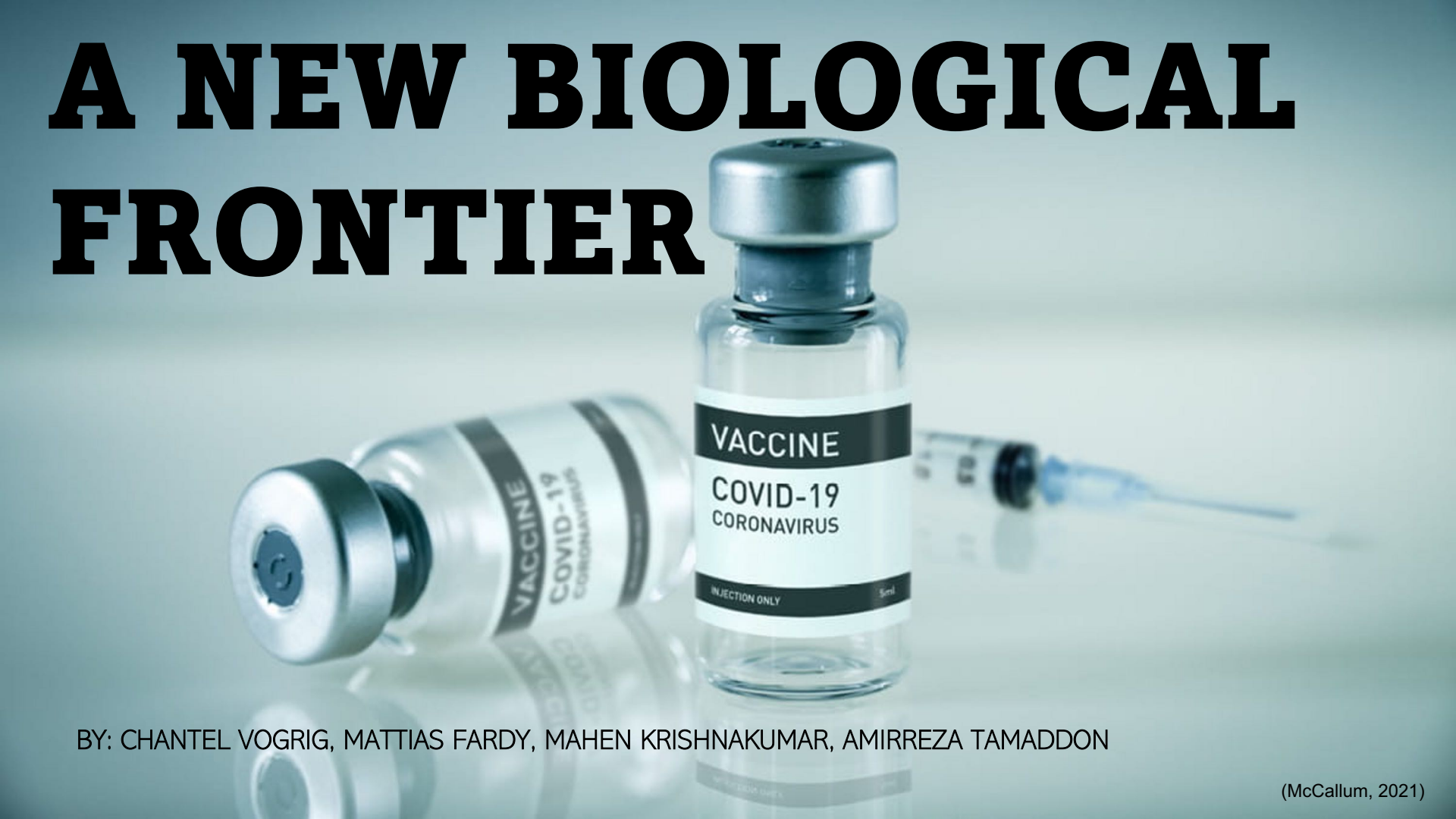
A NEW BIOLOGICAL FRONTIER



BY: CHANTEL VOGRIG, MATTIAS FARDY, MAHEN KRISHNAKUMAR, AMIRREZA TAMADDON

(McCallum, 2021)

Pandemic, Vaccines, and Impacts

Impacts seen across the globe
(as of Nov. 7, 2021)

- 249 million confirmed infections
- 5.04 million confirmed deaths
- 7.24 billion vaccines administered
- (JHU, 2021)

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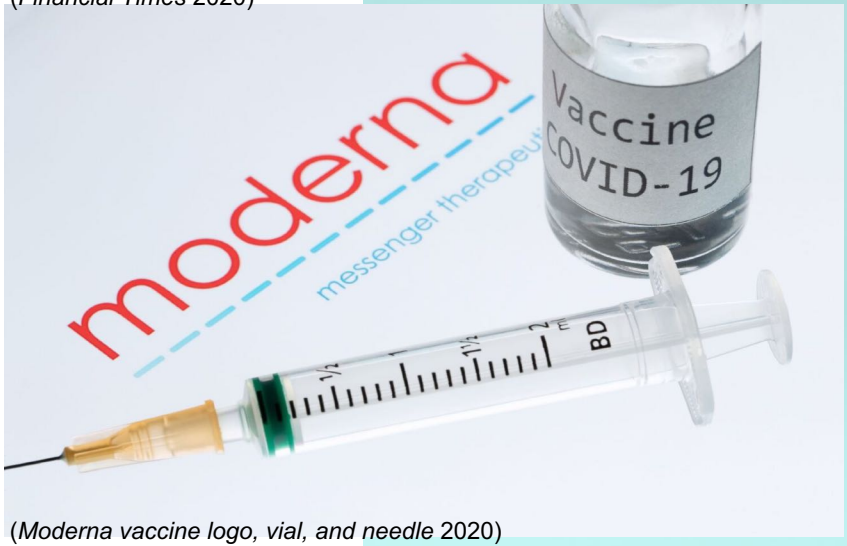
mRNA Vaccines

(*Financial Times* 2020)



(*Moderna vaccine logo, vial, and needle* 2020)

How they work?

mRNA vaccines

How they work?

mRNA vaccines

Vaccine administration

- Intramuscular administration
- Currently need 2 doses of either Pfizer or Moderna to be considered fully vaccinated (CDC, 2021)
- Second dose can be given 3 to 6 weeks after first dose (CDC, 2021)

How they work?

mRNA vaccines

Booster Shots

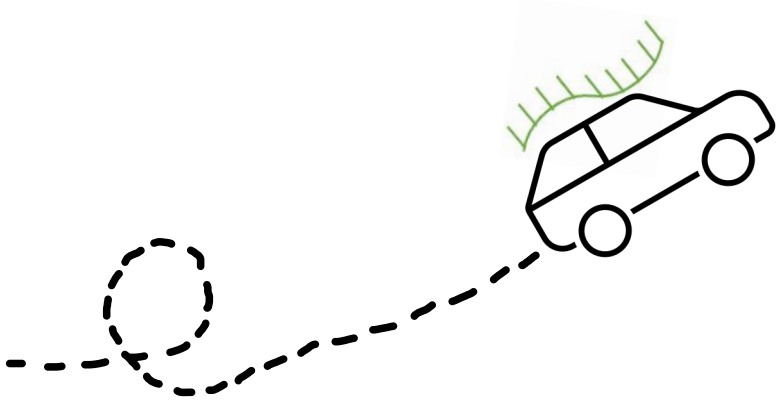
- Due to emerging variants and waning efficacy of vaccines (Tartof et al., 2021)
- Now recommended for those:
- ages 65 and over
- Ages 18 and older with underlying health conditions or who work in high-risk environments
- (CDC, 2021)

