



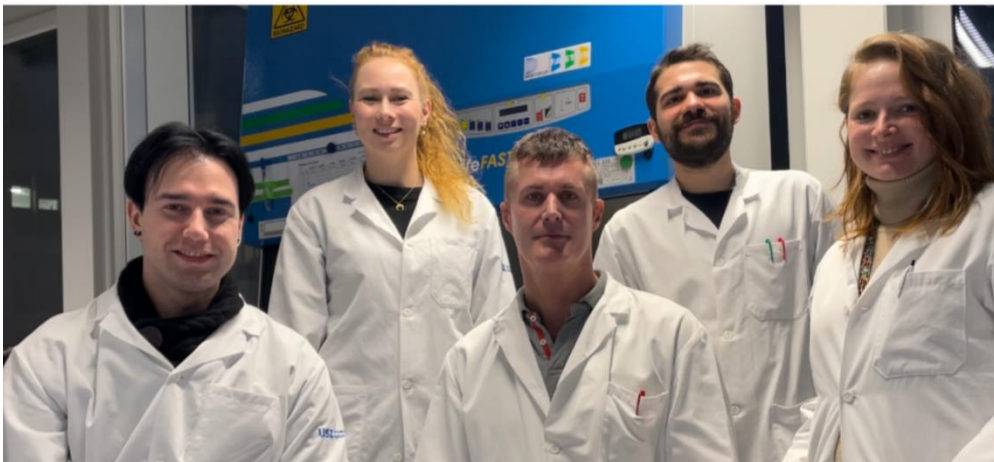
Update on new and evolving vaccine development platforms

