Merck and Moderna announce 3-year results for mRNA-4157/Keytruda 2b study for advanced melanoma patients

- mRNA-4157/Keytruda combination reduced risk of recurrence or death by 49% compared to Keytruda alone in melanoma stage III/IV patients ($p = 0.019$).
- mRNA-4157/Keytruda combination reduced risk of distant metastasis or death by 62% compared to Keytruda alone ($p = 0.015$).
- Initiated phase 3 trials in patients with high risk melanoma and NSCLC.

Information Medicines – a Paradigm Shift?

Gerrit Borchard, PharmD, PhD

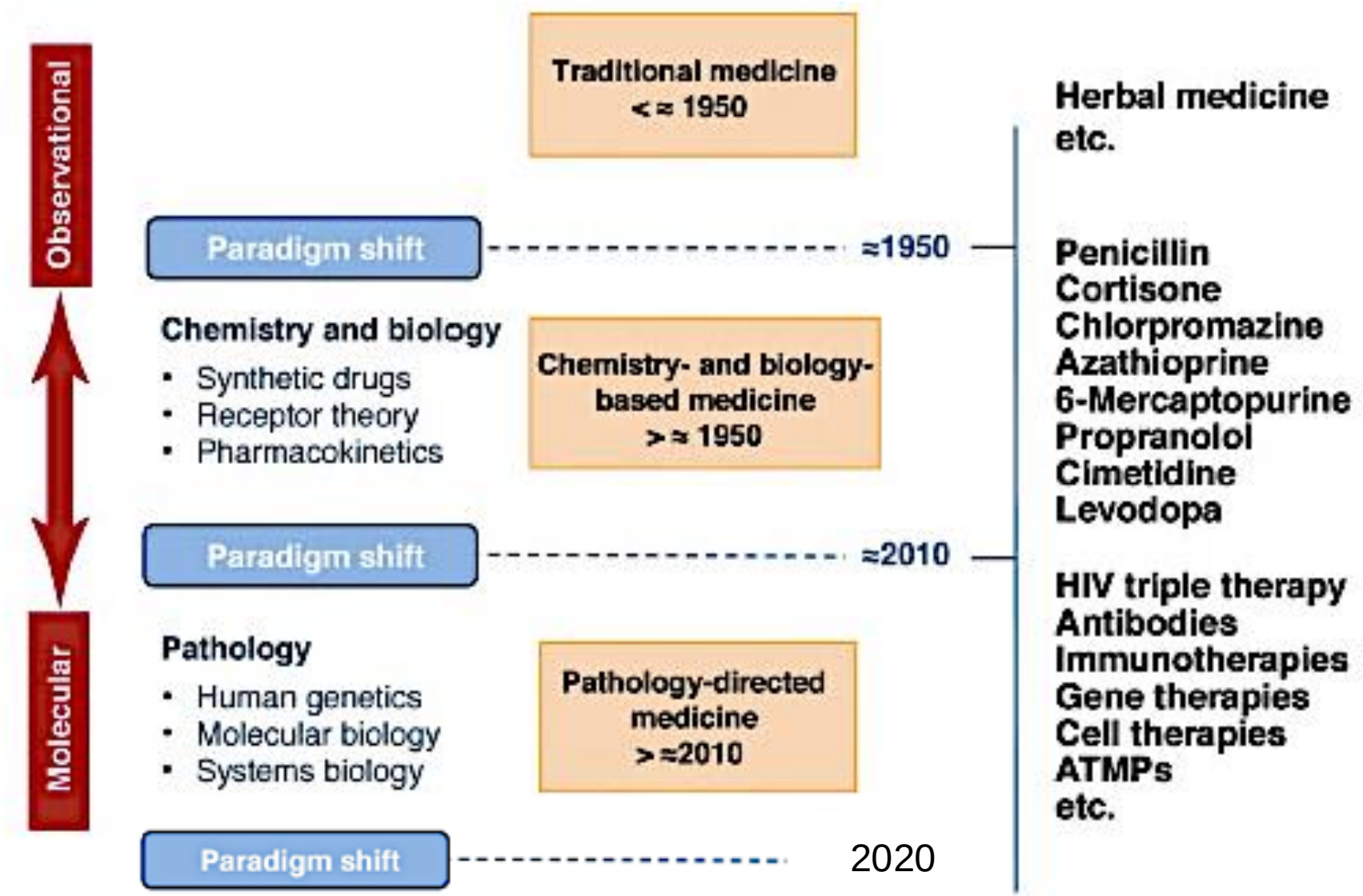
School of Pharmaceutical Sciences, University of Geneva, Switzerland

ISPSO
INSTITUT DES SCIENCES PHARMACEUTIQUES DE SUISSE OCCIDENTALE

edqm
European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

UNIVERSITÉ DE GENÈVE



Next: Information medicine?

01001001	01101110	01100110	01101111	01110010	01101101
01100001	01110100	01101001	01101111	01101110	00100000
01001101	01100101	01100100	01101001	01100011	01101001
01101110	01100101	01110011	00100000	11100010	10000000
10010011	00100000	00001010	01100001	00100000	01010000
01100001	01110010	01100001	01100100	01101001	01100111
01101101	00100000	01010011	01101000	01101001	01100110
01110100	00111111	00001010			

Central Dogma of Biology

DNA

what *can* be made

RNA

what *will* be made

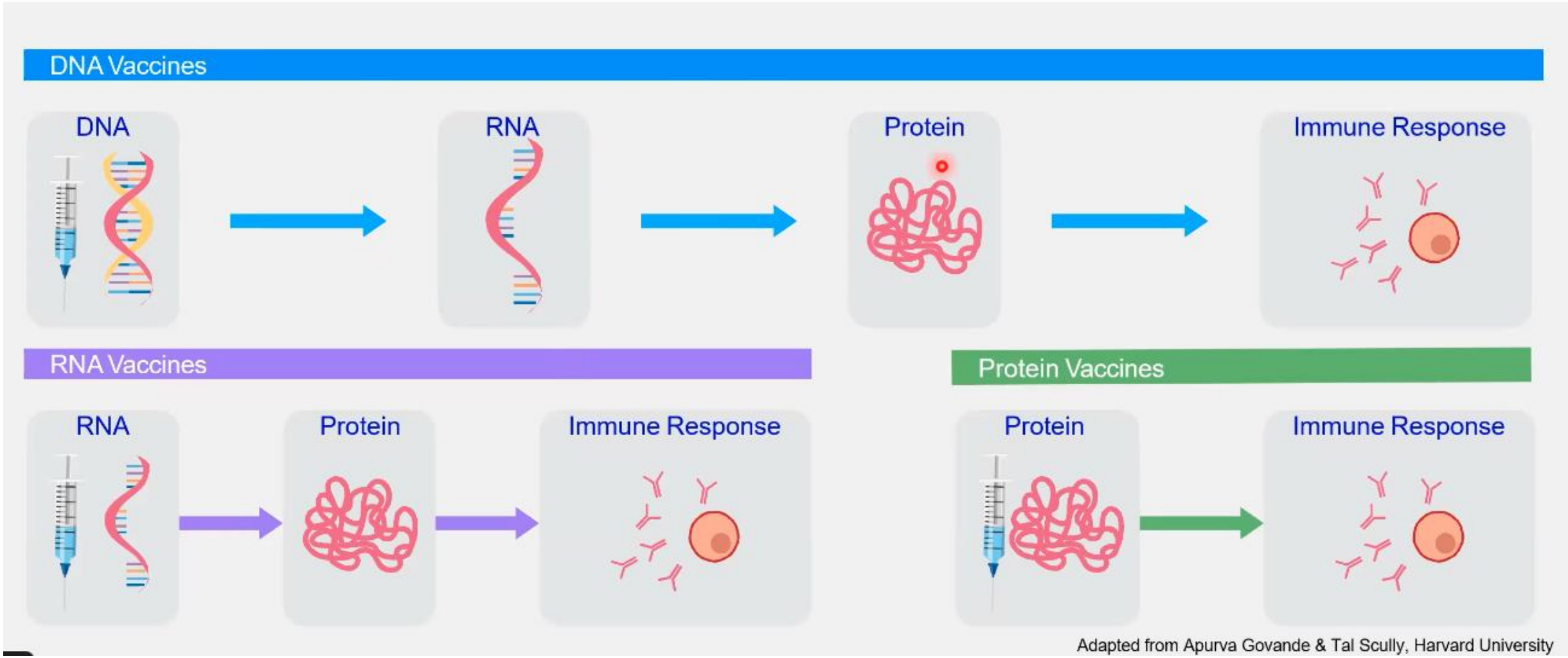
Protein

the product itself

Cells and Tissues

a collection of products together

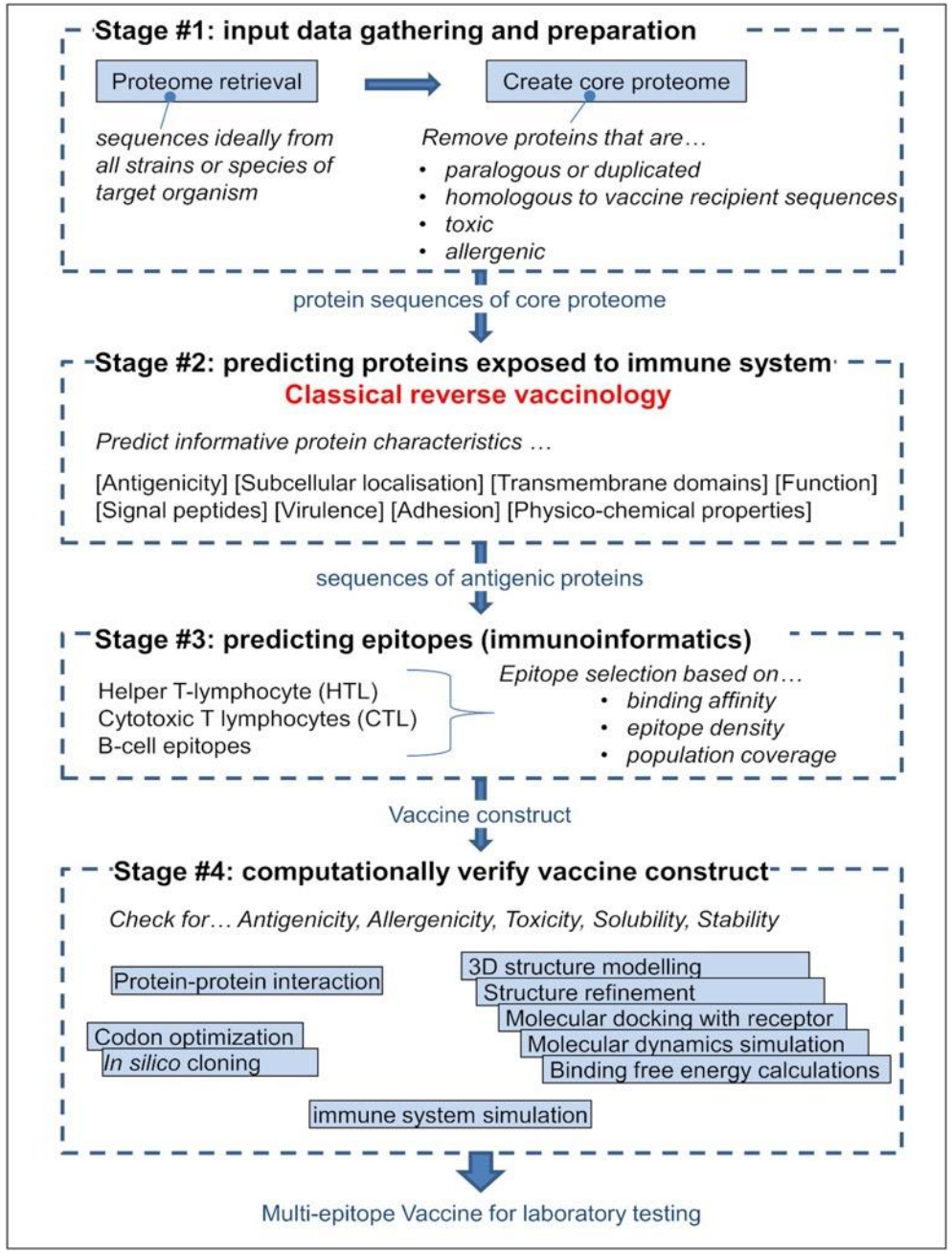
Applying the Central Dogma of Biology to Vaccines