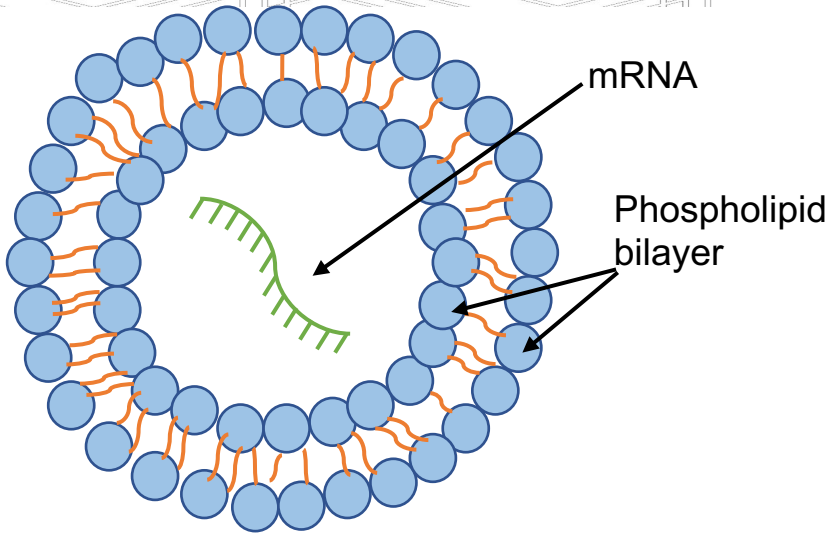
How they work?

mRNA vaccines

Lipid nanoparticle (LNP)

- Spherical phospholipid vesicle
- Contains and carries the mRNA to cells after injection (Malone et al., 1989)
- RNA naturally very unstable
- LNP protects and stabilizes mRNA (Schoenmaker et al., 2021)



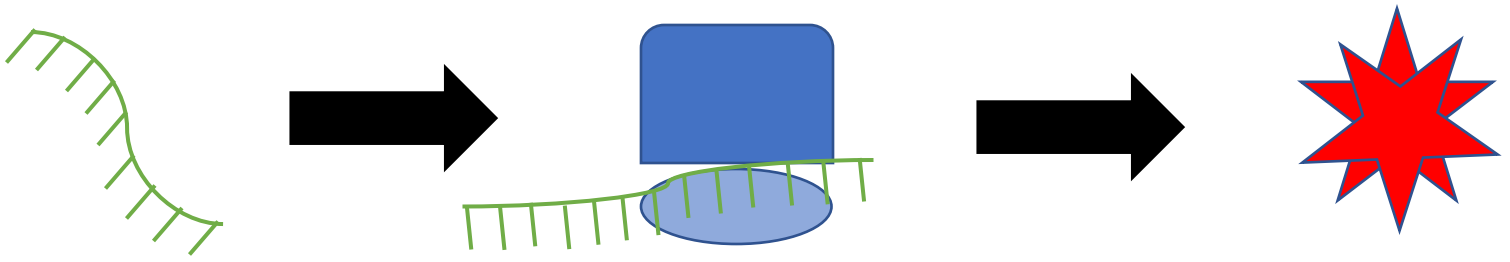


How they work?

mRNA vaccines

Post injection

- mRNA-lipid nanoparticle complex travels to body cells
- mRNA enters the cells cytosol where it can be translated by a ribosome
- This produces a unique protein exclusive to SARS-CoV-2 (Spike protein)
- (Schoenmaker et al., 2021)



mRNA

Translation of mRNA strand by ribosome

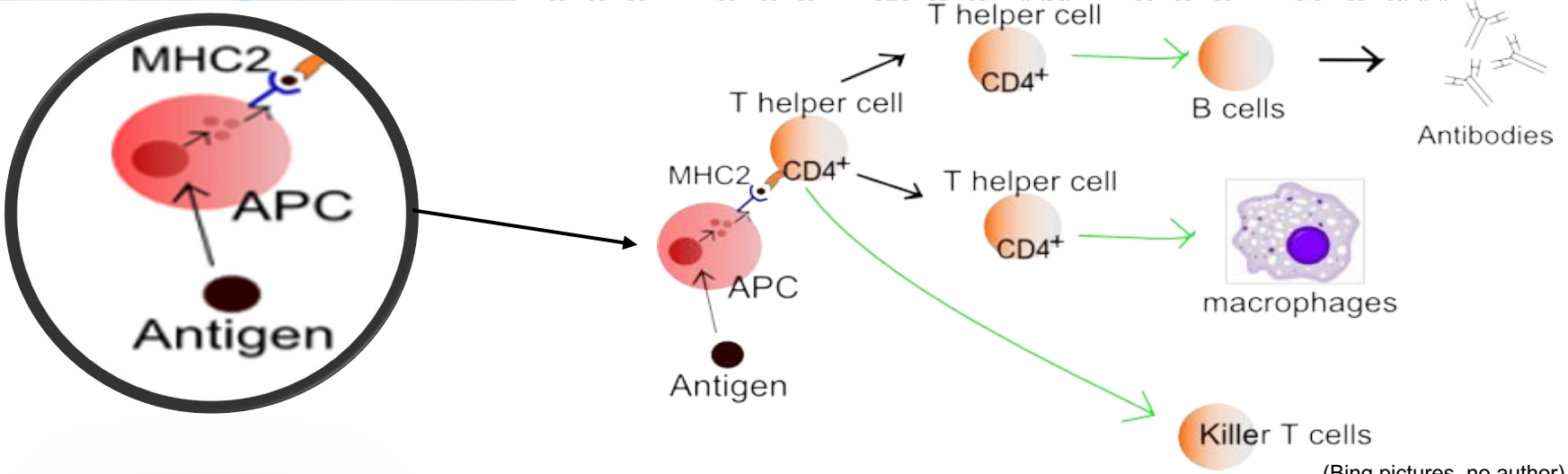
Spike protein

How they work?

mRNA vaccines

The Spike Protein

- Membrane bound after translation in host
- Presented on the outside of host cell membranes
- Becomes target for immune cells (Schoenmaker et al., 2021)