ARVAC, the ALUMNI REFRESHER VACCINOLOGY COURSE 2023

June 2023, 6th

Prof. Steve Pascolo

University Hospital of Zurich, Switzerland



Artist Caroline Schüpbach



**Ecole Normale Supérieure Paris
Pasteur Institute Paris - PhD June 1998**

Post-doc University of Tuebingen 1998-2000

ACADEMIC

PI University of Tuebingen

2000-2007



PI University Hospital of Zurich

2006-



**UniversityHospital
Zurich**



**Universität
Zürich^{UZH}**

INDUSTRY

Co-founder and CSO CureVac GmbH



Founder and CEO of Miescher Pharma GmbH



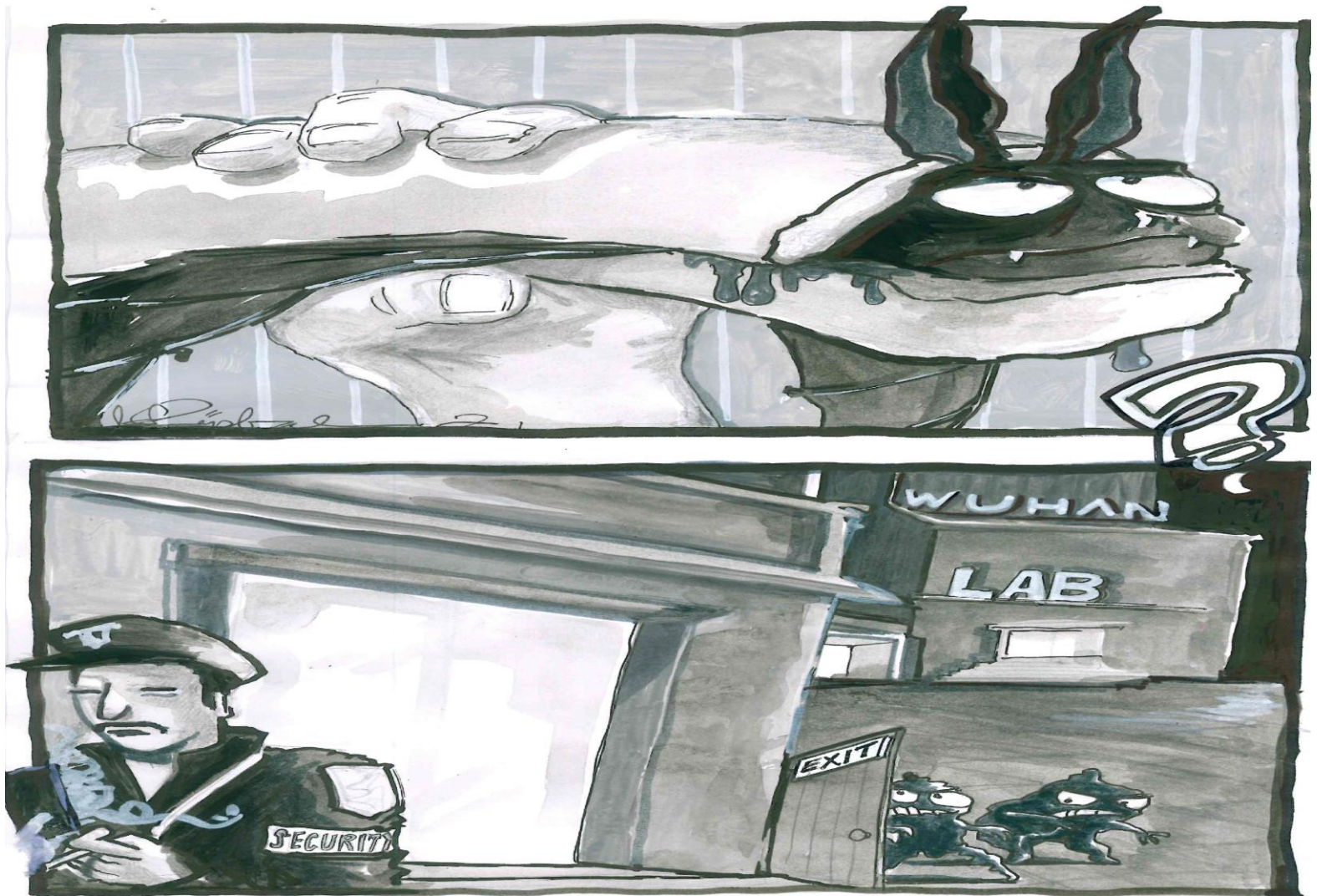
2017: Messenger RNA platform

Synthetic mRNA: the "The Ugly Duckling» of vaccine research till 2020



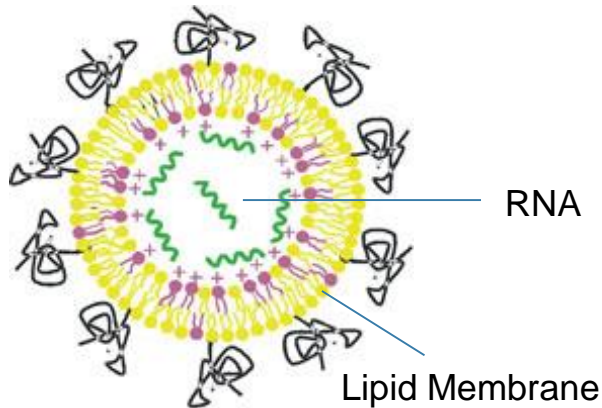
Artist Caroline Schüpbach

2020: SARS-CoV-2 Pandemic

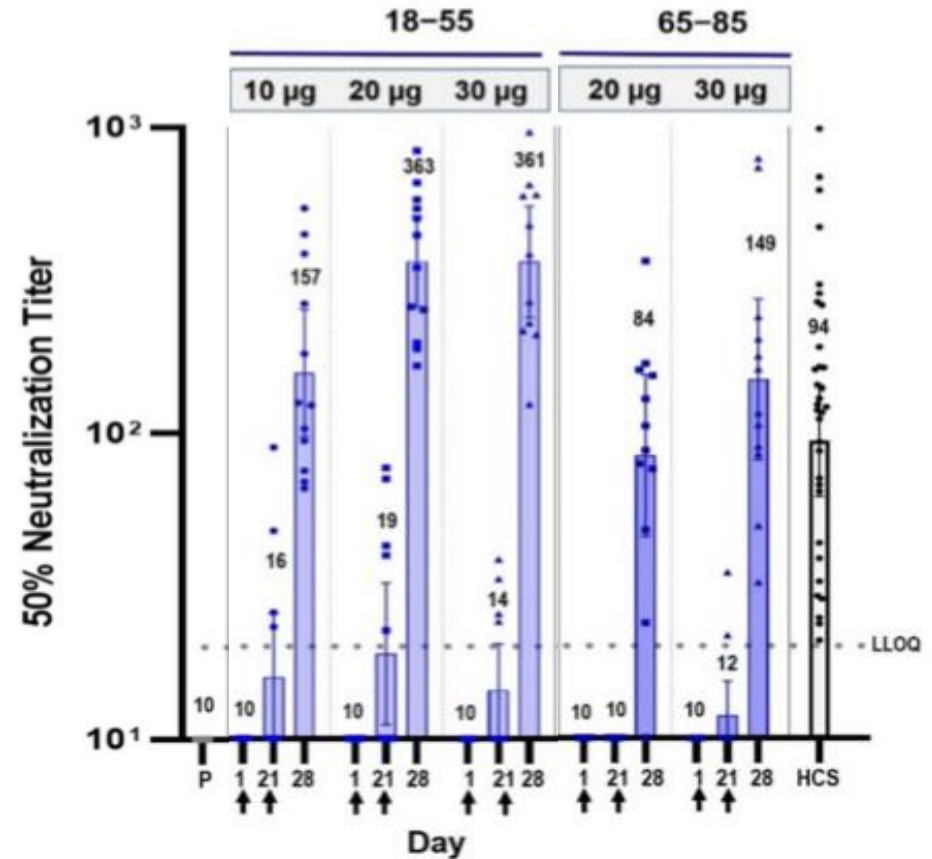


Artist Caroline Schüpbach

Synthetic mRNA vaccine against COVID-19 (Moderna or BioNTech/Pfizer)



March 2021



BioNTech/Pfizer. <https://www.nejm.org/doi/full/10.1056/NEJMoa2027906>

October 14, 2020

Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates

Walsh et al. December 17, 2020, N Engl J Med 2020; 383:2439-2450

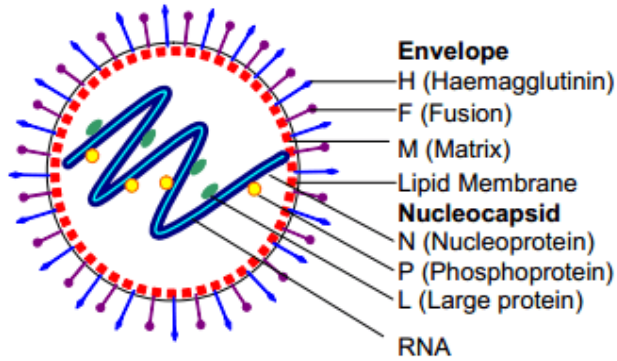
Vaccine preventable diseases (non-exhaustive list)

Disease	Pathogen	Type of vaccine	Injection	Adjuvant	Production	
Tuberculosis	Mycobacterium tuberculosis	Attenuated bacteria	s.c.		bacilli Calmette-Guérin	L I V E
Rubella (German measles)	Rubella virus	Attenuated virus mRNA	s.c.		Human embryonic lung cell line	
Measles (Rubeola)	Measles virus	Attenuated virus mRNA	s.c.		Chick Embryo cells	
Mumps	Mumps virus	Attenuated virus mRNA	s.c.		Chick Embryo cells	
Chickenpox/Varicella-Zoster	Varicella Zoster virus	Attenuated virus DNA	s.c.		Human embryonic lung cell line	
Smallpox (variola)	Variola major virus	Attenuated virus DNA	Prick		Animals	