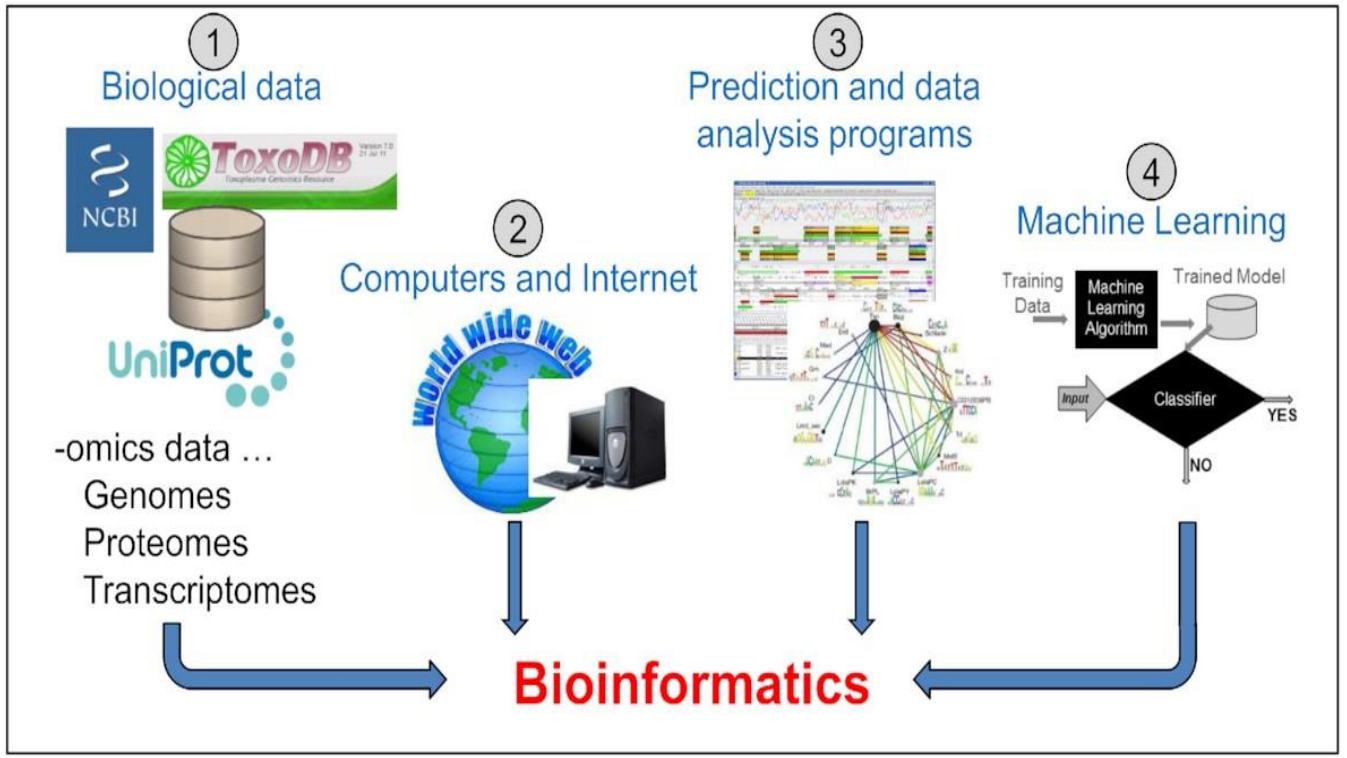


Adapted from Apurva Govande & Tal Scully, Harvard University

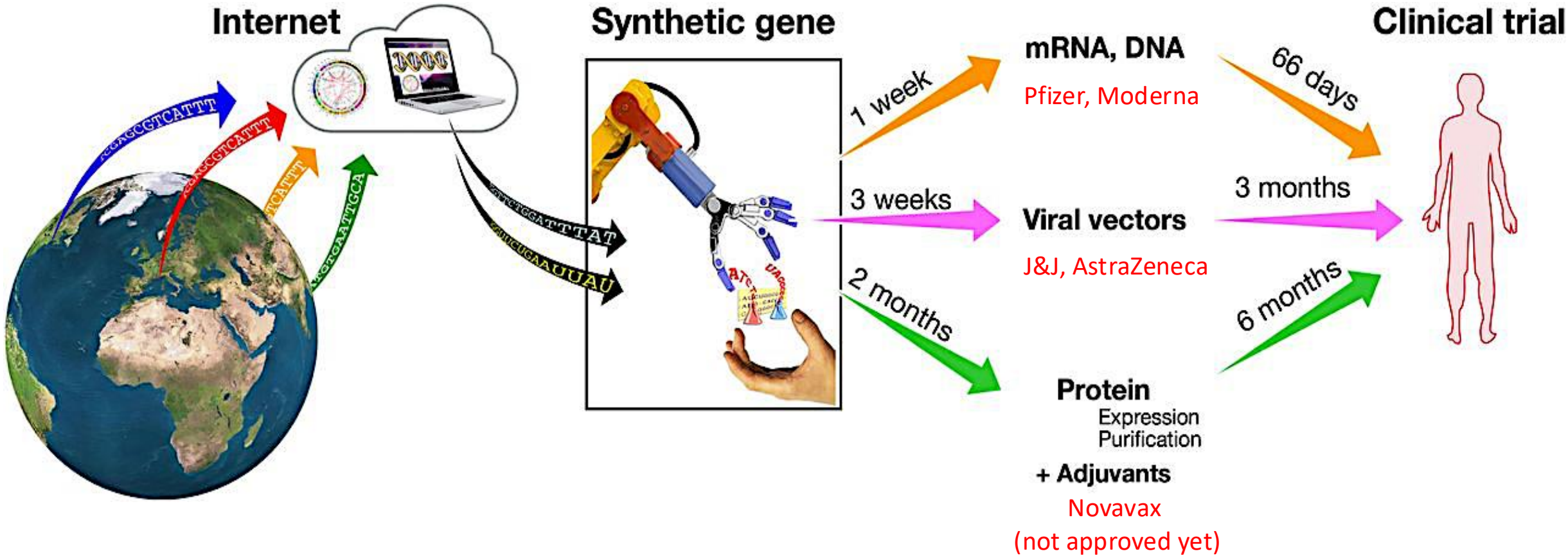
A typical reverse vaccinology workflow.

FEMS Microbiol Rev, Volume 47, Issue 2, March 2023, fuad004,
https://doi.org/10.1093/femsre/fuad004





The technological advances that have come together to develop a COVID-19 vaccine

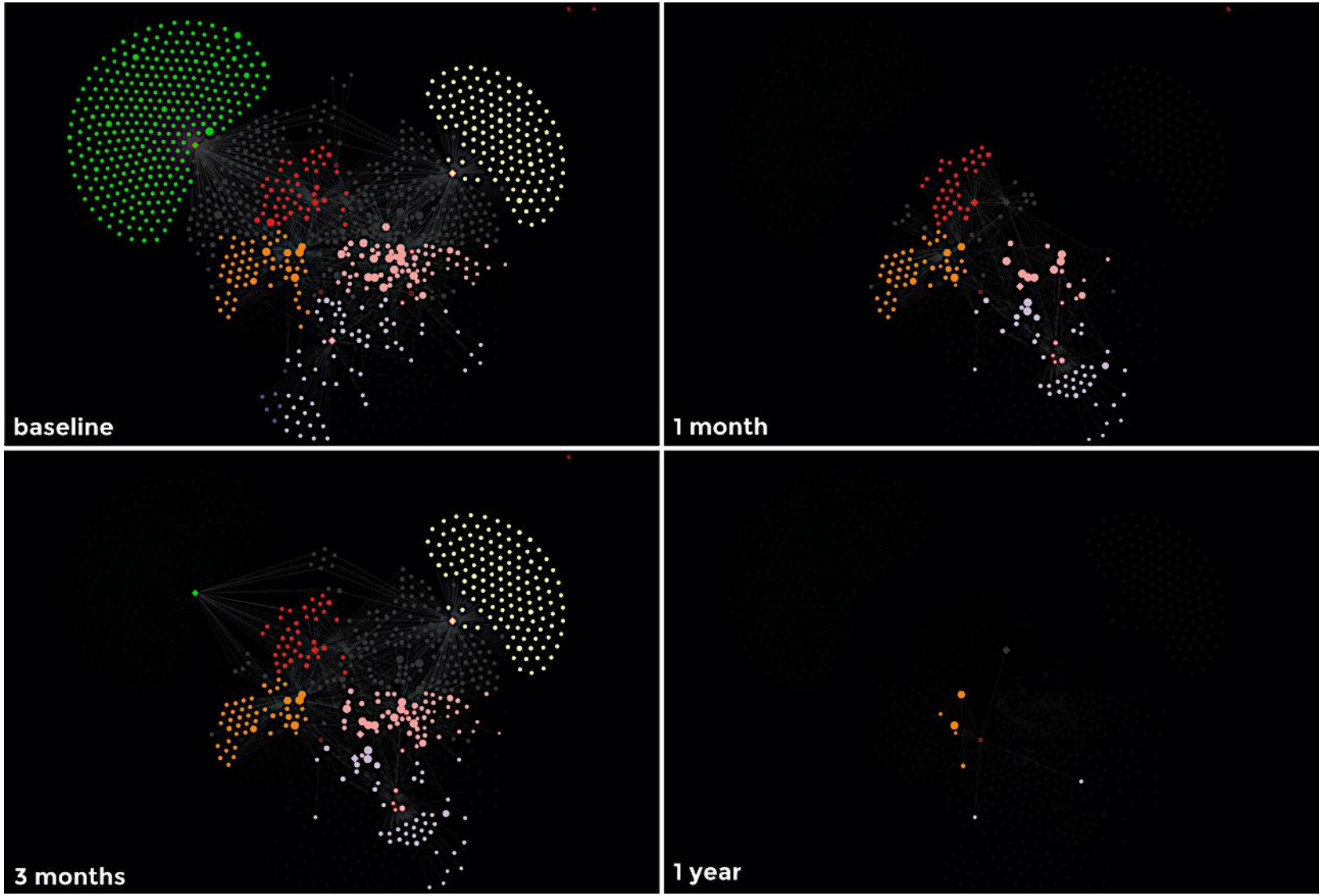


Rino Rappuoli et al. PNAS 2021;118:3:e2020368118

Diseases are complex and dynamic, as well...

Figure 1: An example of the outcome of a bioinformatics analysis combining patient data with the network analysis platform.

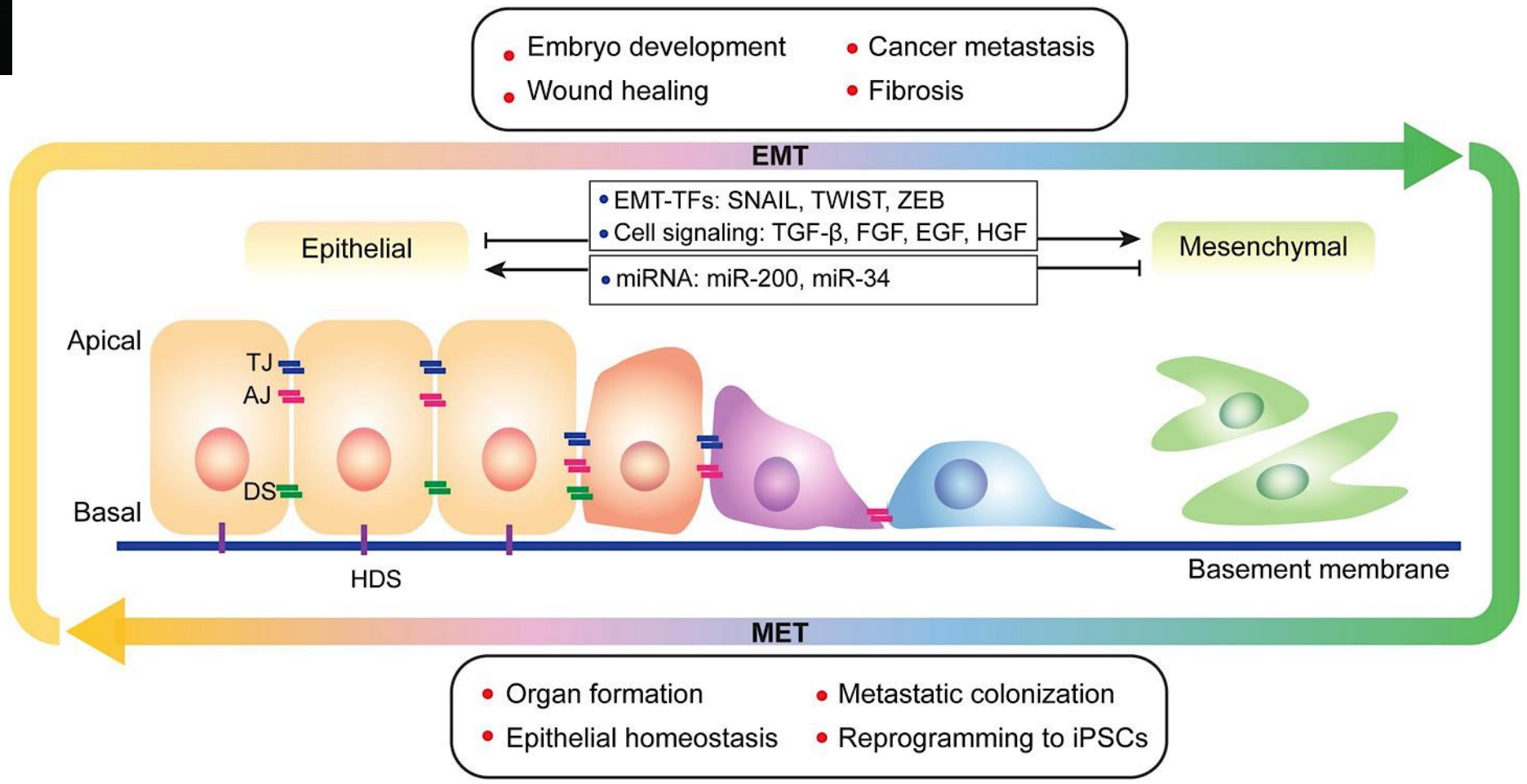
A network model reveals different molecules (nodes, scaled by centrality) and mechanisms (colored network clusters), relevant at different time points after a cardiac event.



What is needed to transfer the information to the patient?

KNOWLEDGE OF NATURE.

Influencing Epithelial – Mesenchymal Transition (EMT) by miRNA Delivery



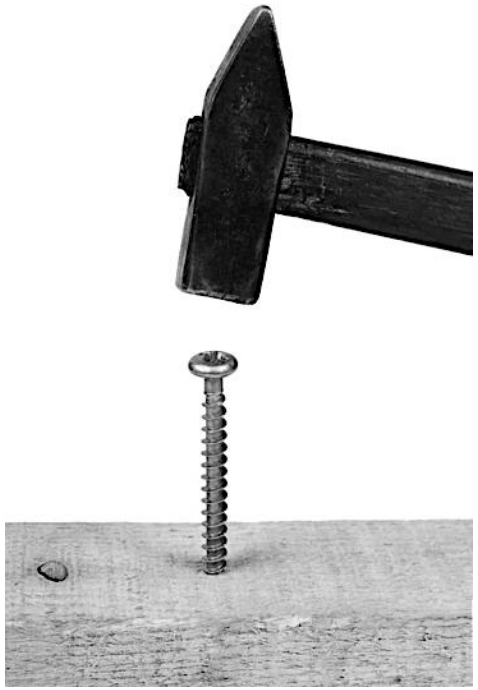
https://www.frontiersin.org/articles/10.3389/fcell.2020.00760/full

SO WHAT?

...and precisely rather personal.

YES?

NOT ALL PROBLEMS ARE NAILS.



You try to screw a screw with a hammer – you’re screwed...

AN APPROACH THAT TAKES INTO ACCOUNT DYNAMIC COMPLEXITY ON A PERSONALIZED SCALE IS NEEDED.

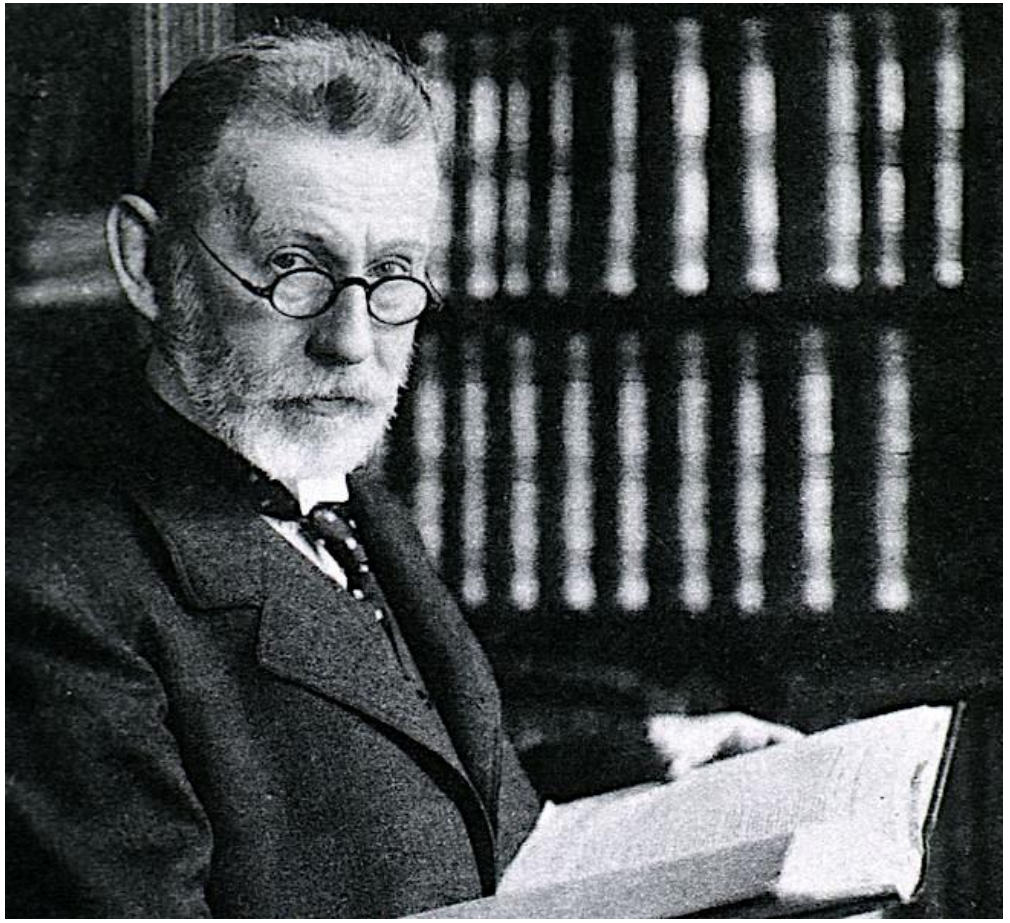
HOW?

We are in need of more complex tools.

