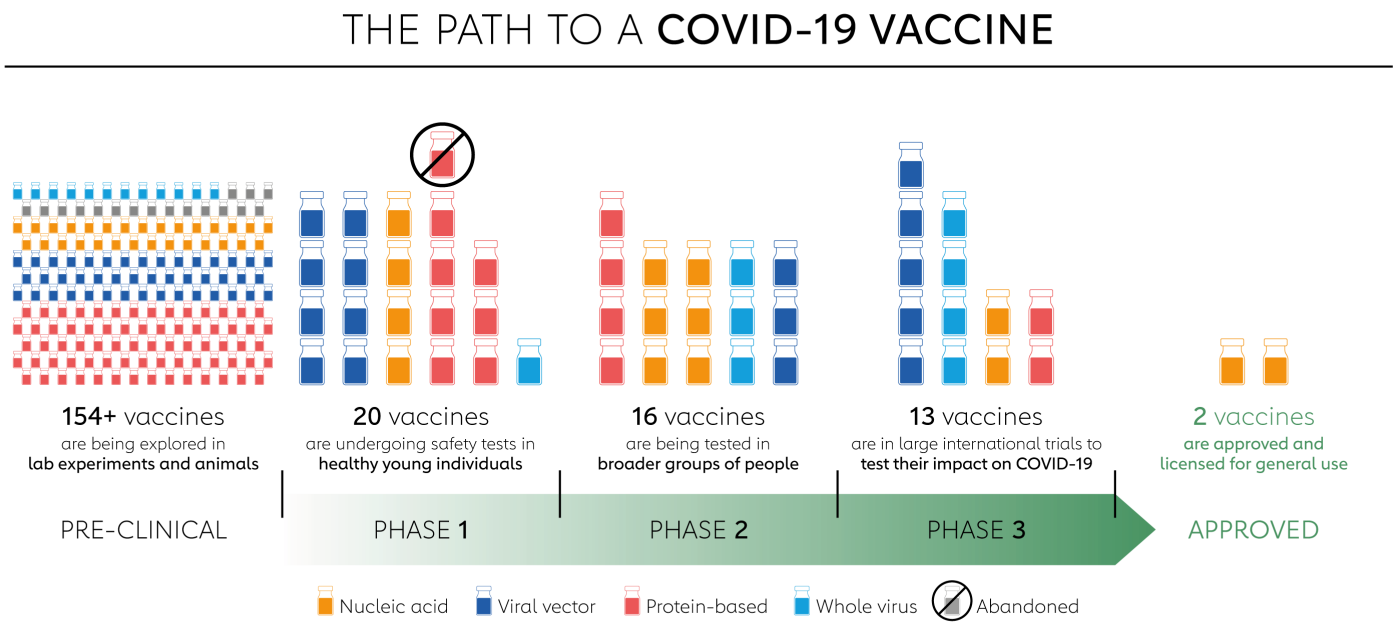
Nucleic Acid Vaccines
Is it all done?

Christian W. Mandl, PhD, MD

Emerging platforms: The winner is…



…mRNA

Specifically: Pseudouridine-modified (ΨU), non-replicating mRNA formulated in lipid nanoparticles

Source: GAVI https://www.gavi.org/vaccineswork/covid-19-vaccine-race (as of Dec 2020)

A brief history of RNA vaccines

(disclaimer: from a personal perspective)

1990 **DNA and mRNA express upon injection in mouse muscle**
Wolff et al., Science 247:1465

1993 **Nucleic acid injection to elicit adaptive immune response**
DNA: Ulmer et al., Science 259:1745
mRNA: Martinon et al., Eur J Immunol. 23:1719

1998 **Infectious and replicating RNA for vaccination**
Mandl et al., Nat Med. 4:1438-40

2000 **CureVac founded by Ingmar Hoerr**

2008 **Pseudouridine modified mRNA**
Karikó et al. Molecular therapy 16: 1833

2012 **Lipid nanoparticles for delivery of Self-Amplifying RNA**
Geall et al. PNAS 109:14604

2013 **First mRNA conference** (Tübingen, Germany)