Rotavirus infection	Rotavirus	Attenuated virus dsRNA	Oral		Vero cells (monkey kidney epithelial cells)	
Yellow fever	Yellow fever virus	Attenuated virus mRNA	s.c.		Eggs	D E A D
Rabies	Rabies virus	Inactivated virus	i.m.		Vero cells (monkey kidney epithelial cells)	
Swine Flu (2009 influenza A (H1N1) pandemic)	H1N1 influenza virus	Inactivated virus	i.m.	MF59 (squalene oil)	Eggs	
Japanese encephalitis	Japanese encephalitis virus	Inactivated virus	i.m. or s.c.		Vero cells (monkey kidney epithelial cells)	
Seasonal influenza	Influenza virus	Inactivated virus	i.m.	Alum/MF59	Eggs	
Hepatitis A	Hepatitis A virus	Inactivated virus	i.m.	Aluminum hydroxide	MRC-5 cells	
Cholera	Vibrio cholera	Inactivated/attenuated bacteria	Oral		Bacteria culture medium	
Poliomyelitis	Polio virus	Inactivated/attenuated virus	s.c. or i.m /oral		Vero cells (monkey kidney epithelial cells)	
Invasive Haemophilus influenzae disease	Haemophilus influenzae type b	Polysaccharide conjugated to tetanus toxoid or mutant of diphtheria toxin	i.m.	Aluminum hydroxide	Haemophilus influenzae type b	
Meningococcal disease	Neisseria meningitidis bacteria	Polysaccharide conjugated to tetanus toxoid or mutant of diphtheria toxin	i.m.		Neisseria meningitidis bacteria	
Invasive pneumococcal disease	Streptococcus pneumoniae	Polysaccharide conjugated to mutant of diphtheria toxin	i.m.		Streptococcus pneumoniae	
Hepatitis B	Hepatitis B virus	Subunit: HBsAg	i.m.	Aluminum hydroxide	Yeast	
Cervical cancer	Human papillomavirus	Subunit: VLPs	i.m.	Aluminum hydroxide	Vero cells (monkey kidney epithelial cells)	
Pertussis	Bordetella pertussis	Pertussis antigens	i.m.	Aluminum hydroxide	Bordetella pertussis	
Tetanus	Bacterium Clostridium tetani,	Tetanus toxoid	i.m.	Aluminum hydroxide	C tetani bacteria	
Diphtheria	Corynebacterium diphtheriae/ulcerans	Subunit: Diphtheria toxoid	i.m.	Aluminum hydroxide	Corynebacterium diphtheriae/ulcerans	

