRCTN89951424	NCT04530396	NCT04505722 NCT04614948	NCT04456595 INA-WXFM0YX	ChiCTR2000034780 ChiCTR2000039000	NCT04560881 NCT04510207 ChiCTR2000034780	CTRI / 2020/11/028976	NCT04611802 NCT04583995 EUCTR2020-004123-16	NCT04646590 ChiCTR2000040153	NCT04636697
Interim reports efficacy	94.1%	95% overall 94% (> 65 years old)	-	62.1% 90% (18 to 55 years old) 🟤	91.4%	-	-	-	79.34% 🟢	-	-	-	-

- 🟡 Company is exploring the development of a lyophilised form of the vaccine that can be stored at 2°C to 8°C.
- 🔴 Study currently temporarily halted to the appearance of an unexpected illness on a study participant
- 🟢 Study was temporarily halted to the appearance of an unexpected illness on a strudy participant. Resumed in most vaccination sites but the US
- 🔵 The number of viral particles per dose has not been disclosed
- 🟤 62.1% of efficacy when vaccine was given as two full doses at least one month apart. 90% of efficacy when vaccine was given as a half dose prime immunization, followed by a full dose boost immunization at least one month apart. Combined efficacy 14 days after the second dose is 70.4%.
- 🟣 Other countries: Argentina, Ecuador, Chile, Panama, Mexico, Costa Rica, Kuwait, Singapure, Switzerland, Bahrein, South Arabia. On December 31st, the WHO granted emergency validation to BNT162b2 / COMIRNATY.
- 🟢 Announced by the manufacturer.

* EMA: European Medicines Agency; UK: The United Kingdom; US: The United States; UAE: United Arab Emirates; EUA:

Nikolaos C. Kyriakidis, Andrés López-Cortés, Eduardo Vásconez González, Alejandra Barreto Grimaldos and Esteban Ortiz Prado, npj Vaccines (2021) 28

THE REAL SOLUTION TO THE PROBLEM:

PFIZER INITIATES PHASE 1 STUDY OF NOVEL ORAL ANTIVIRAL THERAPEUTIC AGENT AGAINST SARS-COV-2

THANK YOU