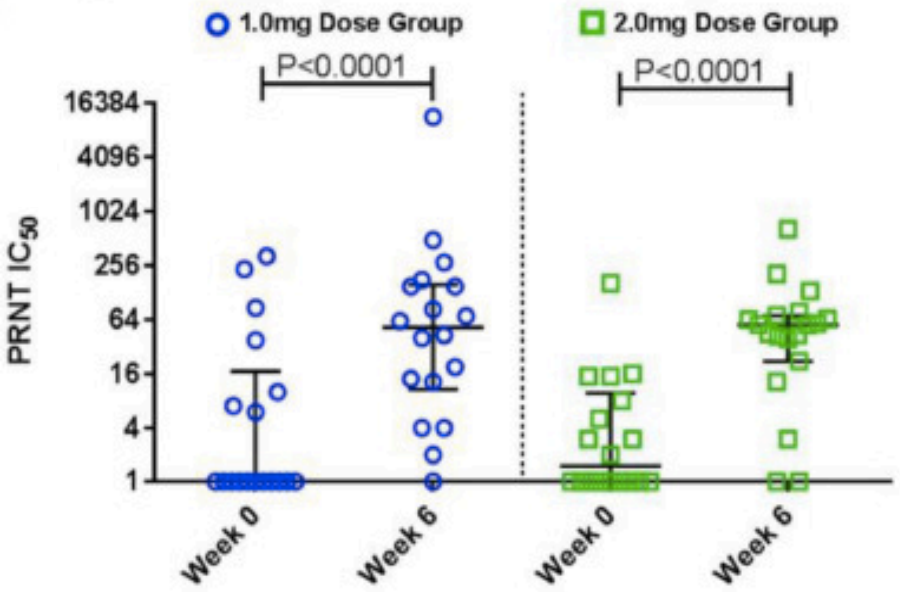
2017 **First clinical ID trials** Influenza, Zika (*Moderna*), Rabies (*CureVac*)

2020 **EUA for vaccines against SARS-CoV-2** (*BioNTech/Pfizer, Moderna*)

How about DNA?

- Electroporation device has strongly increased immunogenicity and consistency, but limits real-world applicability
- Was leading the race with Zika vaccine (7 months from concept to phase 1)
- For SARS-CoV-2:
  - Phase 1 Neutralization titers after 2 immunizations were moderate.
  - Currently in phase 2/3

INOVIO
POWERING DNA MEDICINES



- Tebas, Pablo et al. "Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, Phase 1 clinical trial." *EClinicalMedicine*, 100689. 24 Dec. 2020

Leading RNA Vaccines Against SARS-CoV-2

Company	RNA type	Status	Efficacy (%)	RNA Dosage (µg)	Schedule	Storage Temp. (°C)	Other
Pfizer BIONTECH	mRNA (ΨU)	Approved (EUA)	95	30	2 doses (3 weeks)	- 70	
moderna NIH	mRNA (ΨU)	Approved (EUA)	94	100	2 doses (4 weeks)	- 20 (6 months)	
CUREVAC	mRNA	Phase 2b/3	?	12	2 doses (4 weeks)	2 – 8 (3 months)	
ARCTURUS therapeutics	SAM	Phase 2	?	5 or 7.5	1 or 2 doses	?	Low NT CD8+
Imperial College London	SAM	Phase 1	?	?	?	- 70	Halted (Jan 26)
WALVAX 沃森生物 WALVAX BIOTECHNOLOGY CO., LTD.	mRNA	Phase 1/2	?	5 - 25	?	?	China
gritstone ONCOLOGY	SAM	Phase 1	?	?	?	?	Heterol. boost