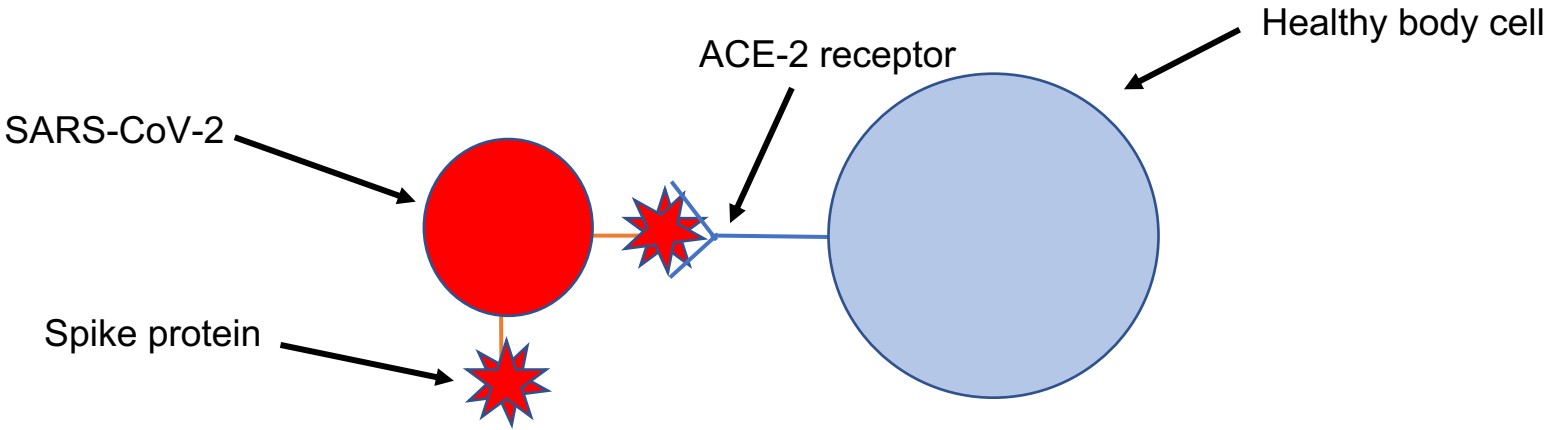
(Bing pictures, no author)

How they work?

mRNA vaccines

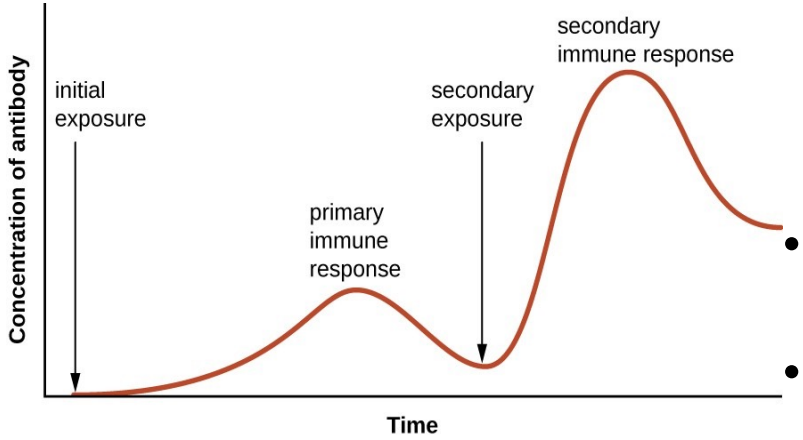
The Spike Protein (in natural infection)

- Receptor binding domain (RBD) of Spike protein binds to the hosts cell ACE-2 receptor (Wrapp et al., 2020)
- RBD and ACE-2 binding results in viral infiltration into healthy cells (Faisal et al., 2021)
- ACE-2 receptor is abundant in the respiratory tract (Pushparajah et al., 2021)



How they work?

mRNA vaccines



(*Antibody concentration v.s. time graph*)

Immune Response

- penetration of needle during intramuscular injection initiates local immune response attracting neutrophils and antigen presenting cells (Schoenmaker et al., 2021)
- T cell activation (Cassaniti et al., 2021)
- Memory B cells and antibodies are generated (Mesin et al., 2020)
- humoral response generated, and memory B cells will recognize the spike protein upon second exposure to actual virus (Mesin et al., 2020)
- Body is better equipped to fight off infection due to immune memory and antibodies
- Faster immune response upon second exposure

First mRNA transfection technique

- 1989
- Robert W. Malone, Philip L. Felgner, and Inder M. Verma first to transfect cells with RNA (Schlake et al., 2012)
- This new transfection technique led to the use of mRNA as a therapeutic

Synthetic mRNA

- Use of bacteriophage RNA polymerase gave scientists the ability to produce large amounts of RNA (Malone et al., 1989)
- In vitro

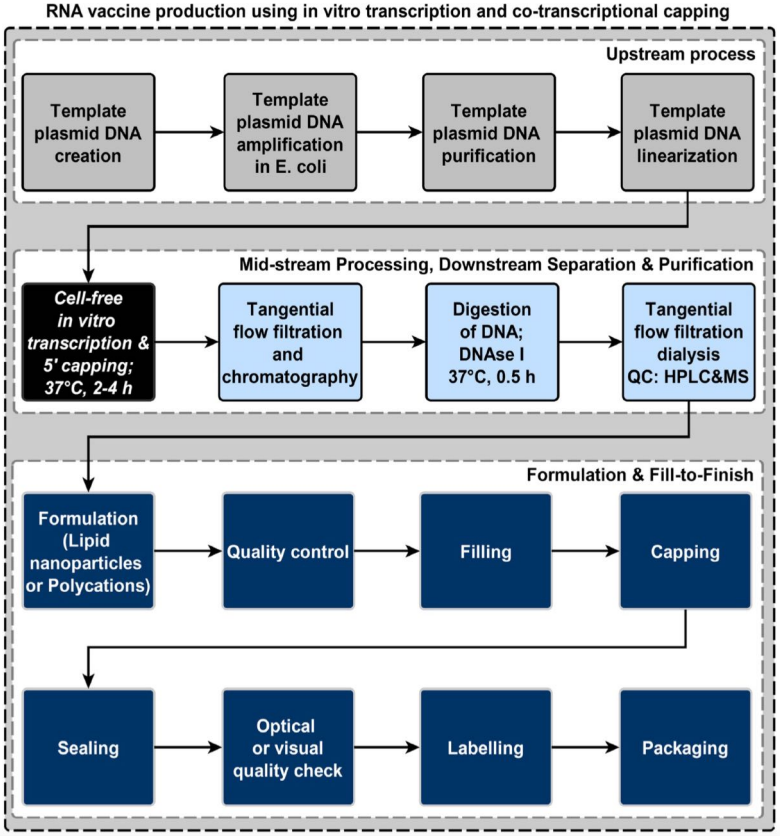
Initial Vaccine Research

- Artificial reconstruction of the virus and Gene Synthesis
  - After the first DNA sequence of SARS-COV-2 was published, scientists were able to synthetically reconstruct the virus using commercially-available gene synthesis, reverse genomics, gene rescue techniques (Thi Nhu Thao et al., 2020).
  - This provided researchers with a head start with recognizing the severity and components of the virus, without having a physical isolated sample of the virus.

- Molecular Docking simulations on Supercomputers
  - These simulations predict the binding strength of the antigen candidate and eliminates the weakly binding candidates (Ahammad & Lira, 2020).
  - Made possible by many-core parallel computer chips.
  - Ability to process billions of potential antigen candidates, in a matter of months compared to days (Acharya et al., 2020)
  - These developments helped to reinvigorate the field of immunoinformatics, a subfield of bioinformatics.