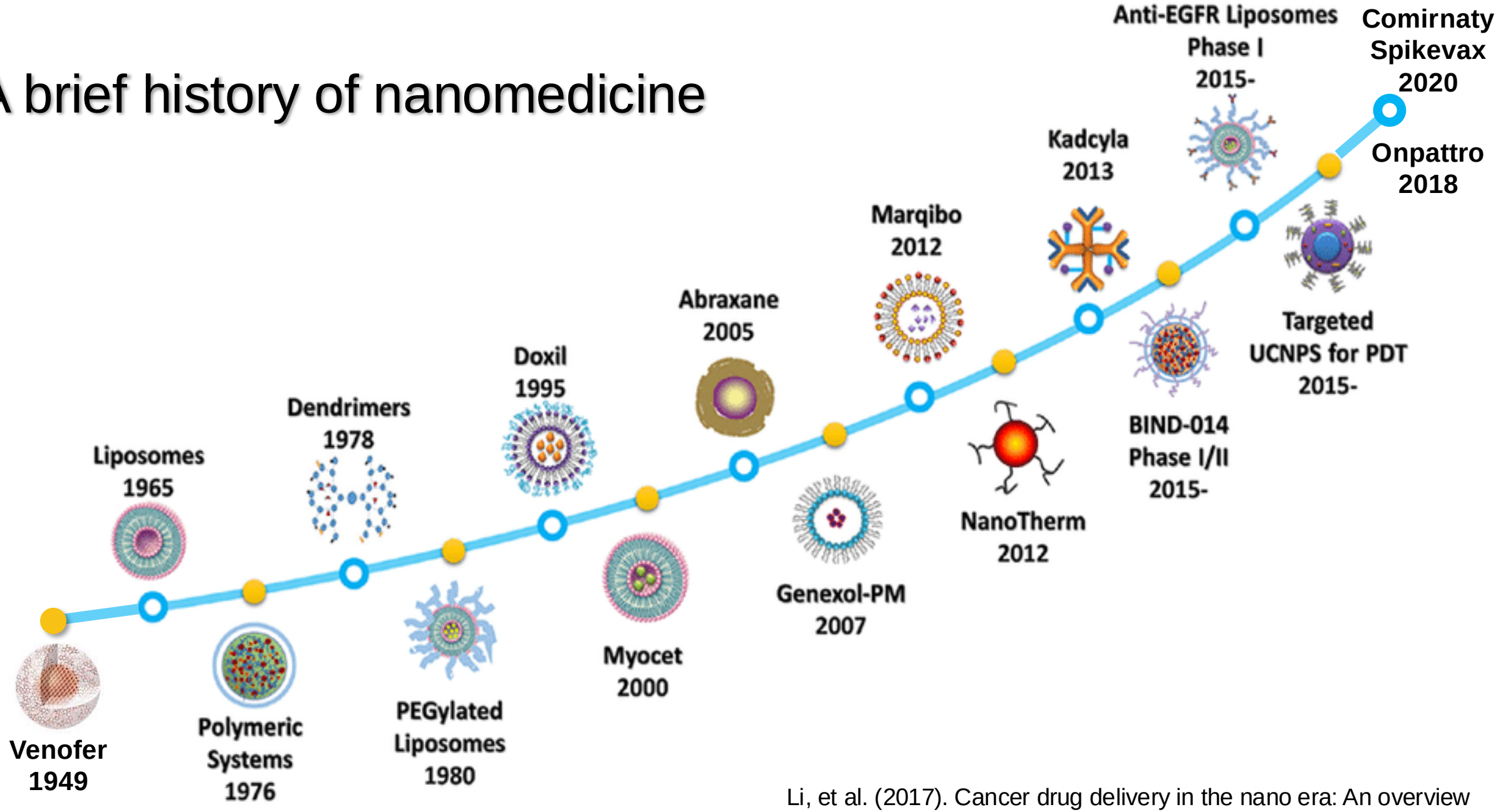
Paul Ehrlich et "*Der Freischütz*"

- *Der Freischütz* is a German opera by Carl Maria von Weber.
- *Freikugeln* ("magic bullets") are supplied by the devil in exchange for the soul of Max, the Prince's gamekeeper.
- These bullets, which hit their target, are used in a shooting competition to appoint the new gamekeeper and win the hand of Agathe, the gamekeeper's daughter.
- Paul Ehrlich (1854-1915) attended this opera in Frankfurt and gave it the concept of "drug targeting".

Honestly Paul, where are we?



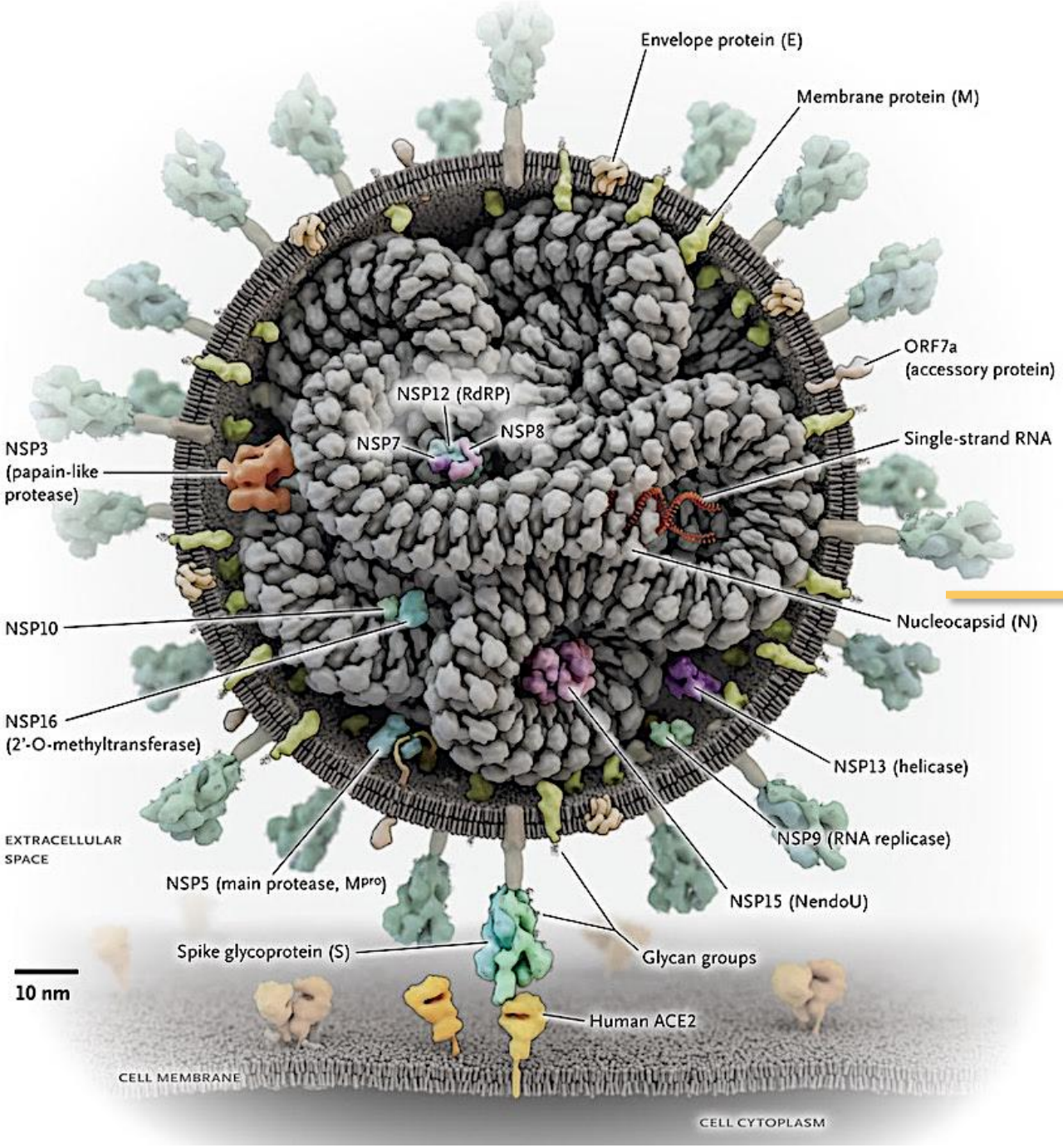
A brief history of nanomedicine



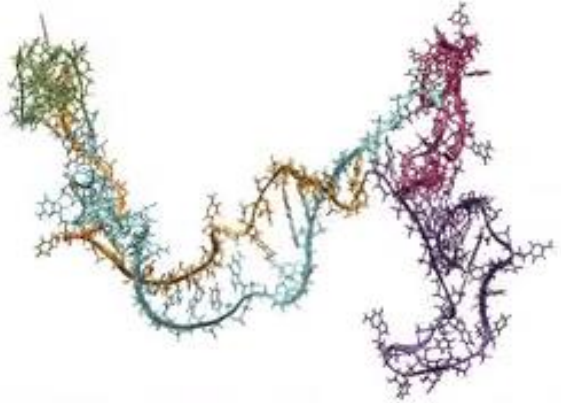
Li, et al. (2017). Cancer drug delivery in the nano era: An overview and perspectives. Oncology Reports. 38. doi: 10.3892/or.2017.5718.

*"**Disappointing outcomes** of nano-sized formulations (nanoformulations) in clinical studies indicate that our overall approach of nanomedicine needs serious reevaluation. (…) we all have to find the reality by absorbing the truth and fight our way out of the egg to **break the ill-conceived illusion of the nanomedicine.**"*

K. Park, J. Control. Rel. 267 (2017) 2-14

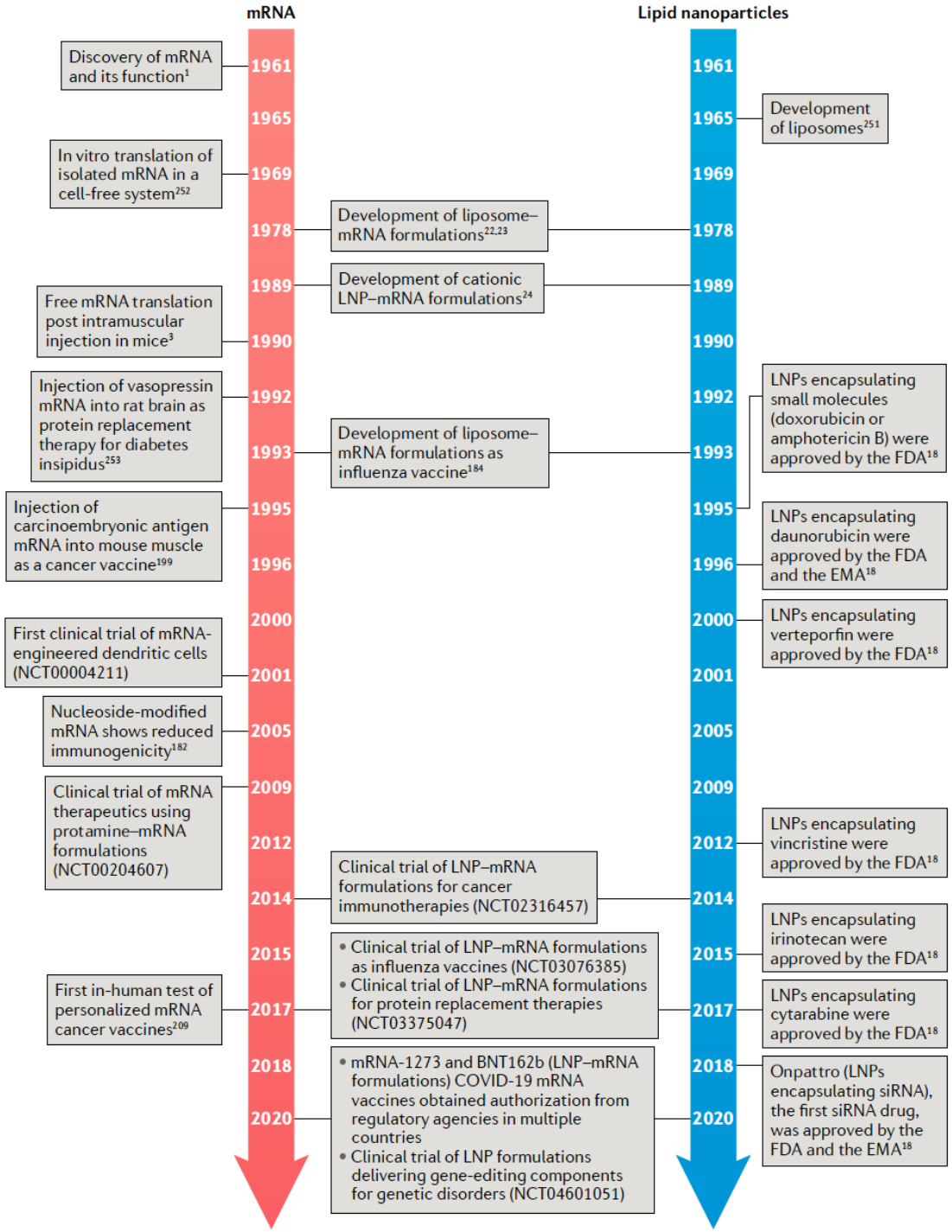


Pfizer/BioNTech's S-protein mRNA



4,284 nucleotides

1388 kDa molecular weight

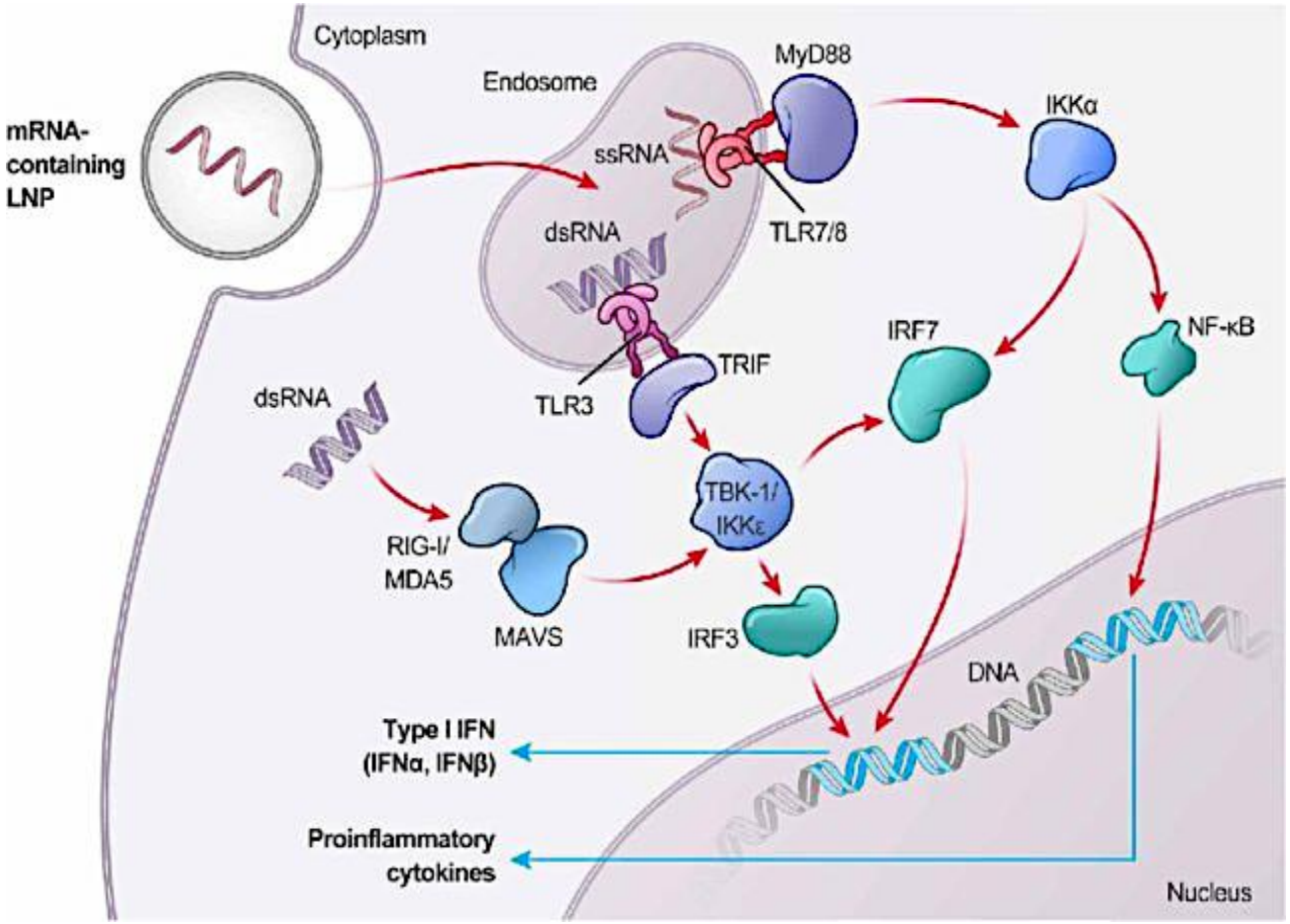


60 years of mRNA...