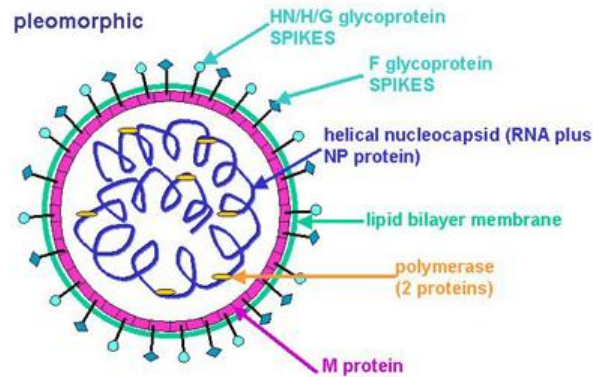
Colored: Basic vaccines suggested in Switzerland

mRNA vaccines: Natural (long used) and synthetic (newly approved)

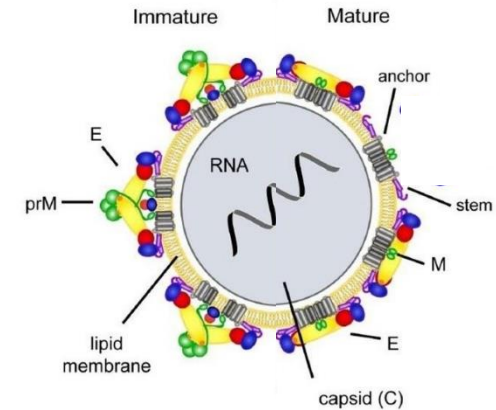
Natural mRNA vaccine



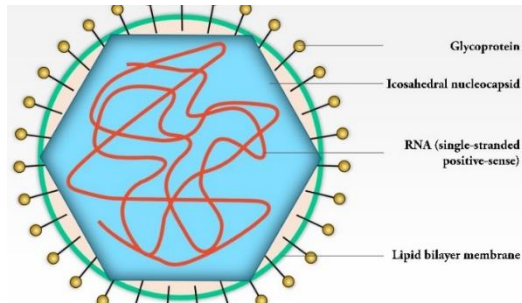
Measles (attenuated Virus)



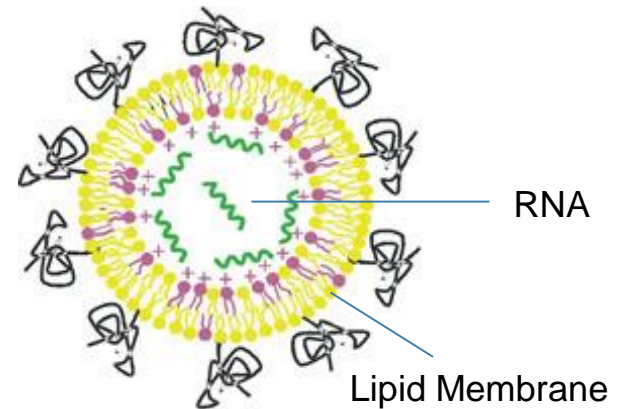
Mumps (attenuated Virus)