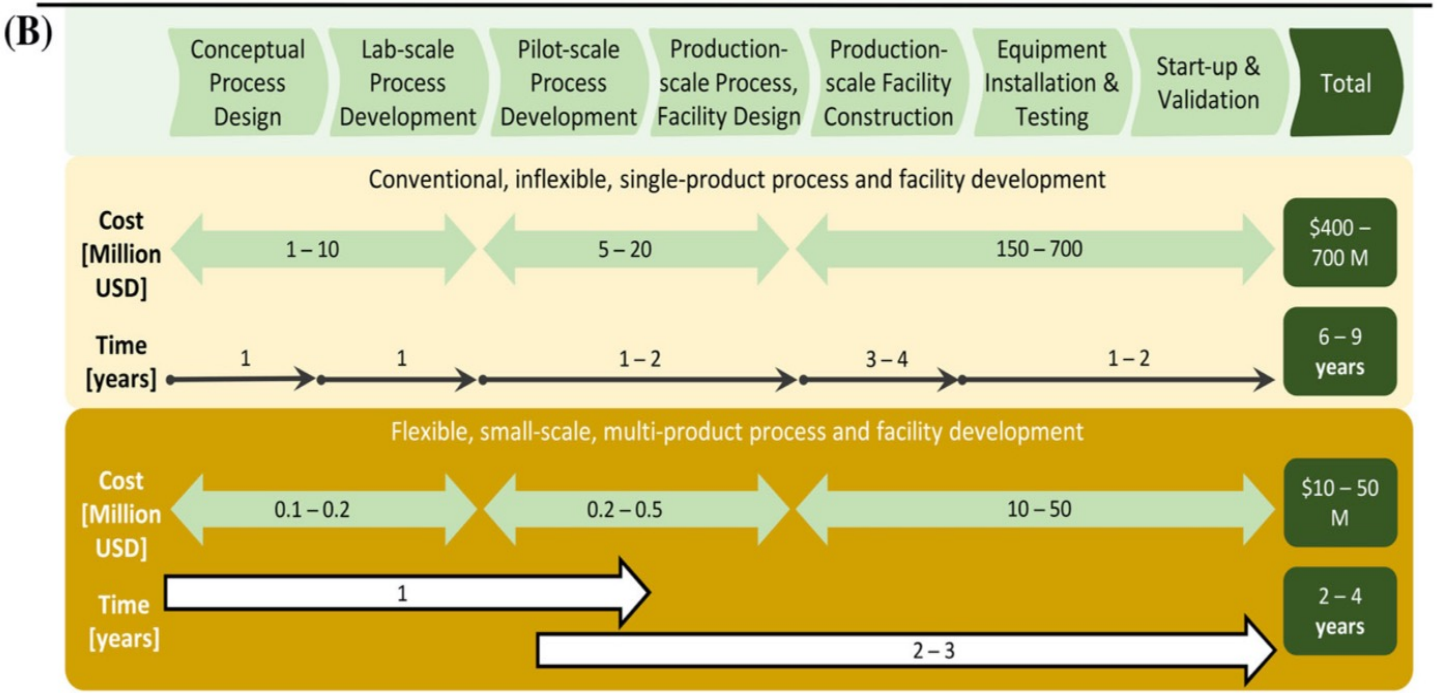
Figure. 1

mRNA Vaccine Development process summary



Note: This figure shows the general process by which RNA vaccines are produced. This example was retrieved from a study which proposed a process to produce self- amplifying RNA vaccines. From “Rapid development and deployment of high-volume vaccines for pandemic response,” by Kis, Z., Kontoravdi, C., Dey, A. K., Shattock, R., and Shah, N. in Wiley Periodicals, *Journal of Advanced Manufacturing and Processing (p. 5)*, 2020, Wiley Periodicals. Copyright 2020 by The Authors.

Figure. 2



Note: This figure compares the mRNA vaccine process development timeline with conventional vaccines. This example was retrieved from a study which proposed a process to produce self- amplifying RNA vaccines. From "Rapid development and deployment of high-volume vaccines for pandemic response," by Kis, Z., Kontoravdi, C., Dey, A. K., Shattock, R., and Shah, N. in Wiley Periodicals, *Journal of Advanced Manufacturing and Processing (p. 5)*, 2020, Wiley Periodicals. Copyright 2020 by The Authors.

RNA based vs. Conventional vaccine process development timeline

Clinical Trials

- large-scale clinical trials are carried out to show that the vaccine is safe and effective.

- Vaccines have been tested on more than 43000 participants in different age groups in 3 phases (U.S. National Library of Medicine, 2020)

Effectiveness

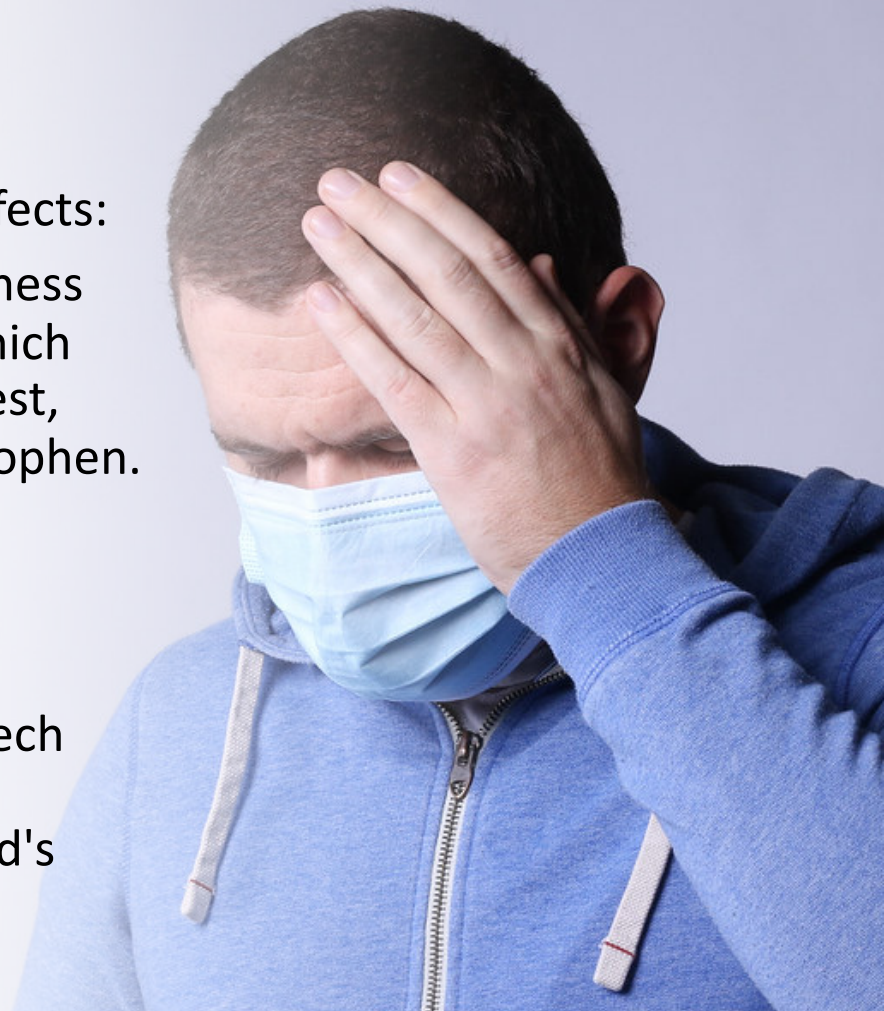
- Different vaccines have different efficacies
- These vaccines are 91-96% effective against severe disease or death from the Alpha variant (Aoun-Barakat et al., 2021)
- The new variants lower the efficacy of the vaccines as they get stronger.