...and its formulation, the “lesser known sister”.

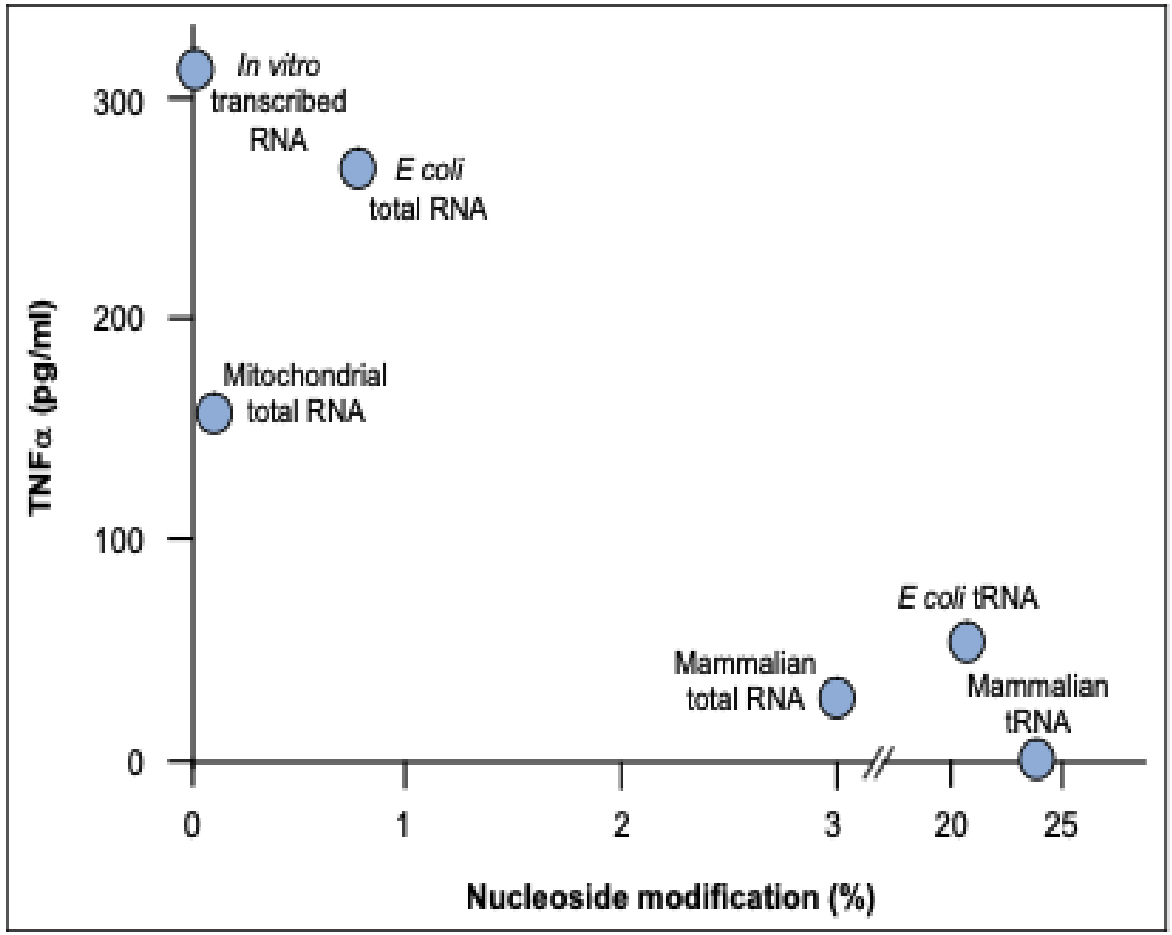
Hou, X., Zaks, T., Langer, R., Dong, Y.
Lipid nanoparticles for mRNA delivery. *Nat Rev Mater* (2021).
https://doi.org/10.1038/s41578-021-00358-0

The mRNA interacts with innate immunity receptors, causing inflammation.



Nelson et al., Sci. Adv. 2020, 6, DOI: 10.1126/sciadv.aaz6893

Modifications of natural nucleosides suppress the immunostimulant activity of RNA.



K. Karikó & D. Weissman, Curr. Opin. Drug Discov. Devel. 2007, 10:523-32.

Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability

Katalin Karikó[1], Hiromi Muramatsu[1], Frank A Welsh[1], János Ludwig[2], Hiroki Kato[3], Shizuo Akira[3] and Drew Weissman[4]

[1]Department of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [2]Laboratory of RNA Molecular Biology, The Rockefeller University, New York, New York, USA; [3]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; [4]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

«I felt like a God!»

Mol. Therap. 16, 2008, 1833-1840, doi: 10.1038/mt.2008.200

2023

2021

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Sciences
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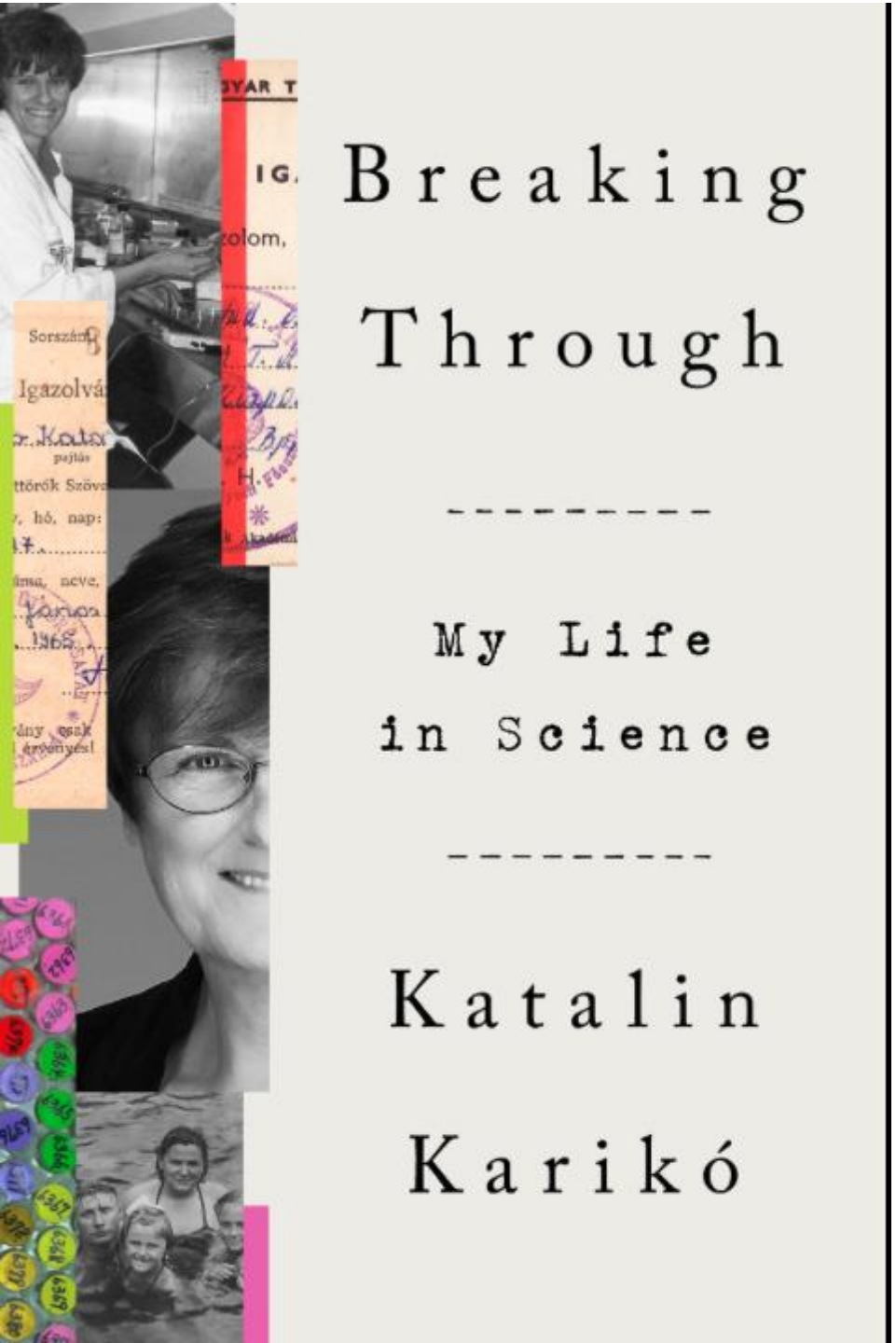
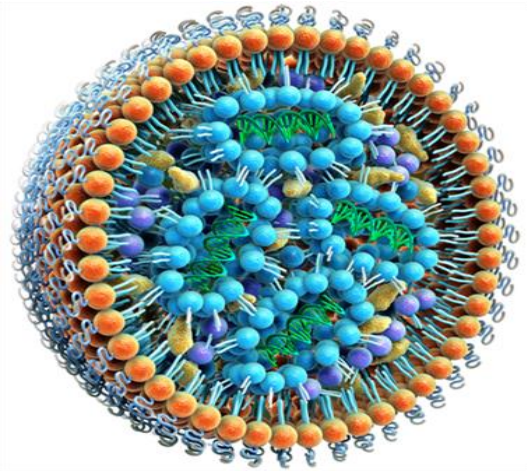


“Our home is simple, small. It is constructed, literally, from the earth that surrounds it: clay and straw, pressed into adobe walls, whitewashed, then covered with a thick roof of reeds.

We live in a single room. The house is larger than this one room, but for most of the year, the other rooms are too cold for anything but storage. We live where the heat is.”

What are we talking about?



Step 1: DNA template

- prepare DNA (*E. coli*, cell-free)
- purify linear DNA template
- Freeze product

Step 2: mRNA

- prepare mRNA (cell-free)
- purify mRNA
- Freeze product

Step 3: Drug product

- formulate LNP
- buffer exchange by TFF
- filter-sterilize
- fill & finish, freeze

At the onset of the pandemic, very few companies were able to manufacture GMP grade DP!

Storage ?

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UNIVERSITÉ DE GENÈVE

Chula VRC
Chula Vaccine Research Center
Faculty of Medicine, Chulalongkorn University

Freeze-drying a monovalent mRNA-LNP dengue serotype 1 vaccine

A. Ramos Barros[1], Aya Halmi[1], C. Khawsang[2], E. Prompetchara[2],C. Ketloy[2], G. Borchard[1]

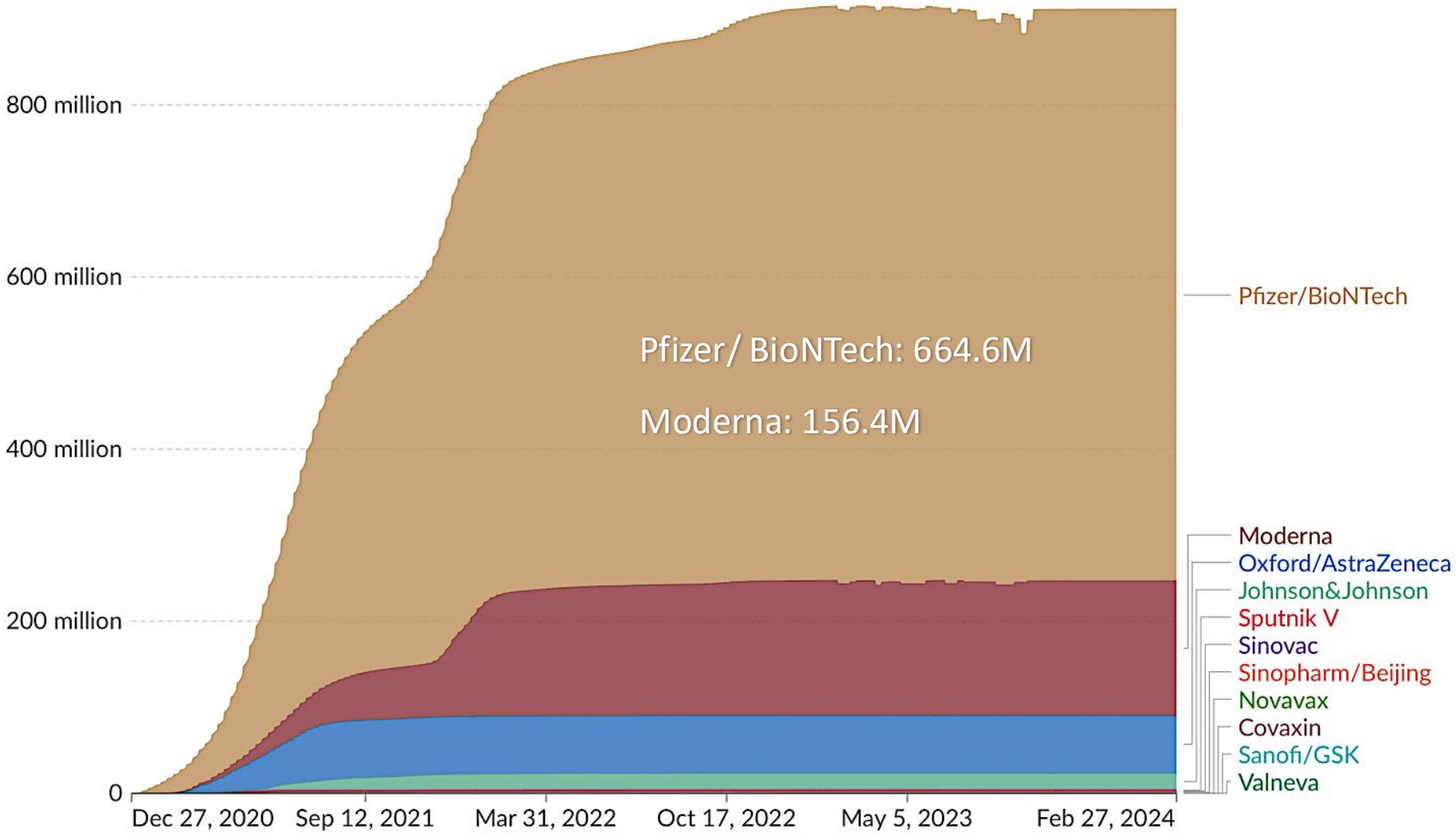
[1]Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Rue Michel-Servet 1, Geneva, Switzerland

[2]Chula Vaccine Research Center (VRC), Faculty of Medicine, Chulalongkorn University,1873 RamaIV Rd., PathumWan, Bangkok, 10330, Thailand

COVID-19 vaccine doses administered by manufacturer, European Union

Our World in Data

All doses, including boosters, are counted individually.