Is it all done?
There is plenty of room for innovation

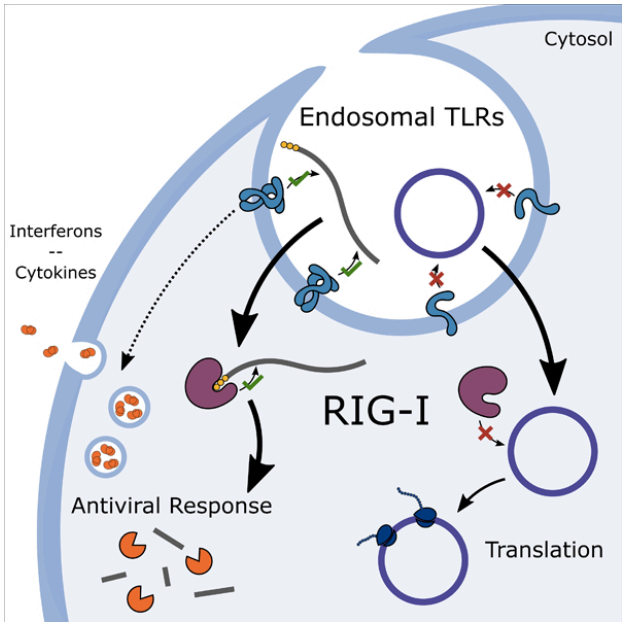
- Thermostability
  - Increase shelf life, eliminate need for deep freezing
- Packaging capacity (RNA/particle)
  - To allow expression of complex antigens or complementing RNAs
- Dose reduction
  - To reduce costs and reactogenicity
- Improve immune response
  - Magnitude and breadth; T cell responses; longivity
- Reduce reactogenicity
  - Local and systemic reactions similar to other licensed vaccines

Innovation: RNA

Source: BioNTech F-1 sec.gov

- Modified versus non-modified RNA
  - Unmodified RNA needs careful dosing
  - Lower doses with non-modified (CureVac: Rabies vaccine at 1 µg)
  - Purification is key
- Self amplifying versus non-replicating RNA
  - Lower dose requirement because of amplification in vivo
  - Stronger T cell responses (CD8)
  - Explore the universe of positive-sense RNA viruses
  - Synthetic replicons (Replicate Bioscience)
  - Trans-amplifying replicons
  - Infectious RNA (dose << 1 µg)

Innovation: RNA



Wesselhoeft, RA et al. Molecular cell 74 (2019): 508

Circular mRNA

- Generated by self-splicing
- By-passes RNA sensors – no innate stimulation
- No terminal degradation – longer expression

RNA Printer

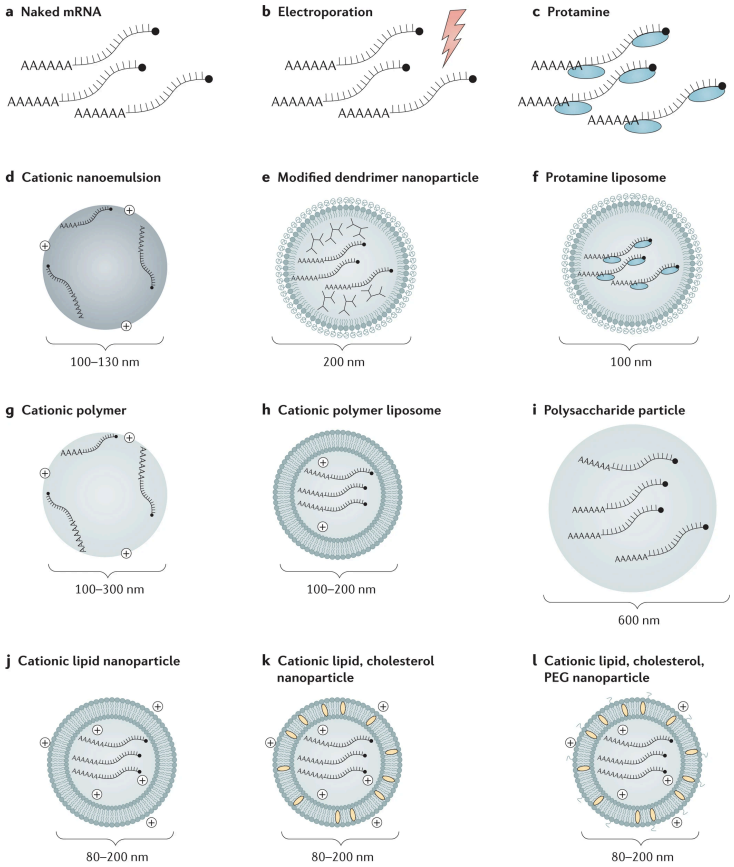
- CureVac and Tesla joined forces for mobile, decentralized production
- Personalized medicine
- Rapid response

Elon Musk in Berlin Sep 3, 2020; picture: REUTERS

Innovation: Delivery

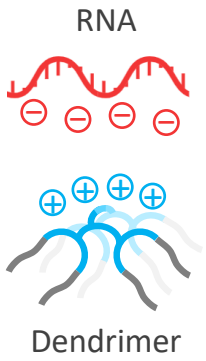
Lipid nanoparticle delivery has evolved as the gold standard