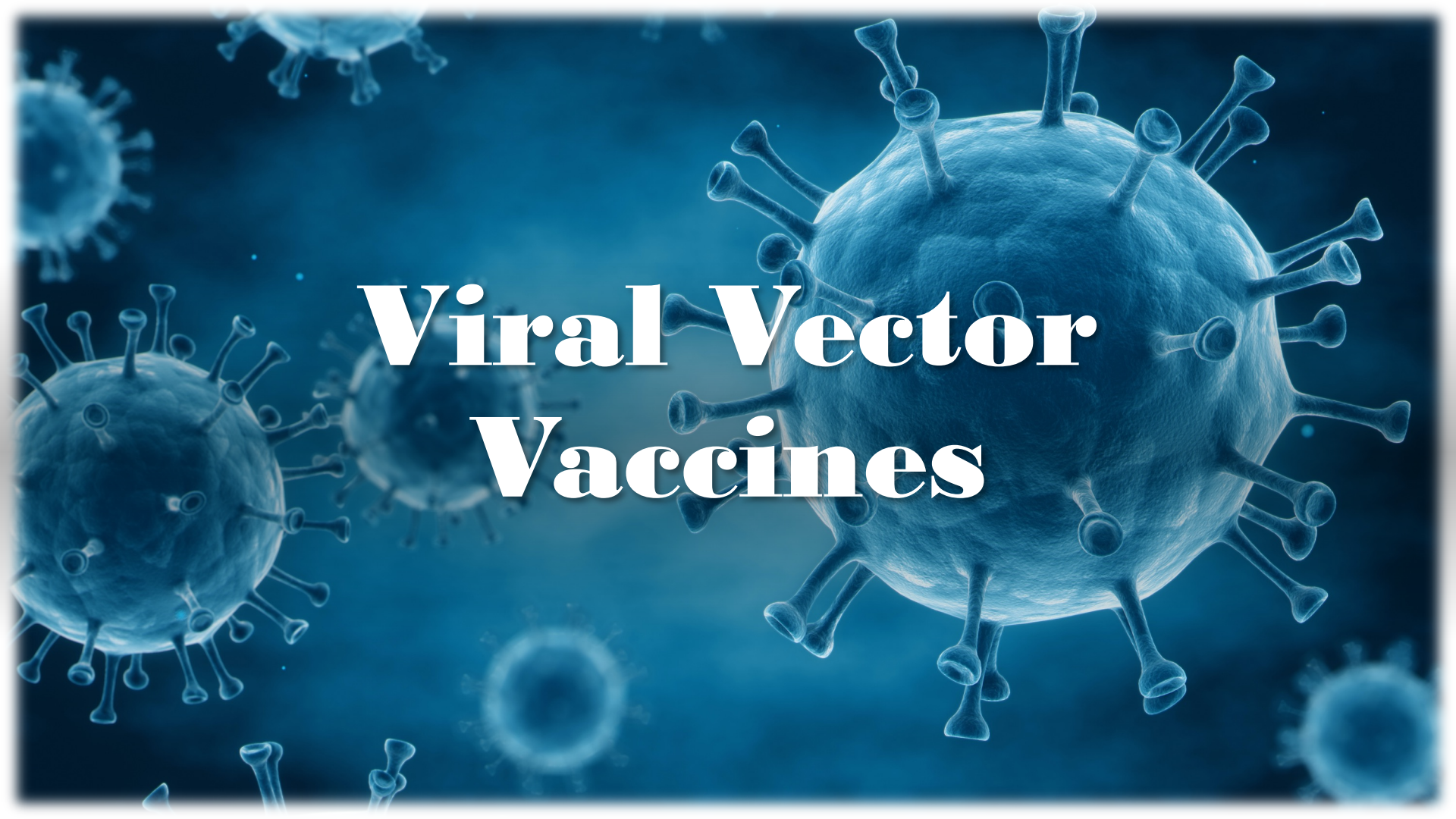
(Nebraska Medicine. 2021)

Side effects

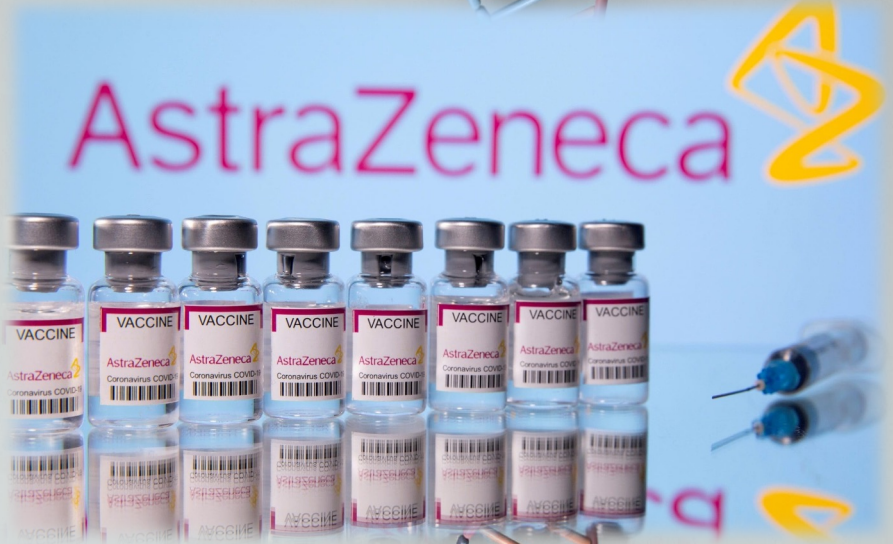
- mRNA vaccines mostly have similar side effects:
  - Chills, headache, pain, tiredness, and redness and swelling at the injection site, all of which generally resolve within a day or two of rest, hydration, and medications like acetaminophen.

- Rare side effects:
  - people may develop myocarditis and pericarditis after getting the Pfizer/BioNTech vaccine. Myocarditis and pericarditis are inflammatory heart conditions (Ireland's Health Services. 2021)




Viral Vector
Vaccines

(*Medscape* 2021)



(*The Telegraph*, 2021)

How they work?