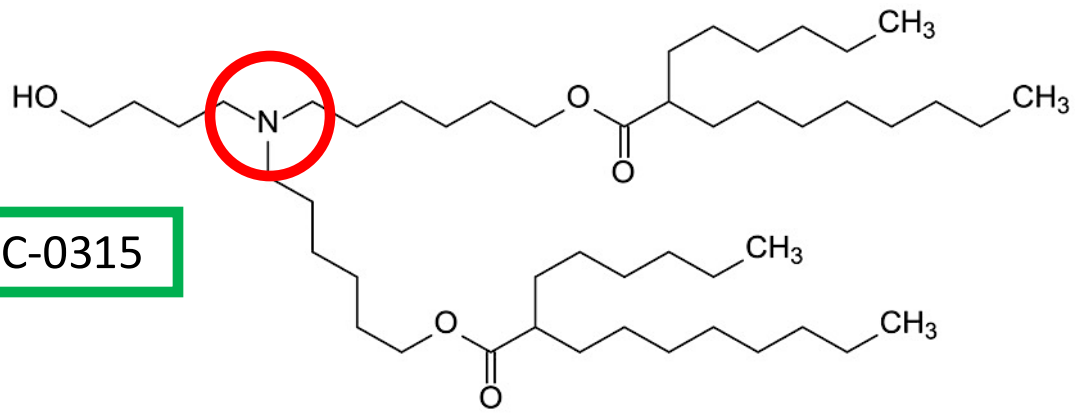
Pfizer/ BioNTech: 664.6M

Moderna: 156.4M

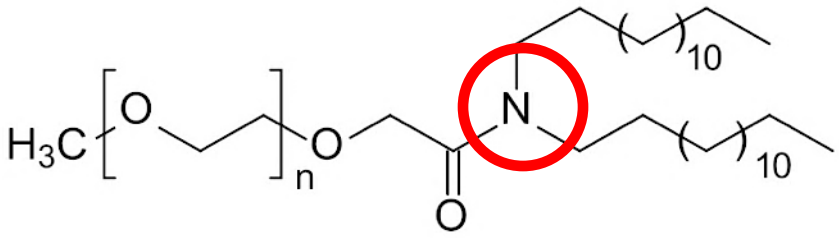
Data source: Official data collated by Our World in Data

OurWorldInData.org/covid-vaccinations | CC BY

Comirnaty's formulation

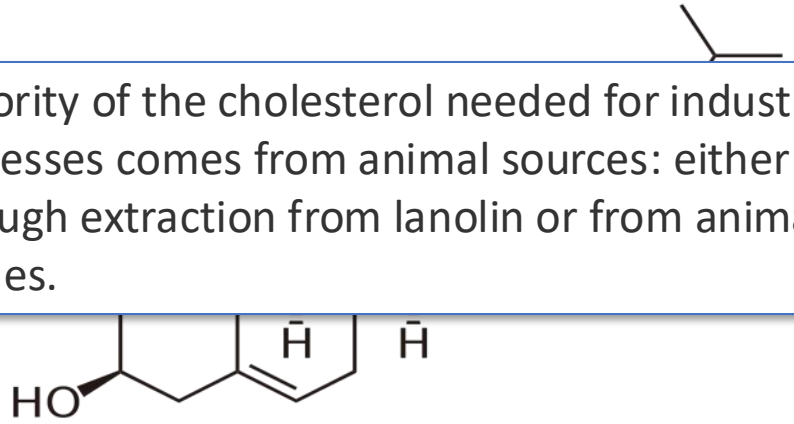


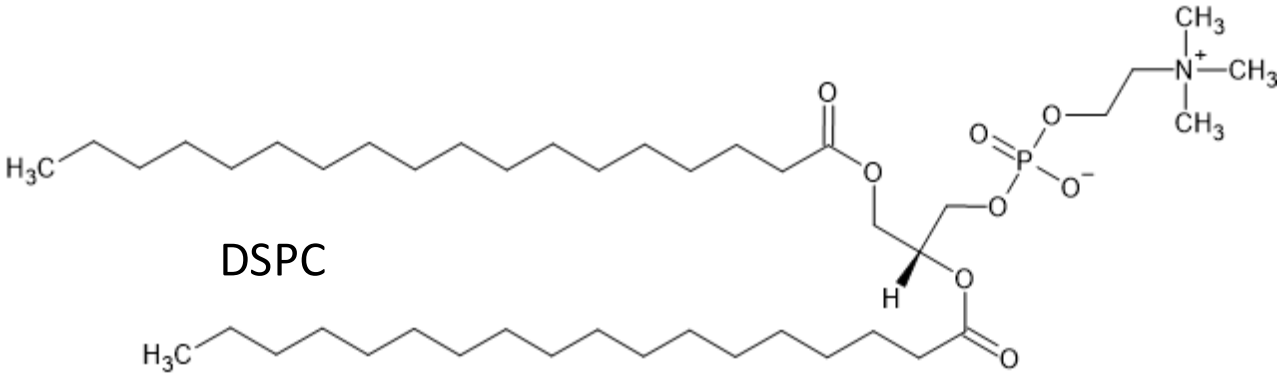
ALC-0315



ALC-0159

Majority of the cholesterol needed for industrial processes comes from animal sources: either through extraction from lanolin or from animal tissues.



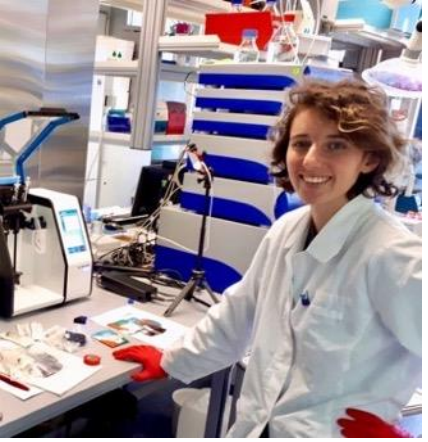


DSPC

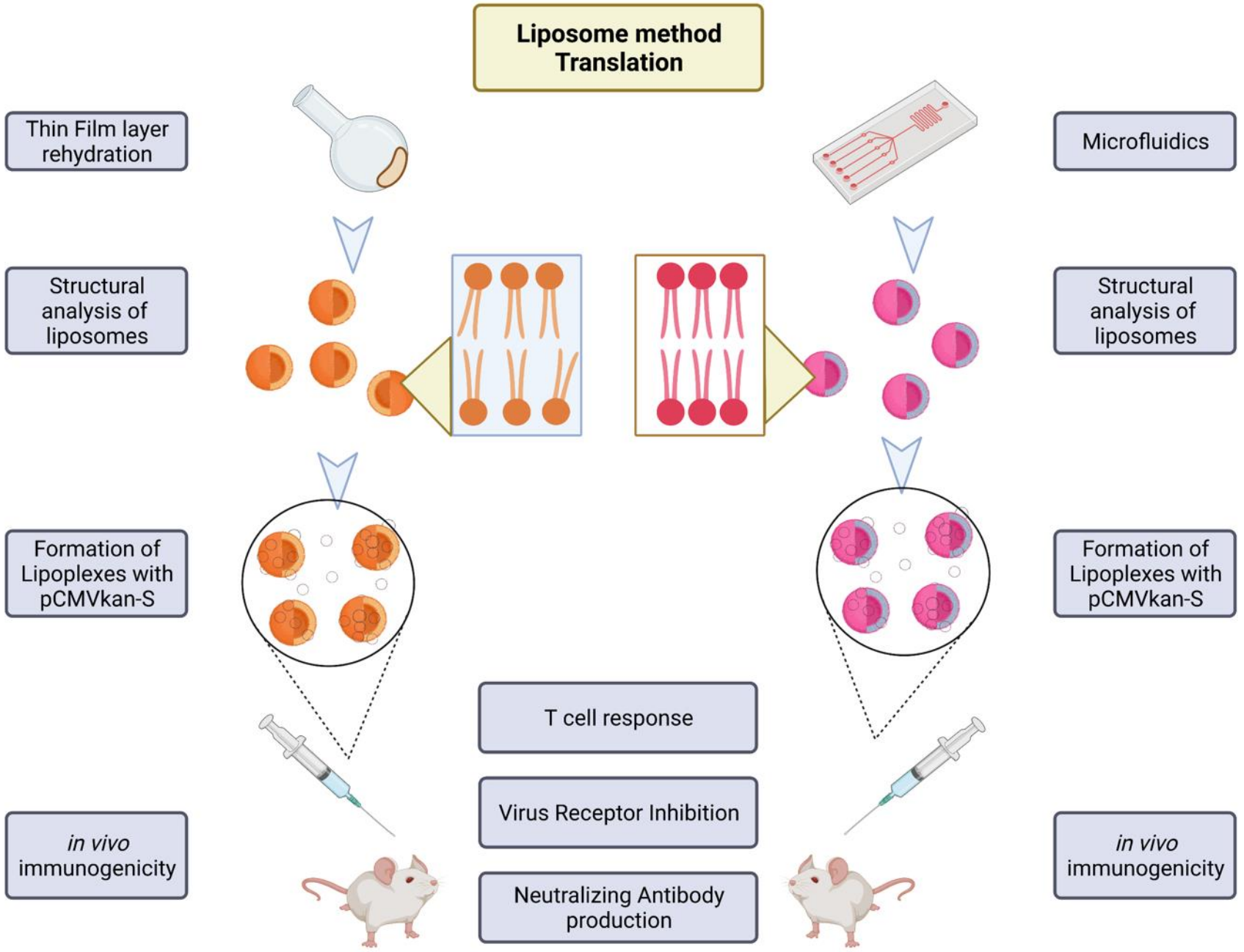
0.2 mg/dose = 130 tons in EU doses alone

How do you source and assure quality of these compounds for billions of doses?

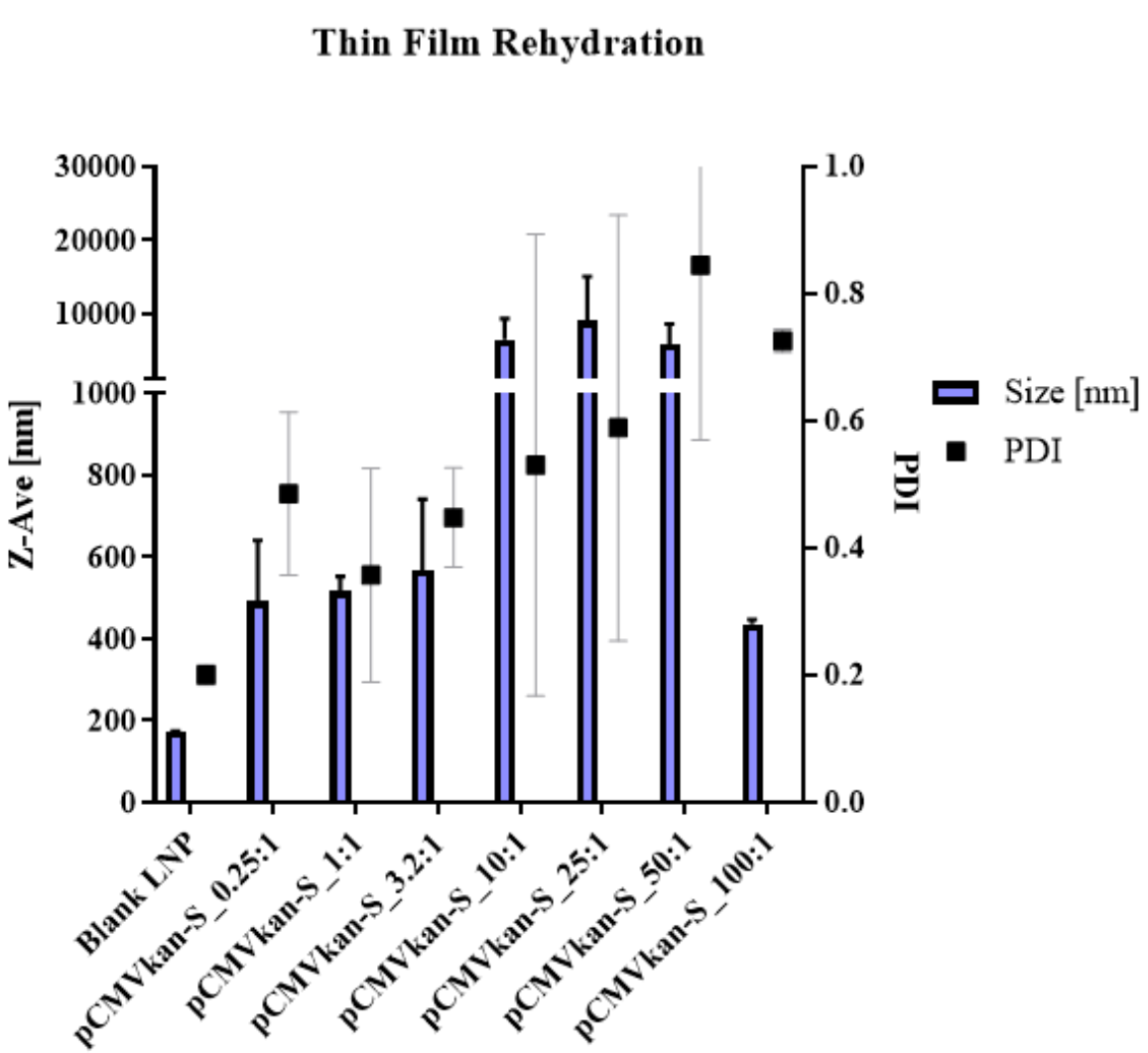
KCl, KH_2PO_4, NaCl, Na_2HPO_4, sucrose, aq. ad inj.