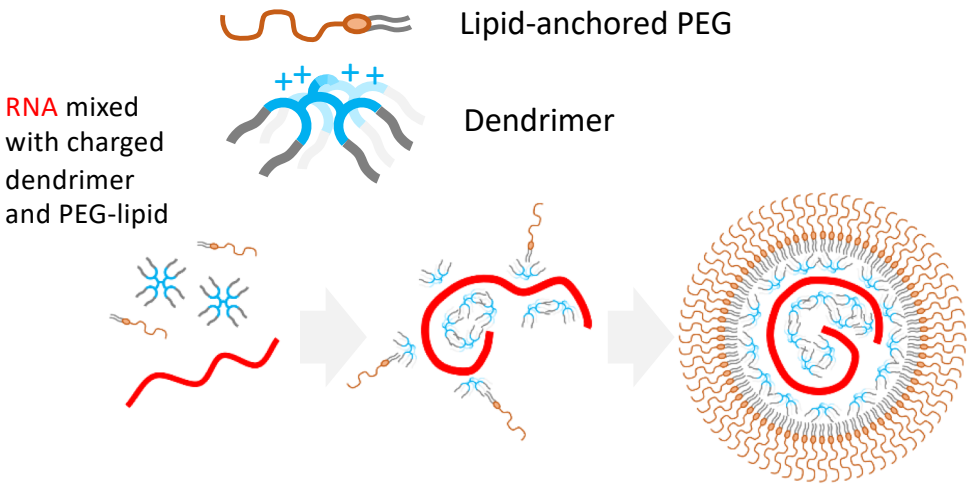
- Originally developed for small siRNA
- **2012**: Replicons delivered by LNPs (Geall, A. J. et al. *Proc. Natl Acad. Sci. USA* **109:** 14604).
- **2015**: Modified mRNA delivered by LNPs (Pardi, N. et al. *J. Control. Release* **217:** 345).
- **Limitations**:
  - Reactogenicity of components (lipids; allergic reactions to PEG?)
  - Limited packaging capacity
  - Limited temperature stability



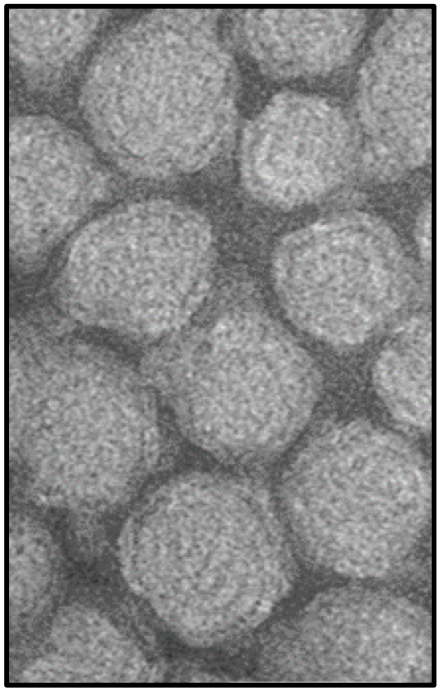
Source: Pardi et al., *Nature Reviews Drug Discovery* 17: 261 (2018)

Innovation: Dendrimer Delivery





- **Large class of molecules with defined branching repeats ("snowflake-architecture")**
- Adaptable chemistry to meet different application needs
  - Stability
  - Charge density – packaging capacity
  - Degradability



Multilamellar nanoparticles
~100-150nm in diameter

Where to go from here?
How broad can the technology be?

- Rapid response against emerging pathogens – YES
- New viral targets – RSV, herpes viruses (complex antigens)
- Non-viral targets – bacteria (cannot replace glycoconjugates), parasites
- Replace existing vaccines – influenza (will need better immunogenicity), rabies
- Heterologous prime-boost
  - To increase magnitude, breadth and longevity of immune response
  - Reduce reactogenicity
  - Logistically challenging, but pandemic may shift perception:
- RNA-encoded antibodies (passive immunization, immunotherapy)
- Non communicable disease targets:
  - Cancer vaccines
  - Immunotherapy
  - Cardiovascular
  - Gene editing
  - Gene replacement