Viral vector vaccines

How they work?

viral vector vaccines

Vaccine administration

J&J's Janssen

- Intramuscular administration
- Johnson & Johnson's Janssen vaccine requires one shot
- CDC recommends getting a booster shot of any covid vaccine 2 months after first janssen shot (CDC, 2021)

How they work?

viral vector vaccines

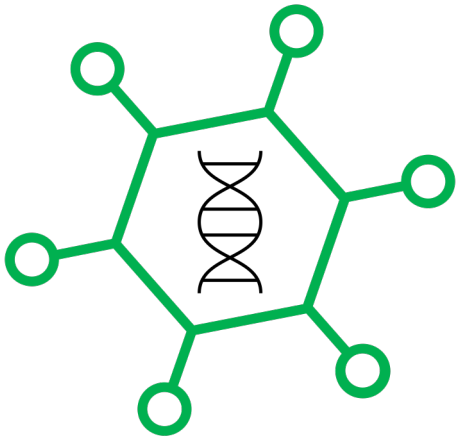
Vaccine administration

AstraZeneca

- Intramuscular administration
- 2 doses needed in Canada
- Health Canada recommends an mRNA vaccine as a second dose (*Government of Canada* 2021)

How they work?

viral vector vaccines



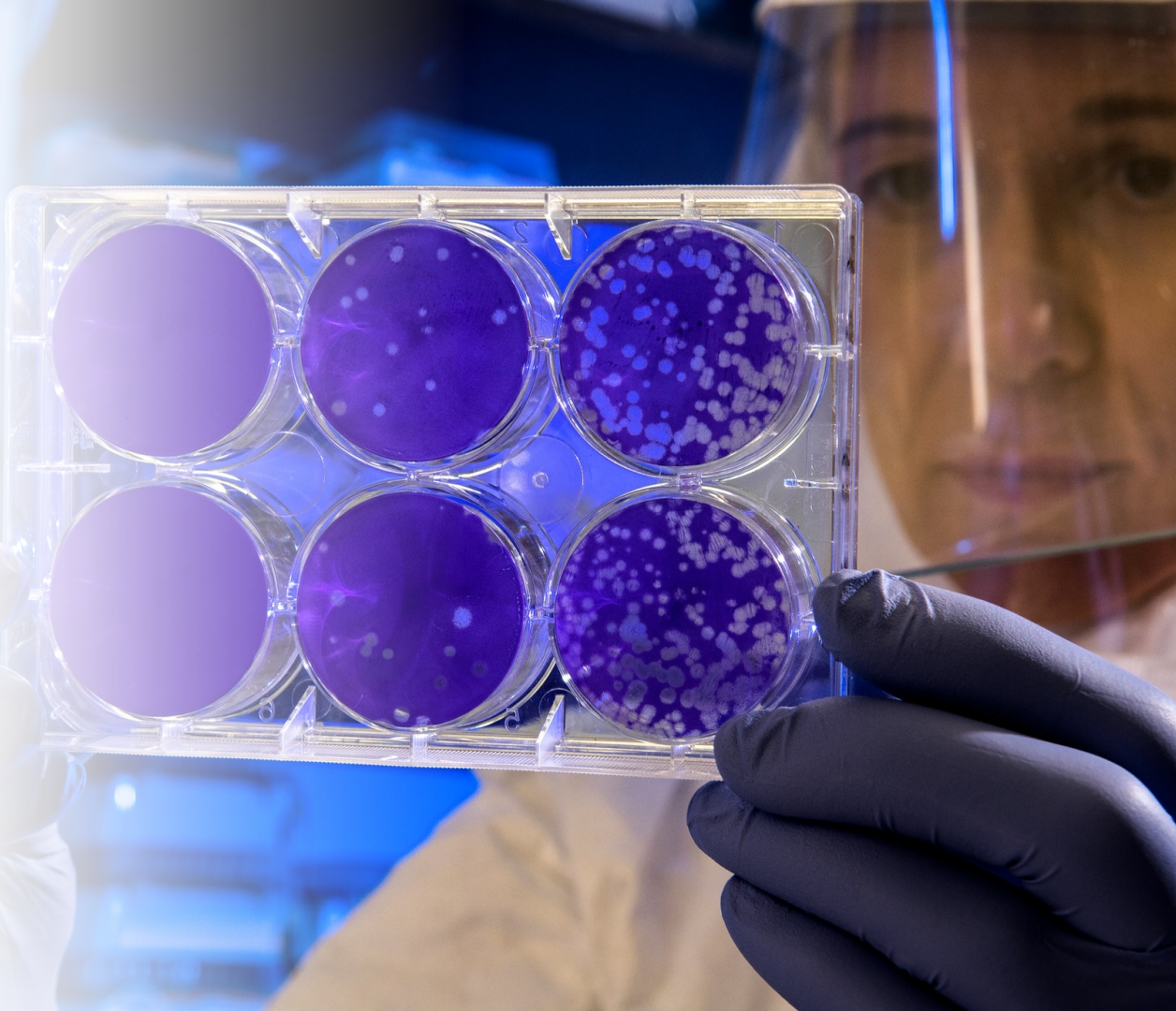
Viral vector

- Uses another virus to carry the genetic information of the virus we want to vaccinate against
- This carrier virus is called a vector!
- Genetic information encodes for the antigen (spike protein)
- Covid 19 viral vector vaccines use non-replicating vectors
- They cannot replicate, therefore, efficacy is lower
- Humoral and cell mediated immune responses generated
- (Pushparajah et al., 2021)

Adenovirus (ad)-vector Vaccine Development Process

- Isolate DNA plasmid of SARS-COV-2 and select the target genes to be expressed.
- Isolate the DNA plasmid of ad.
- Obtain a cell line that is susceptible to adenoviruses (e.g. HEK293).

(Luo et al., 2019)



- Construct the ad vector by deleting the ad self-replicating genes and substituting them with the antigen coding gene. (Mendonça et al., 2021)

- Determine/Check the level of expression of the target gene in the new plasmid.

1. Insert GFP into the plasmid to check for gene expression.
2. Infect the cell line and check for target gene expression.
3. Repeat/modify process until target gene expression is verified.

- Test for immunogenicity of the ad-vector.

1. This could be done using model organisms, usually mice would be used as test subjects.
2. Further testing would require clinical trials.

(Luo et al., 2019)

Clinical Trials

- These Vaccines have been tested on more than 23000 participants in different age groups (The Lancet. 2021)

Effectiveness

- These vaccines are 74 - 85 % effective against severe disease or death from the Alpha variant (Aoun-Barakat et al., 2021)

Efficacy

- Efficacy can refer to different things.
- Efficacy can refer to how protected someone is against an outcome, like severe disease, hospitalization or death.
- In data from trials presented to FDA, the percentages of persons with serious outcomes were small in both the vaccinated and unvaccinated groups, vaccinated people were well protected from hospitalization and death from COVID-19. (Nebraska Medicine. 2021)

Asha Publication. 2019

Side effects

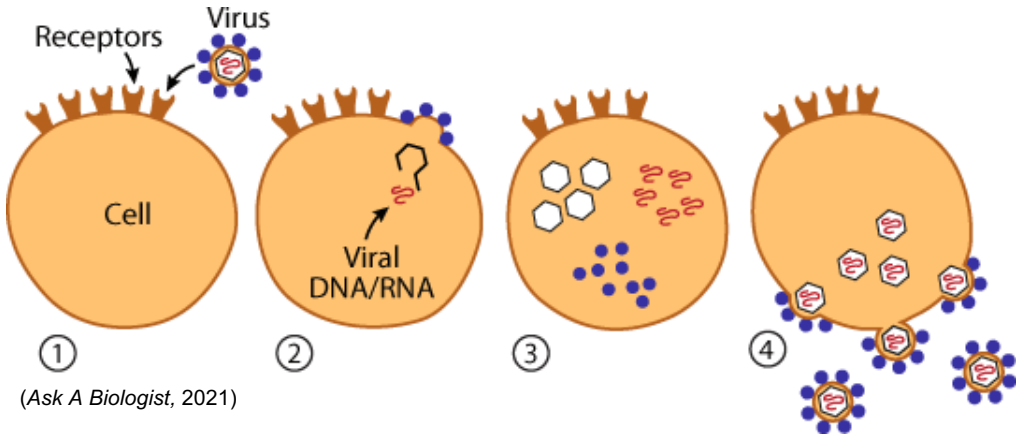
- Some countries suspended using it after a small number of recipients developed blood clots.