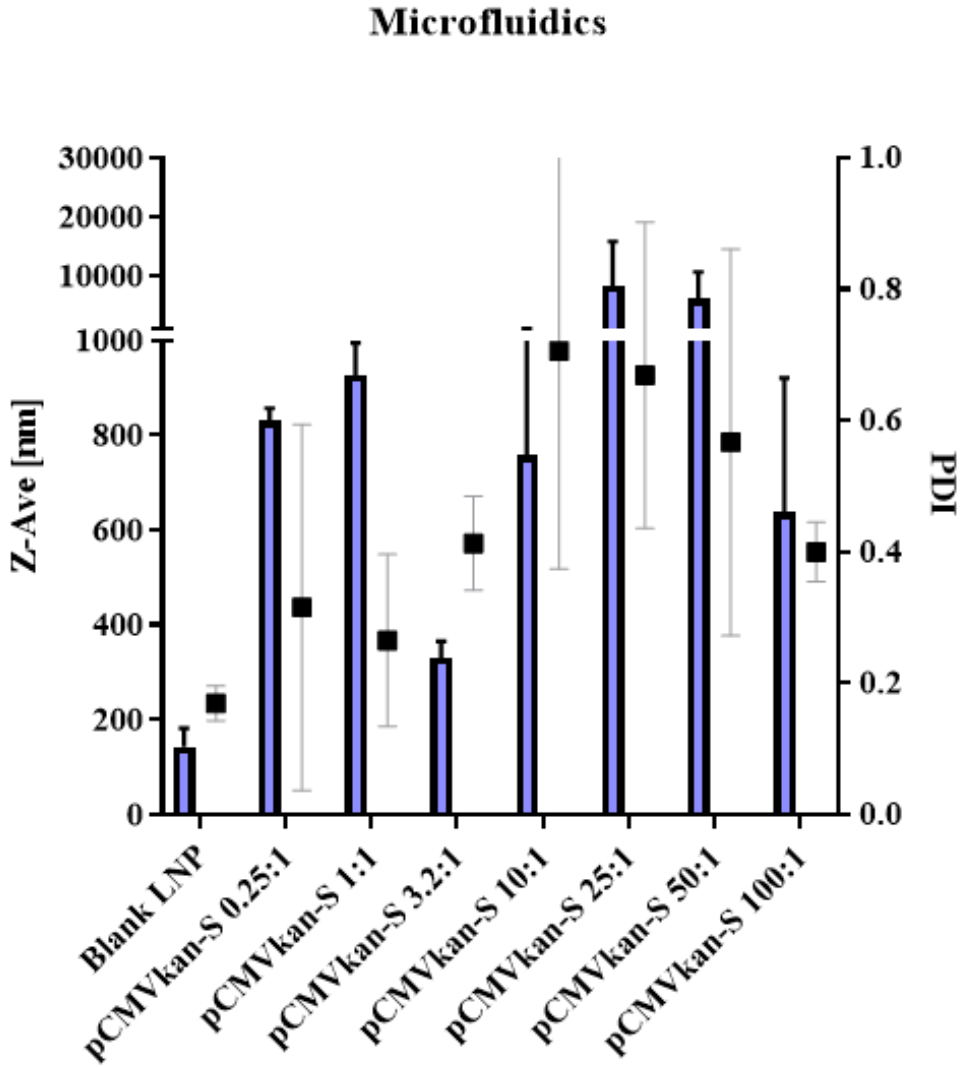


Project structure: liposomal Covid-19 DNA vaccine



DLS results of DOTAP4/pCMVkan-S complexes at different N/P ratios

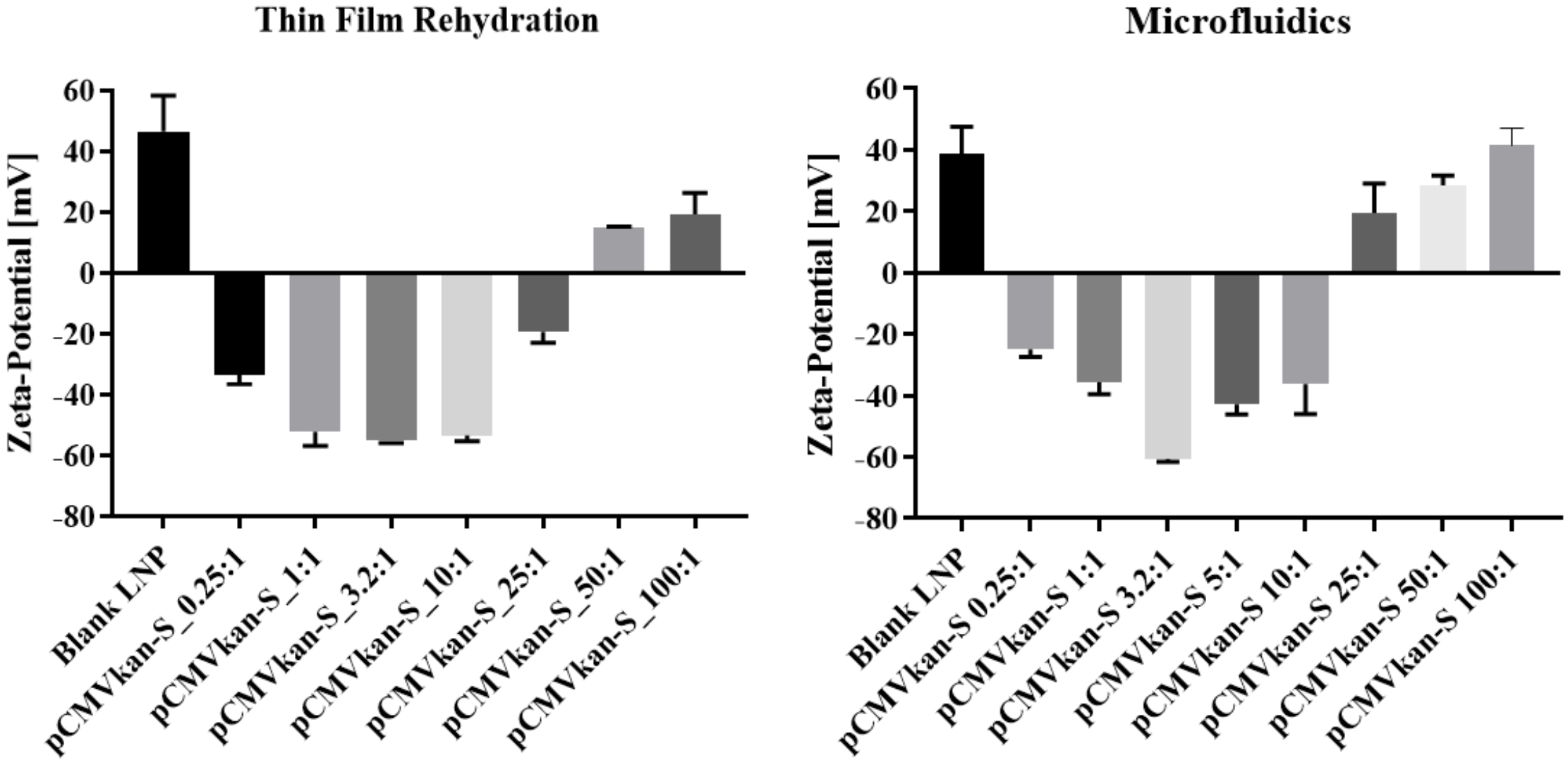




Mean ± SD (N=3).

Peletta et al., Pharmaceutics 14 (2022) 1427

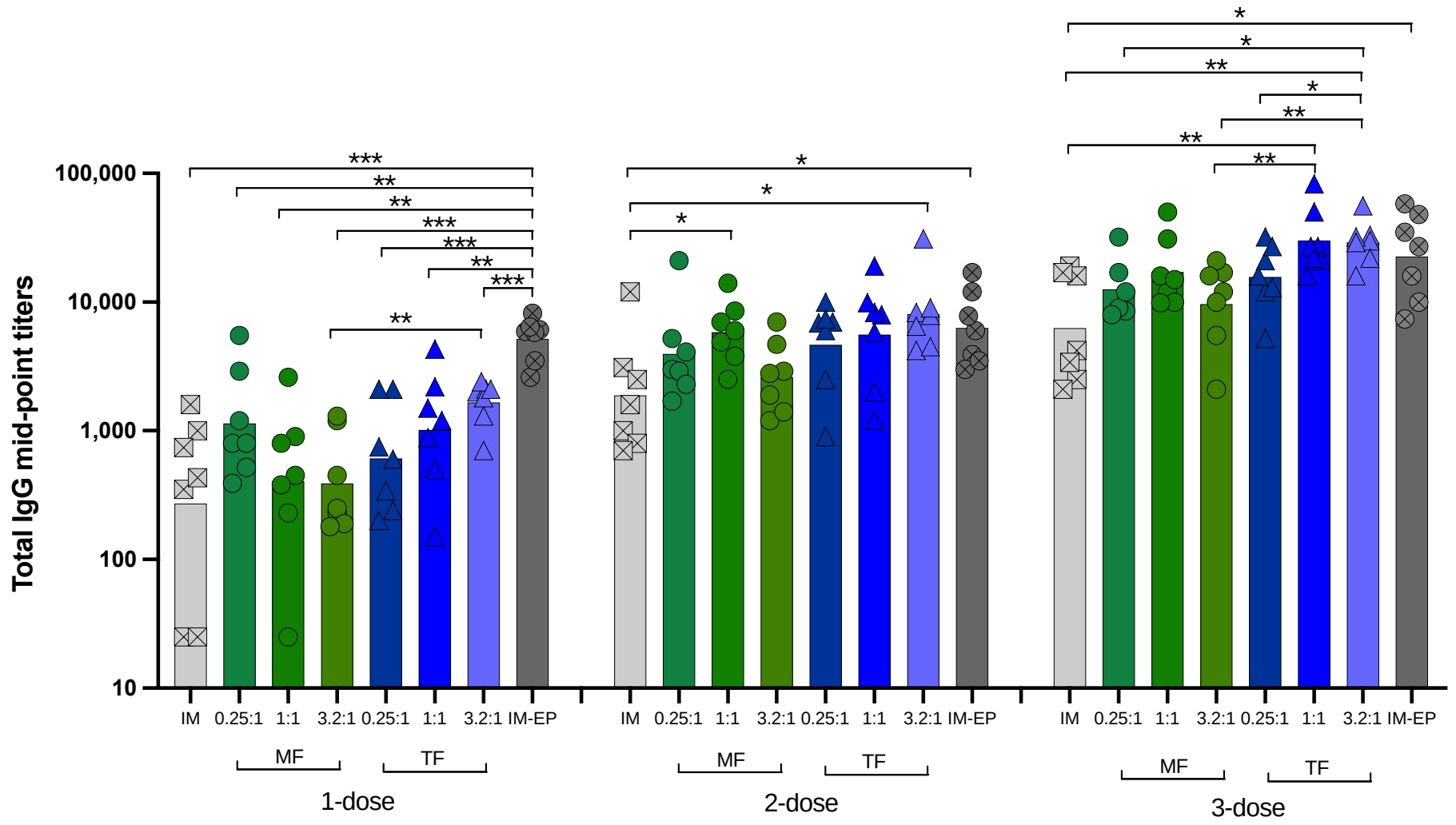
Zeta-potential of DOTAP4/pCMVkan-S complexes at different N/P ratios



Peletta et al., Pharmaceutics 14 (2022) 1427

mean ± SD (N=3).

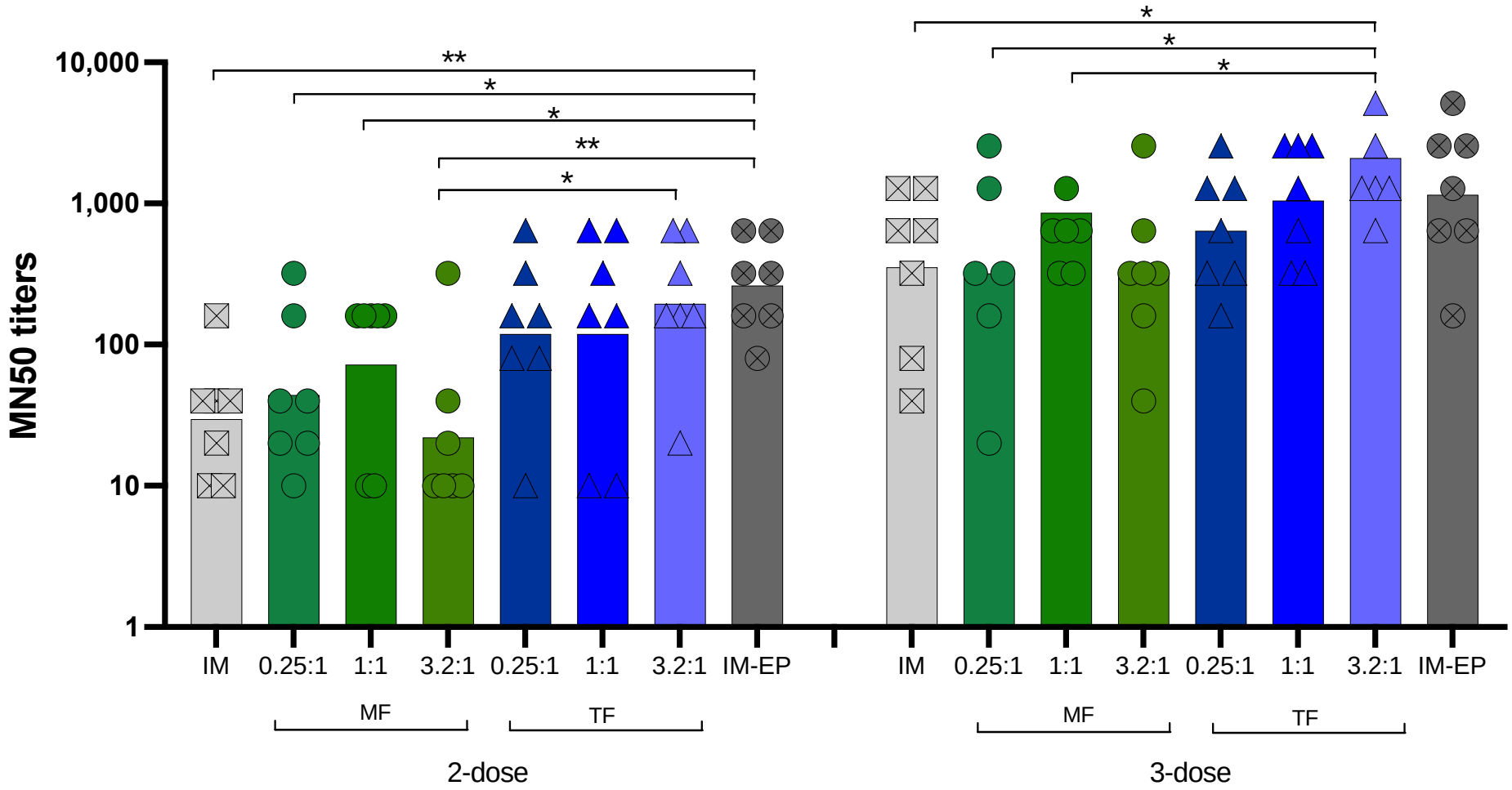
Midpoint titers of SARS-CoV-2 spike-specific total IgG



Peletta et al., Pharmaceutics 14 (2022) 1427

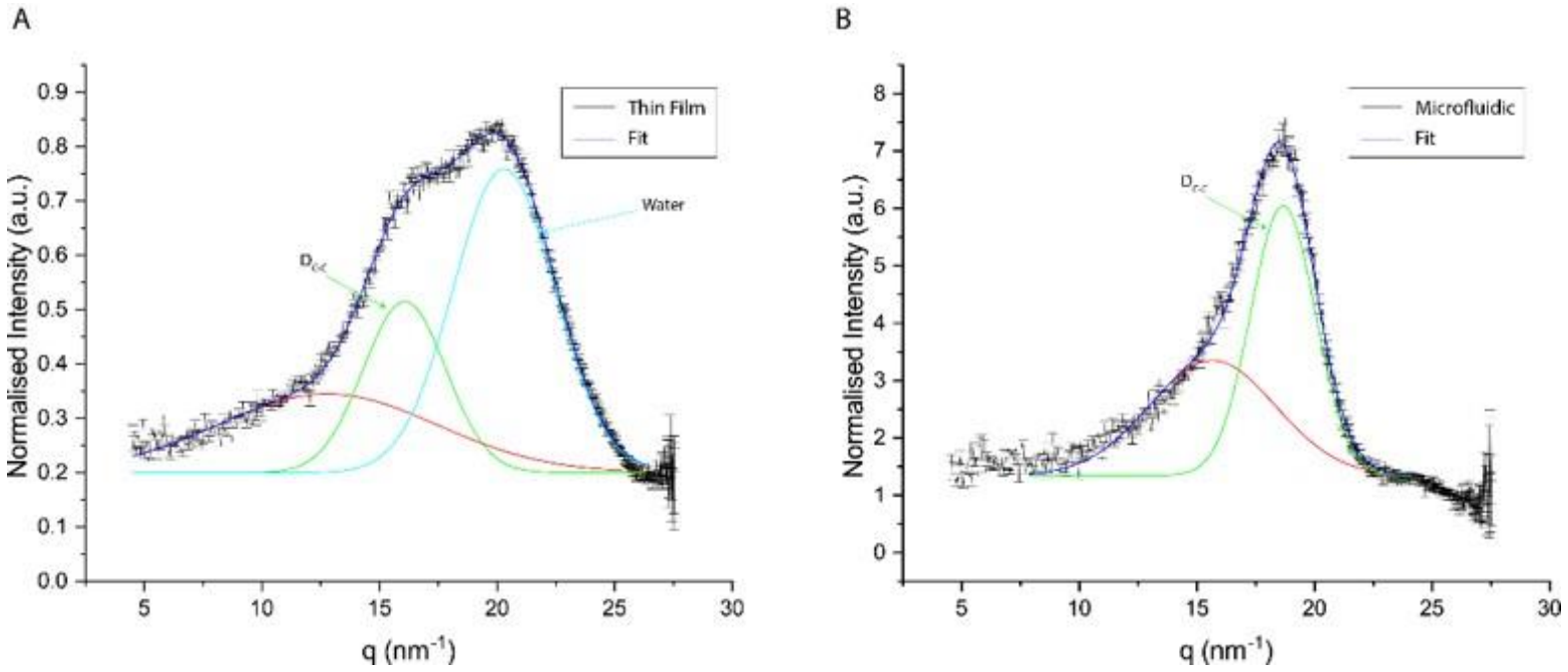
Weeks 2, 4, and 6 in mice immunized intramuscularly with naked pCMVkan-S (IM), pCMVkan-S formulated with DOTAP4 at N/P ratios of 0.25:1, 1:1, and 3.2:1 manufactured by thin film rehydration (TF) or microfluidics (MF) methods and by using electroporation device (IM-EP). Each bar represented GMT of the midpoint IgG titers in each group (n = 7). *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Live-virus neutralization assay

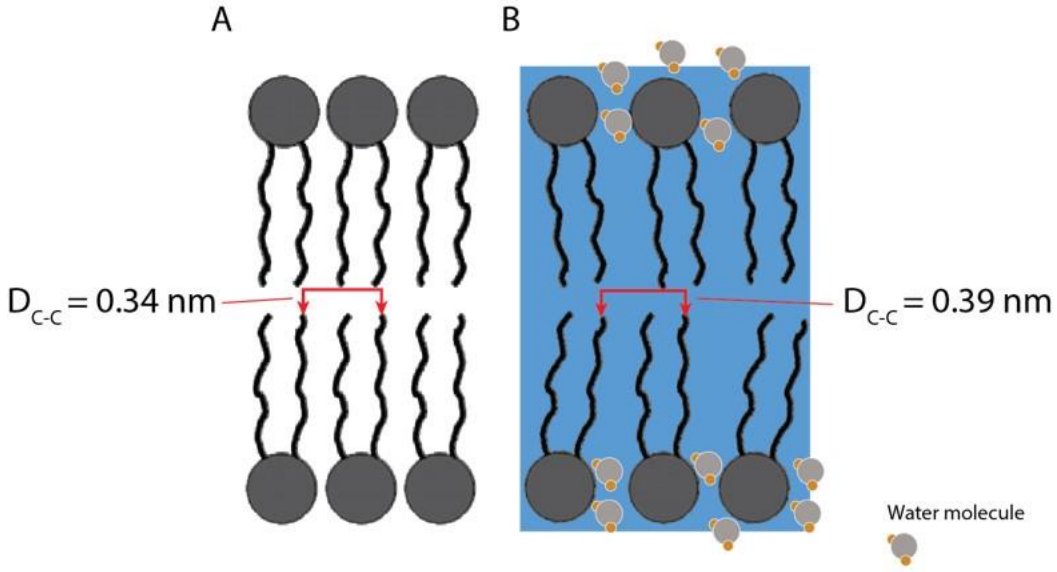


Peletta et al., Pharmaceutics 14 (2022) 1427

2- and 3-dose vaccine immunized i.m. with naked pCMVkan-S (IM), pCMVkan-S formulated with DOTAP4 at N/P ratios of 0.25:1, 1:1, and 3.2:1 manufactured by thin film rehydration (TF) or microfluidics (MF) methods and by using electroporation device (IM-EP). Each bar represented GMT of the MN50 titers in each group (n = 7). * and ** indicate $p < 0.05$ and $p < 0.01$, respectively.



Fitting results of wide-angle X-ray scattering (WAXS) peak in both A: MF and B: TF samples



Schematic representation of the lipid structure in liposome mono-bilayers prepared via A: microfluidics, B: thin-film re-hydration (blue square represent high hydration level).

The inter-chain distance differences as well as the degree or disorder was exaggerated in the schematic to highlight structural differences of both preparation methods.

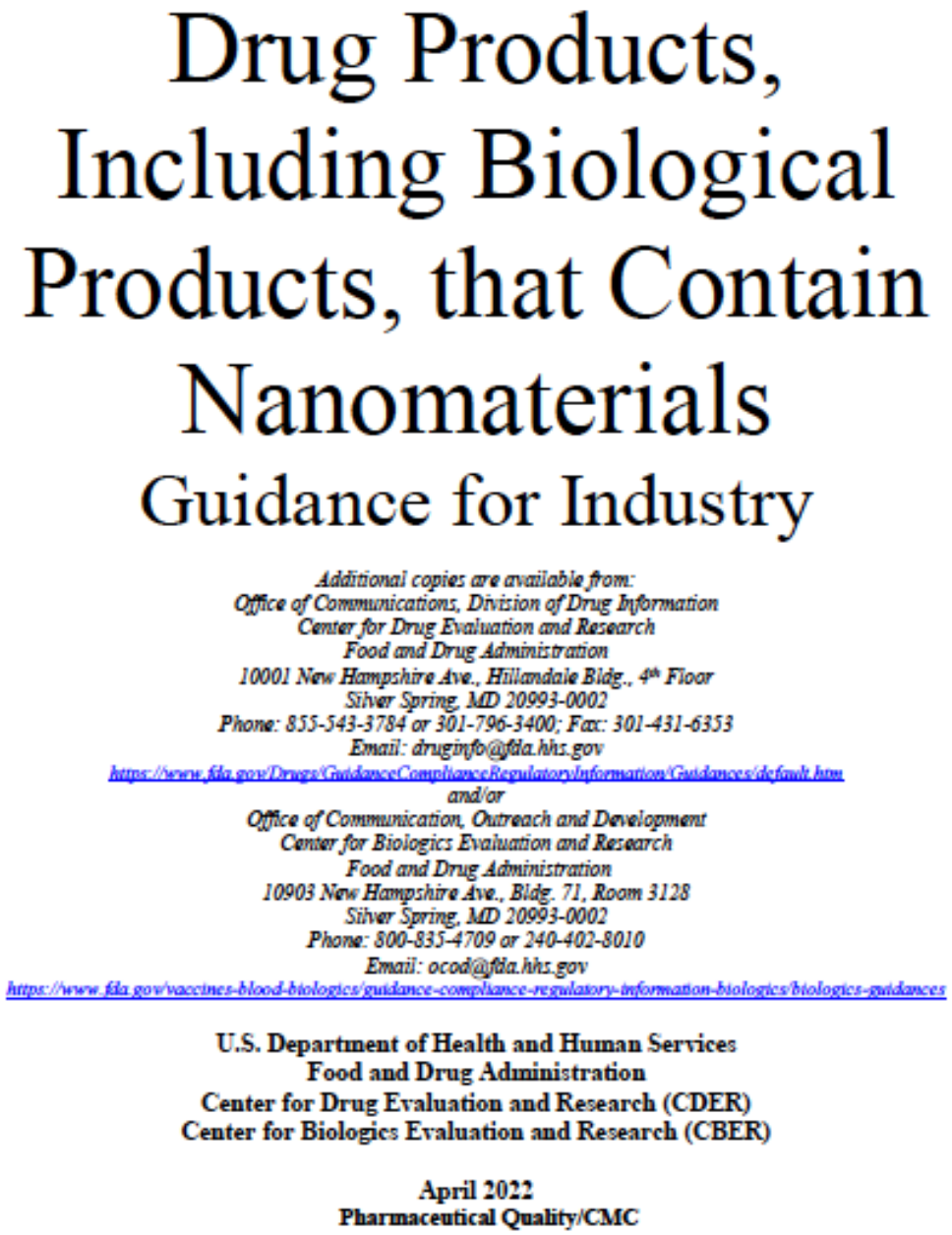
How to regulate information medicines to ensure their safety and efficacy?

Regulatory view(s)...

	Drug Substance (DS)		Drug Product (DP)	
Name	**EMA**	**FDA**	**EMA**	**FDA**
Onpattro	Double-stranded siRNA (ds-siRNA) patisiran sodium	Double-stranded siRNA (ds-siRNA) patisiran sodium	LNP (four lipids) + DS	LNP (four lipids) + DS
Spikevax	modified mRNA	modified mRNA + two lipids*	LNP (four lipids)	LNP (two lipids) + DS
Comirnaty	modified mRNA	modified mRNA	LNP (four lipids) + DS	LNP (four lipids) + DS

*PEG2000-DMG and SM-102 mentioned as “starting materials” for drug substance

Hemmrich, E., McNeil, S. Active ingredient vs excipient debate for nanomedicines. *Nat. Nanotechnol.* **18**, 692–695 (2023)



Drug Products, Including Biological Products, that Contain Nanomaterials

Guidance for Industry

Additional copies are available from:
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353
Email: druginfo@fda.hhs.gov
https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
and/or
Office of Communication, Outreach and Development
Center for Biologics Evaluation and Research
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 71, Room 3128
Silver Spring, MD 20993-0002
Phone: 800-835-4709 or 240-402-8010
Email: ocod@fda.hhs.gov
https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

April 2022
Pharmaceutical Quality/CMC

FDA draft guidance: Quality attributes

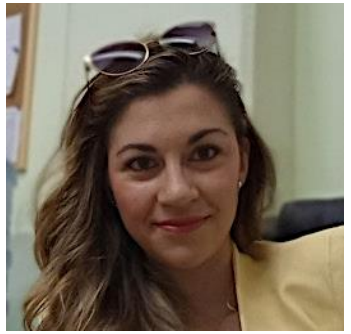
Always:

- Chemical composition
- Average particle size and distribution
- Shape and morphology
- Physical and chemical stability
- Free API, *in vitro* release kinetics

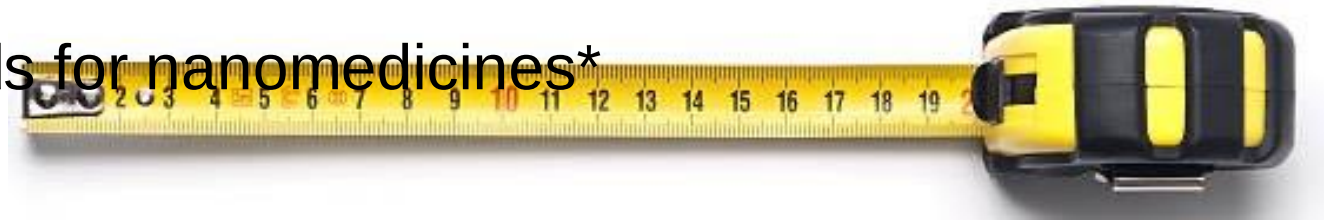
Potentially:

- Structural attributes related to function
- Surface and coating properties
- Porosity, density
- Particle concentration
- Crystal form
- Impurities, **sterility** and **endotoxin levels**

Characterization of CQAs: How?



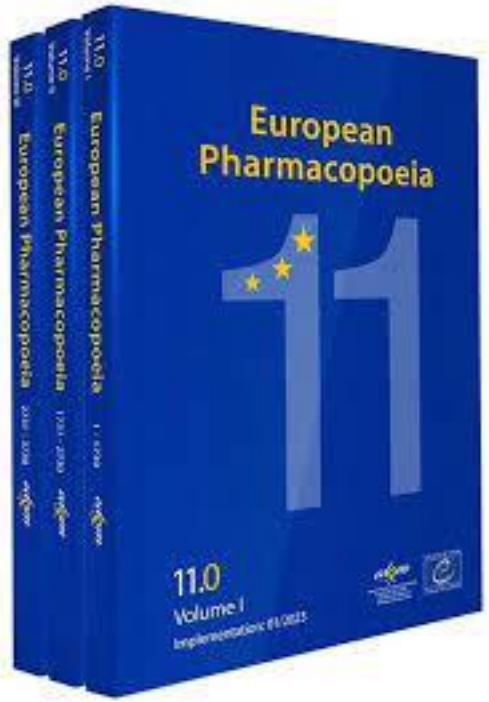
- FDA (draft) guidances on nanomaterials, liposomes, iron carbohydrate, etc.
- EMA reflection papers
- FDA/EMA tell you what you might do, but do not say how…
- Academic labs usually do not establish SOPs (they should)
- Information not harmonized, “Everybody is developing their own terminology” (JRC-NIST workshop 2018, Ispra, Italy)*
- Lack of available reference standards for nanomedicines*



*Halamoda-Kenzaoui et al., *Regul Toxicol Pharmacol* 106 (2019) 187-196

“...mRNA vaccines are nanomedicines...”

CASSS CMC Strategy Forum Europe 2021, October 2021



European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

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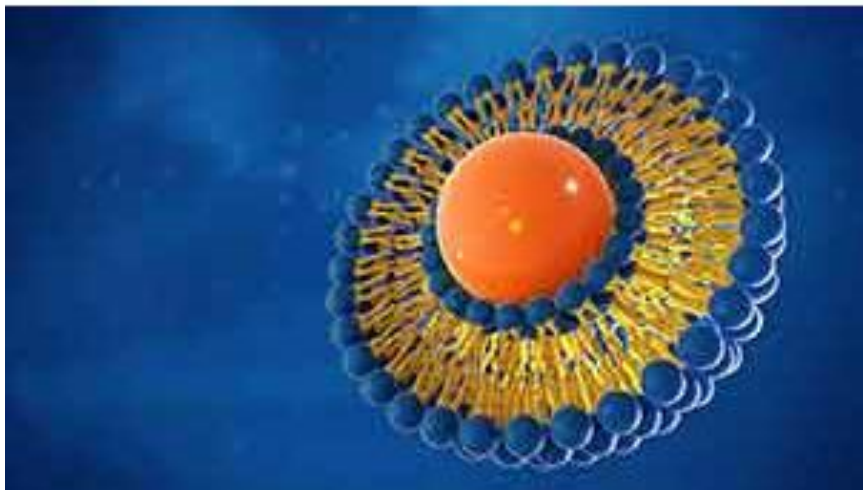
Quality requirements for nanomedicines: which role for the European Pharmacopoeia?



7-8 June 2022

Council of Europe premises

40 registrants (67 including speakers & EDQM staff) from **15** countries: 5 academia, 15 authorities, 16 industry

Outcomes

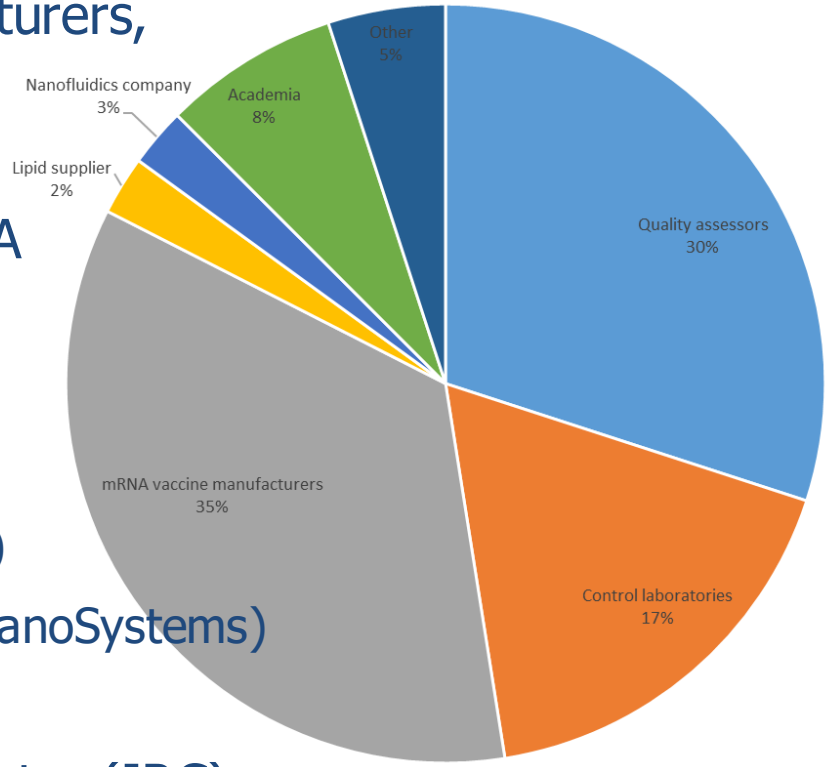
- Creation of a Working Party on mRNA vaccines (mRNAVAC)
- Appointment at November 2022 session of the Ph. Eur. Commission
- Develop a consolidated strategy for future standards addressing these vaccines and their components
- The ideas and proposals put forward on this topic during the recent EDQM Symposium on Nanomedicines will be taken into account

https://www.edqm.eu/en/-/quality-requirements-for-nanomedicines-what-role-should-the-european-pharmacopoeia-play-

mRNAVAC Working Party – composition

- Experts appointed at COM 174
- **43 Members** from various areas of activities: vaccines, mRNA, nanomedines / nano-formulation
- Regulatory authorities, national control labs, mRNA vaccine manufacturers, lipid supplier, nanofluidics company, academia
- Regulators/NCLs:
  - European regulators but also US FDA, Health Canada, TGA, TFDA

    → Global effort!
- Industry:
  - 14 experts from 6 mRNA vaccine manufacturers (Moderna, Pfizer / BioNTech, eTheRNA, GSK, Sanofi-Pasteur, Novartis)
  - 1 lipid supplier (Lipoid GmbH), 1 nanofluidics company (Precision NanoSystems)
- 6 Group 15 experts including its Chair (S. Andersen)
- 1 representative from the European Commission's Joint Research Centre (JRC)
- Chair: G. Borchard



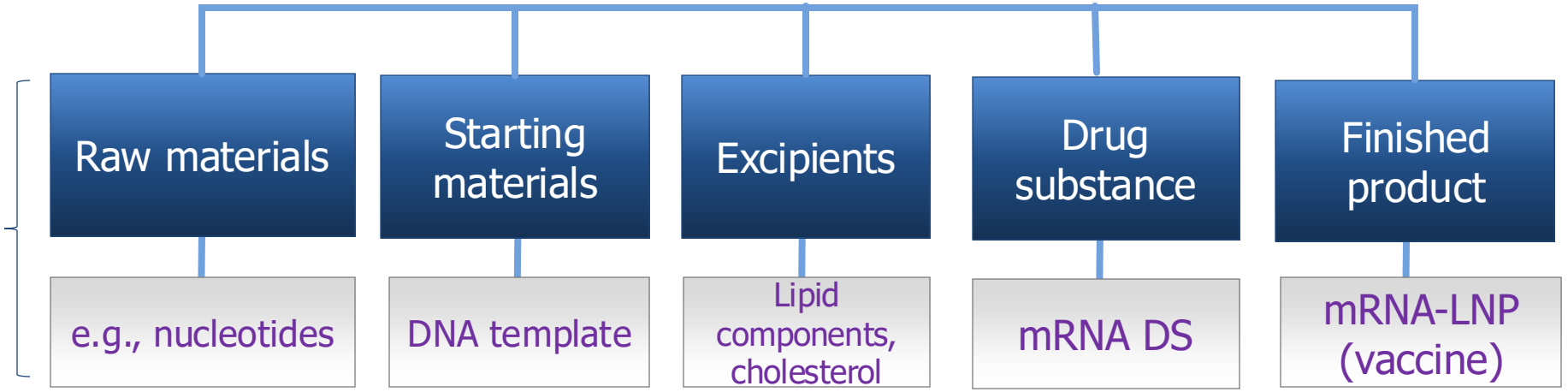
mRNAVAC Working Party – country distribution

Reminder

16 countries, 4 continents

What role can the Ph. Eur. play in setting standards for mRNA vaccines?