- Other side effects are almost same as the mRNA vaccines which are:

Tenderness, pain, warmth, redness, itching, swelling or bruising at the injection site, all of which generally resolve within a day or two (Aoun-Barakat et al., 2021)

Quick Recap:
Viruses insert genetic material into the cell, hijacking its machinery to create more components for more viruses.

RNA-based vaccines aim to do the same thing but with a desired protein instead (Le Page, 2021).



(*Ask A Biologist*, 2021)

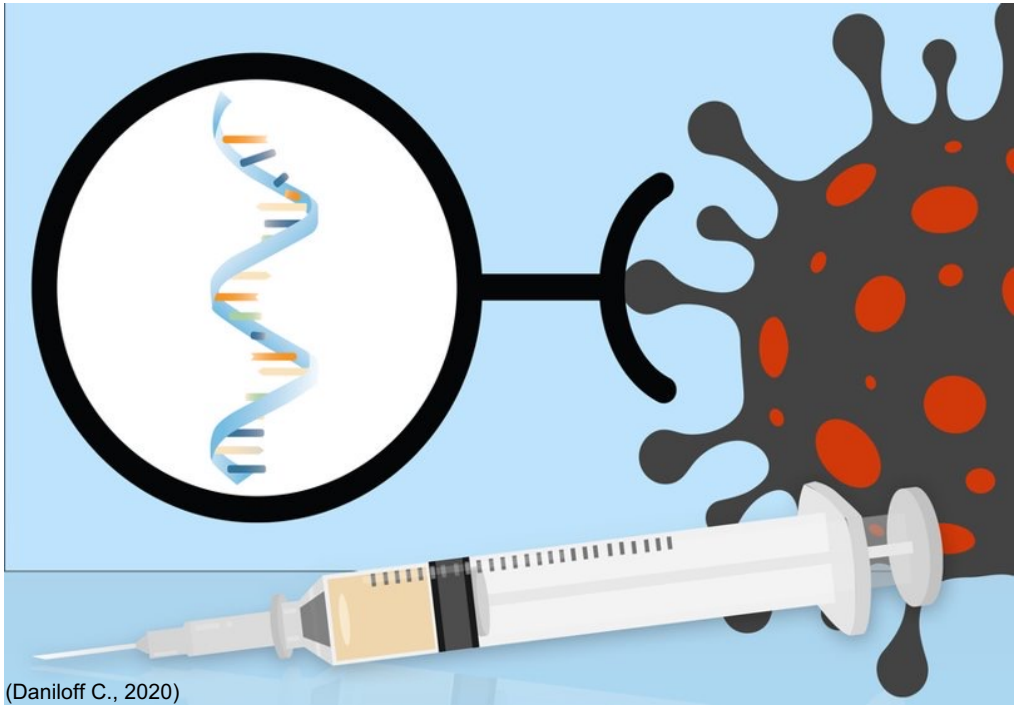
What Does This Mean for the Future?

Firstly, traditional vaccines spread to the entire body

viral vector vaccines allow for more localized delivery of mRNA
(Rohovie et. Al., 2017)

Secondly, RNA vaccines are drastically faster to produce.

Inserting synthesized RNA means scientists can code for desired proteins (Le Page, 2021).



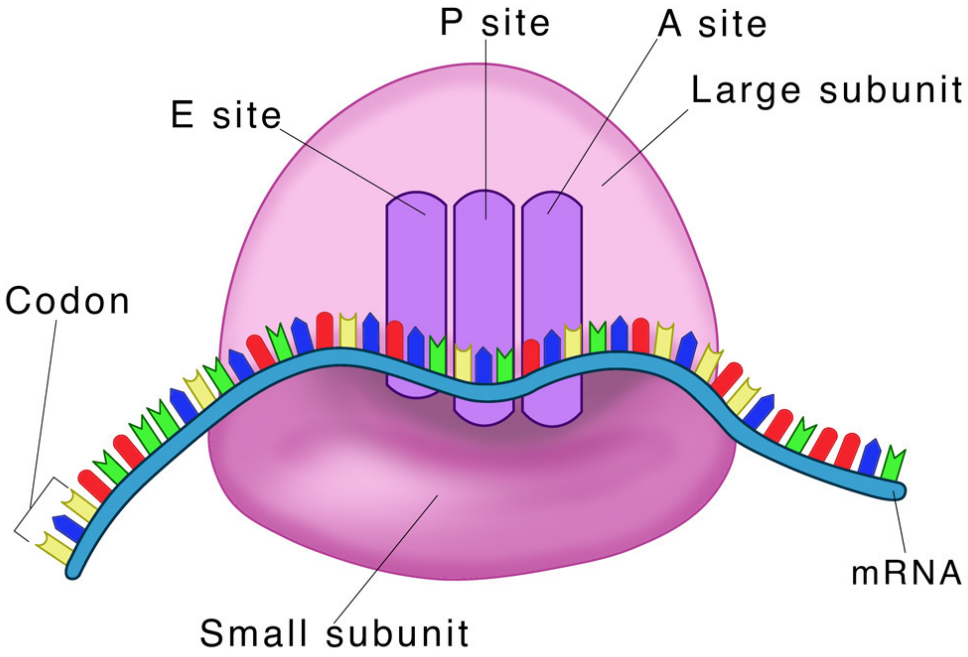
(Daniloff C., 2020)

The ability to make a target protein means potential cures for:

genetic diseases
- non-functioning enzymes due to inheritance or mutation could be replaced