1) Areas for standardisation

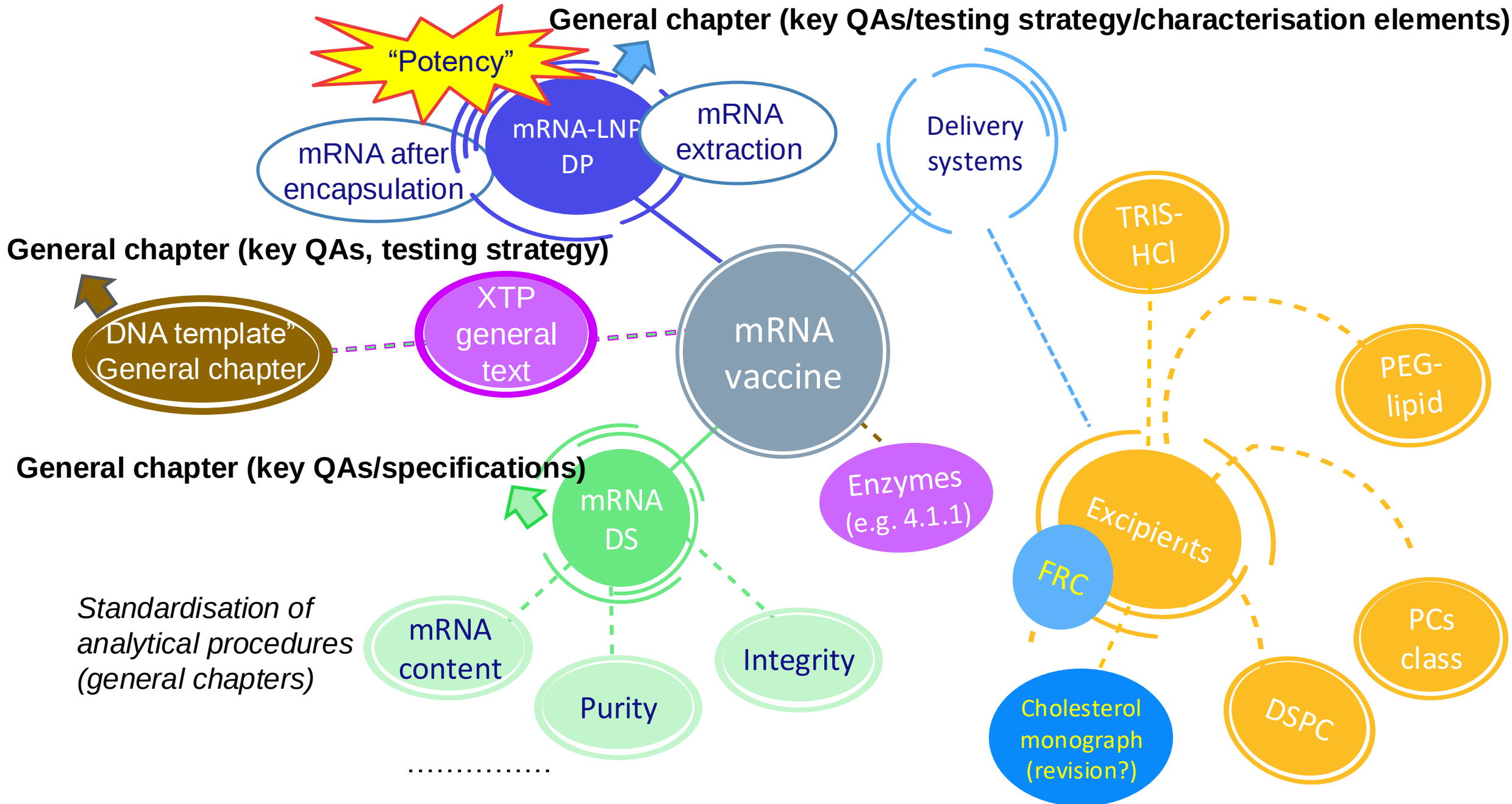


→ *Additional info: feedback from EDQM Nanomedicines Symposium*

2) Format / Type of Ph. Eur. text

→ *Additional info: induction training*

mRNA Vaccines: Proposed "Roadmap" (from 1st mRNAVAC WP meeting, Feb. 2023)



mRNA Vaccines: Elaboration of chapters 5.36, 5.39 & 5.40

5.40. DNA TEMPLATE FOR THE PREPARATION OF mRNA SUBSTANCES

DNA template for the preparation of mRNA substances (5.40.)

mRNA substances, DNA templates for the preparation of (5.40.)

1. DEFINITION

A DNA template is a linear double-stranded DNA used as a starting material for the manufacture of mRNA substances for the production of mRNA vaccines for human use. The linear DNA template is transcribed *in vitro* using a cell-free enzymatic reaction to produce the corresponding mRNA substance.

The DNA template may be a linearised plasmid DNA that has been produced in bacteria or may be derived enzymatically using a cell-free process. For the latter, different technologies such as PCR or rolling circle amplification can be used.

Regardless of the production method, the linear DNA template contains the promoter sequence for the RNA polymerase used for mRNA transcription, the sequence to be transcribed into the mRNA, which consists of the 5′ and 3′ untranslated regions (UTR), the open reading frame for the encoded antigen and, if appropriate, the poly(dA:dT) tract for the poly(A) tail.

Certain aspects of this general chapter may apply regardless of the intended use
the mRNA that is transcribed from the linear DNA template.

2. PRODUCTION

2.1. *GENERAL PROVISIONS*

Production of plasmid DNA is based on a bacterial cell-bank system. Plasmid DNA is amplified in bacterial cells and then purified as the circular form. In order to be used for *in vitro* transcription, the circular plasmid DNA is then linearised with a suitable restriction endonuclease.

Production of DNA by enzymatic technologies based on cell-free amplification of DNA can also be used. This starts with a small quantity of DNA to be amplified (input DNA). Some technologies give rise to a covalently closed DNA form that then has to be linearised as for plasmid DNA, others produce a linear form with the appropriate 3′ end required for mRNA transcription. To ensure the consistency of the input DNA, a master DNA stock is established.

2.2. *LINEARISED PLASMID DNA*

Plasmid construction. The plasmid is composed of:

– the plasmid backbone that contains multiple restriction endonuclease recognition sites for insertion of the genetic insert and the bacterial elements necessary for plasmid production (such as selectable genetic marker(s) for the selection of cells that carry the recombinant plasmid) and the recognition sequence for the endonuclease used for linearisation;

5.39. mRNA SUBSTANCES FOR THE PRODUCTION OF mRNA VACCINES FOR HUMAN USE

mRNA substances for the production of mRNA vaccines for human use (5.39.)

1. DEFINITION

mRNA substances for the production of mRNA vaccines are single-stranded mRNA molecules encoding one or more target antigens for induction of an immune response against an infectious agent. They are used as active substances for the production of prophylactic vaccines against infectious diseases.

mRNA substances are produced by a cell-free enzymatic process (referred to as *in vitro* transcription) using a suitable DNA template encoding the required antigen sequence.

The sequence of the mRNA may contain one or more open reading frames that encode the target antigen(s), flanking untranslated regions (UTRs), a 5′ cap (or alternative) and a 3′ poly(A) tail. The mRNA may contain naturally occurring nucleosides (modified or unmodified) and synthetic nucleosides. The mRNA backbone may be optimised.

In the case of

2. PRODUCTION

2.1. *GENERAL PROVISIONS*

The production method for a given mRNA substance must have been shown to yield consistently comparable batches. Substance specifications and relevant in-process tests and limits are set.

Process validation.

The production process is validated for the following aspects, including (but not limited to):

– consistency of the production process on an appropriate number of batches;
– adequate removal of product- and process-related impurities (for example, enzymes, DNA template and dsRNA if applicable);
– reusability of purification components (for example, chromatographic resin if applicable or tangential flow filtration membrane lifetime), with limits or acceptance criteria being set as a function of the validation.

Characterisation.

The mRNA substance is characterised in order to determine its structure, physico-chemical properties, purity and ability to be translated into the protein that it encodes.

5.36. mRNA VACCINES FOR HUMAN USE

mRNA Vaccines for human use (5.36.)

1. DEFINITION

mRNA vaccines for human use are preparations containing mRNA molecules compatible with the cellular protein translation machinery encoding for antigens capable of inducing a specific and active immunity in humans against an infecting agent or the toxin or antigen produced by it.

A suitable delivery system is necessary for the effective protection and administration of the mRNA substances. The scope of this general chapter is limited to lipid nanoparticle (LNP)-based delivery systems.

mRNA vaccines using LNPs as delivery system may contain one or more mRNA substances encapsulated in LNPs. LNPs are noncovalent, multicomponent assemblies, heterogeneous in their size, composition, and surface properties of the LNP subpopulations. They are composed of lipid and lipid-like components capable of encapsulating mRNA to ensure the desired product stability. The purpose of the LNPs is to protect the mRNA from enzymatic degradation by nucleases and enable cytosolic delivery of the mRNA.

PRODUCTION

GENERAL PROVISIONS

Production of mRNA vaccines using LNPs as a delivery system is based on self-assembly of the lipid and RNA (see 5.39 *mRNA substances for the production of mRNA vaccines for human use*) components resulting in encapsulation of the mRNA substance. This may be achieved by introducing a solution of the lipid components in a suitable solvent into a solution containing one or more mRNA substances in a suitable buffer, via a mixing system that is capable of controlling the flow rate, and thereby the mixing rate, and the ratio of the components. The resulting mRNA-containing LNP dispersion is further processed through a suitable purification process (e.g. ultrafiltration/diafiltration) to ensure adequate removal of product- and process-related impurities, medium exchange, and concentration adjustment.

Process validation

The production process is validated for the following aspects, including (but not limited to):

– consistency of the production process during mixing of the lipids and mRNA, the formation of RNA-containing LNPs, purification, formulation, final bulk vaccine production, and fill and finish steps;

– acceptable operational range for various processing parameters to ensure consistency in the quality of the product;

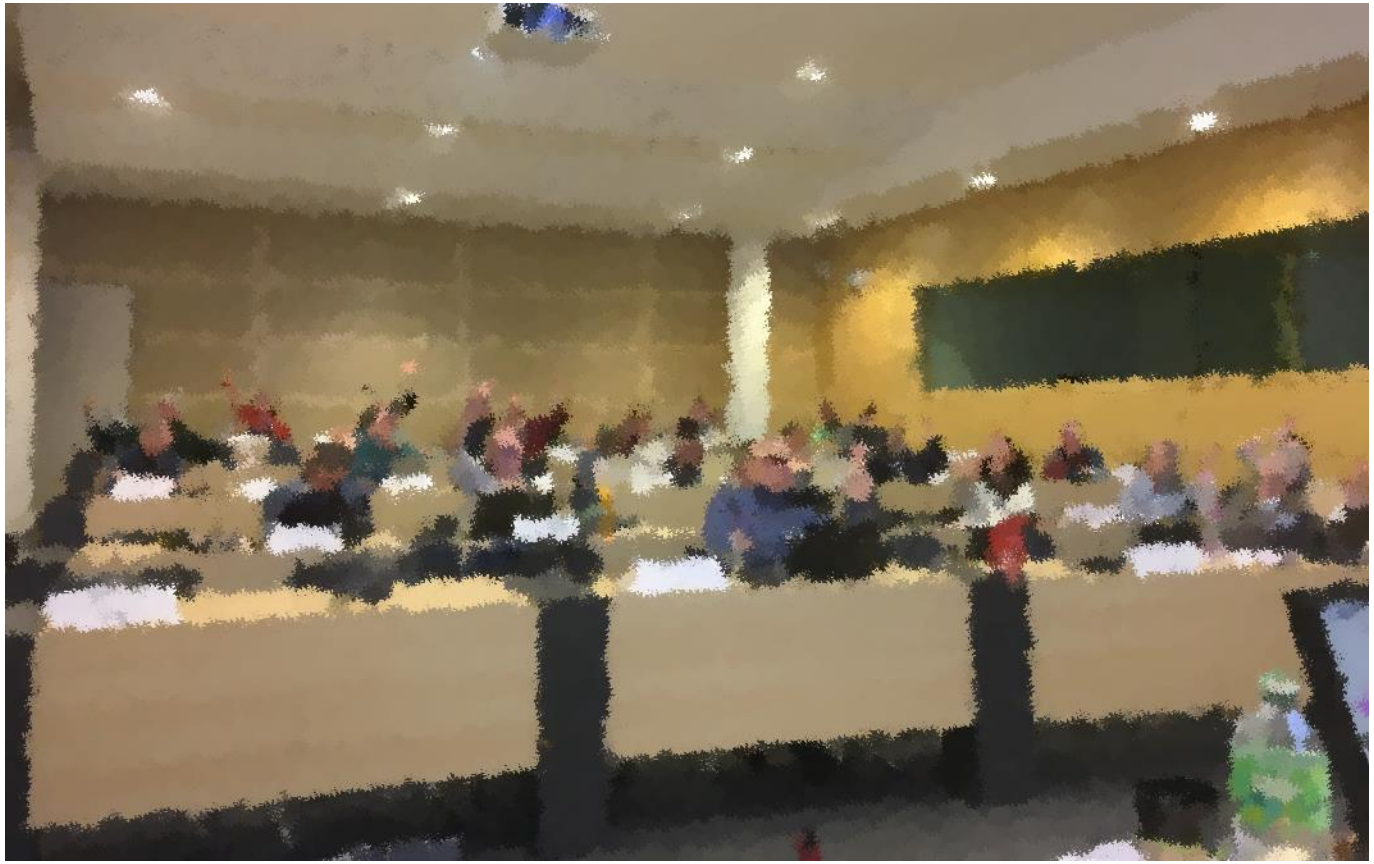
– critical processing steps and their acceptance criteria, including the manufacture of any intermediates;

Texts published in Pharmeuropa!

mRNA vaccines: Current status

- Drafted the 3 proposed general chapters (mRNA-LNP DP, mRNA DS, DNA template) in dedicated sub-teams

- Continue the discussions on other topics
  (incl. excipients, raw materials, standardisation of analytical procedures and reference standards)




Merci!