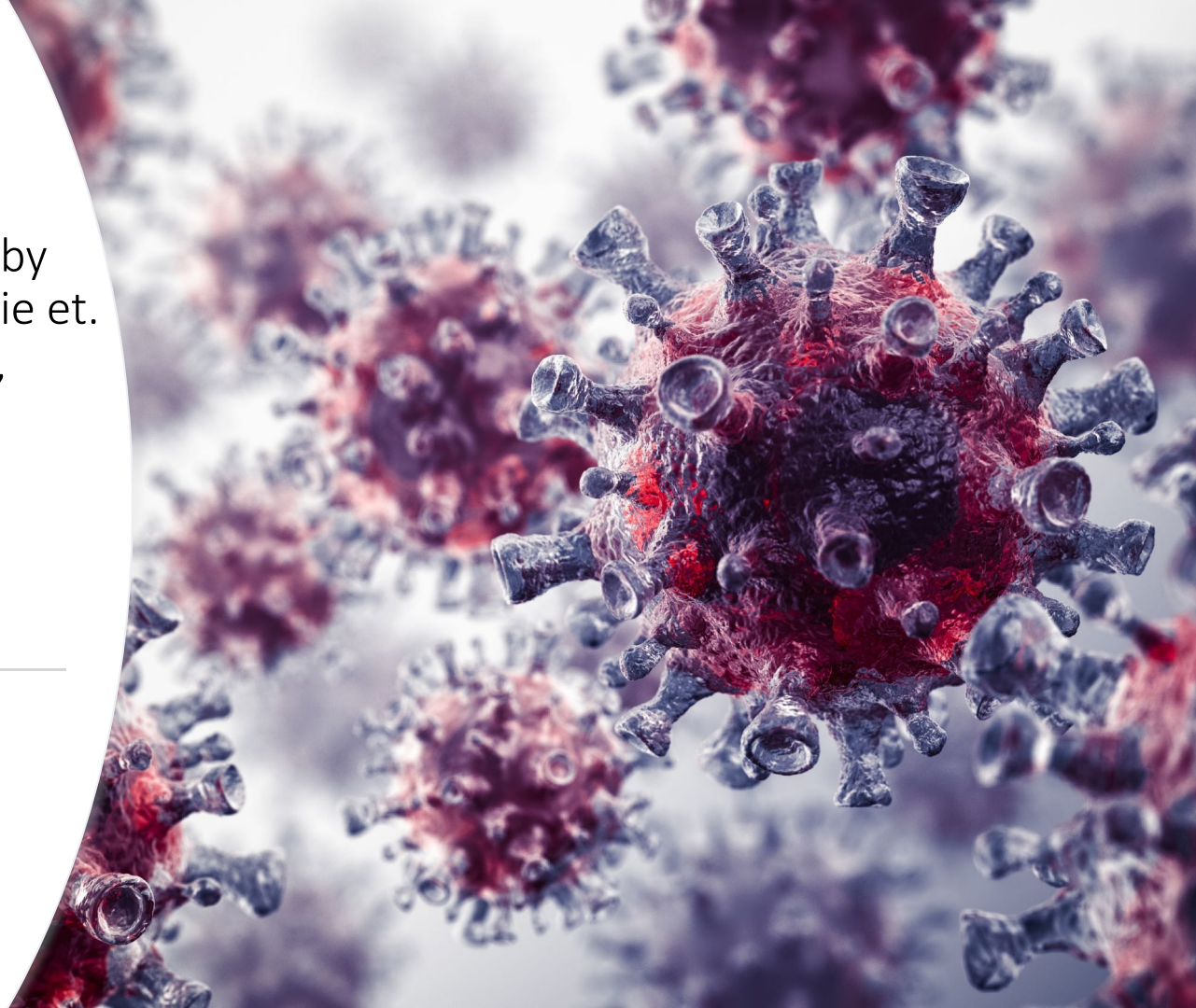
Tissue damage
- specific proteins could stimulate cell growth in areas of permanent damage (heart attack)

(Le Page, 2021) (Damase et. Al., 2021)

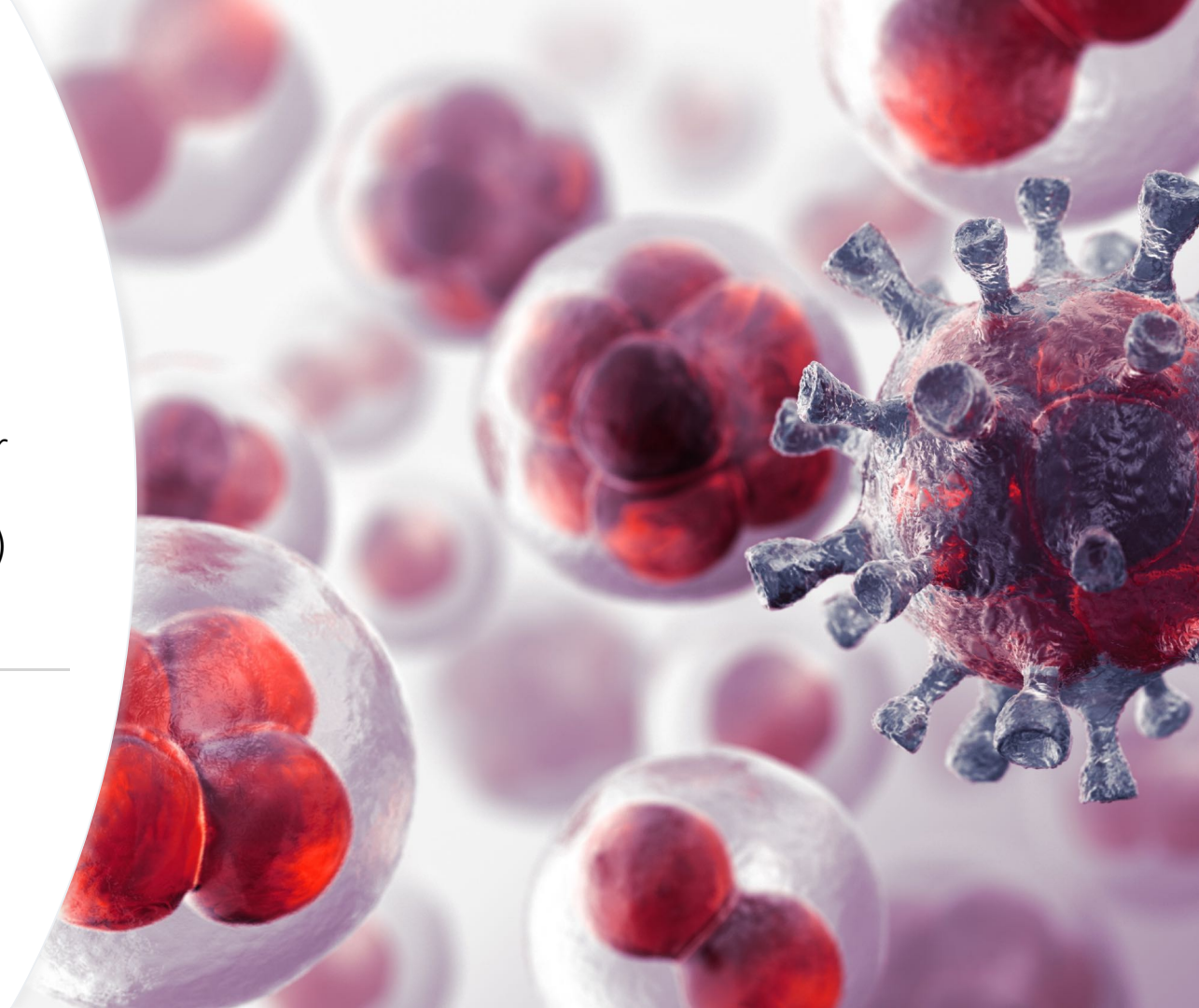


([Ribosome], n. d.)

The ability to target specific organs for RNA delivery is made possible by virus-like particles (Rohovie et. Al., 2017) (Damase et. Al., 2021)



Viral capsids can target specific cells due to ligand-receptor binding. Viruses' surface can be modified to present ligands only certain cell receptors will bind to for endocytosis (Rohovie et. Al., 2017) (Damase et. Al., 2021)



What this means for the future of vaccinations:

- They will be able to target specific tissues for RNA delivery, mitigating the risk of complications elsewhere in the body

- smaller concentration of vaccine needed since RNA is being localized to affected area (Rohovie et. Al., 2017) (Damase et. Al., 2021).

The End

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