Yellow Fever (attenuated Virus)



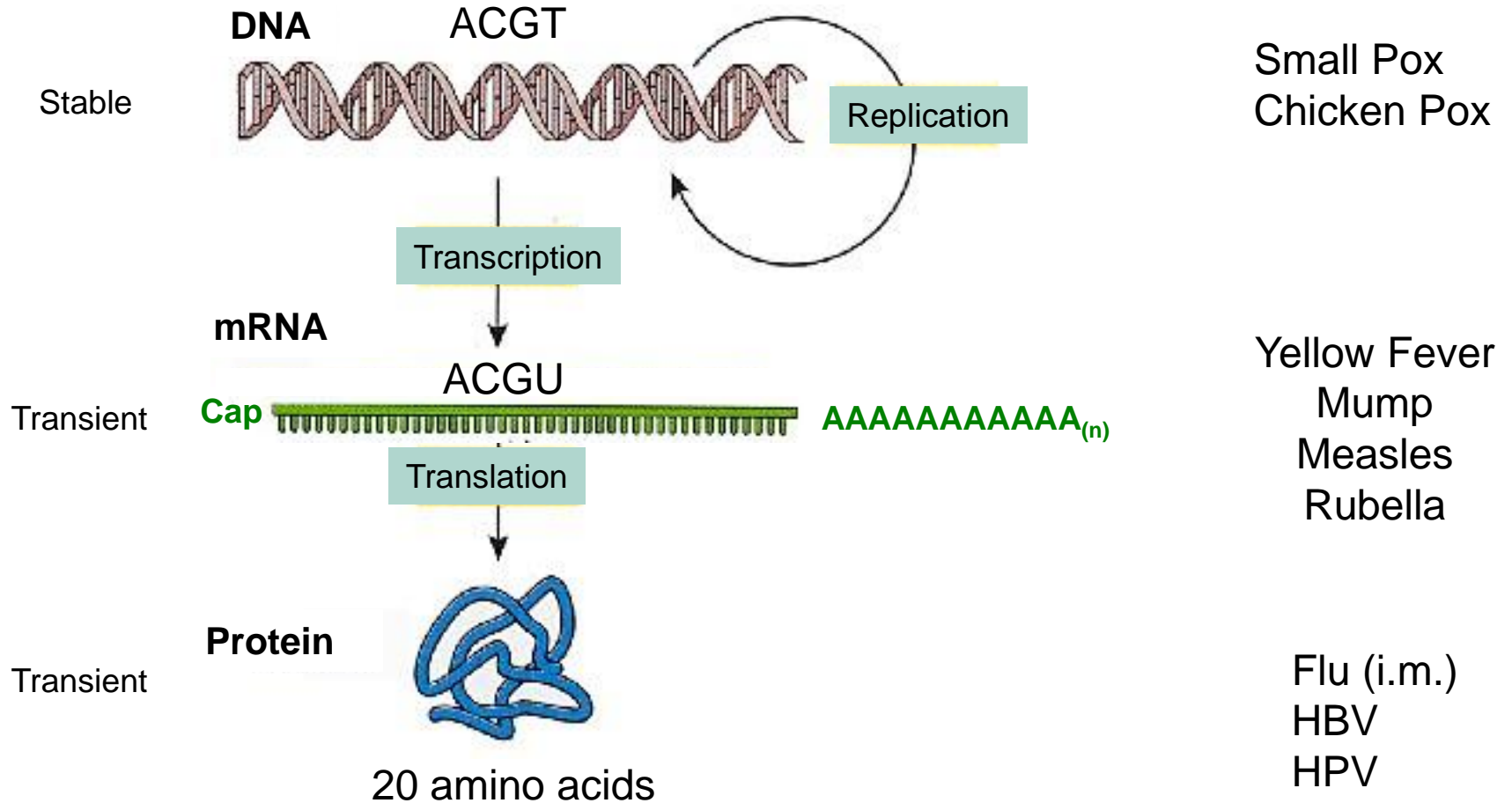
Rubella (attenuated Virus)

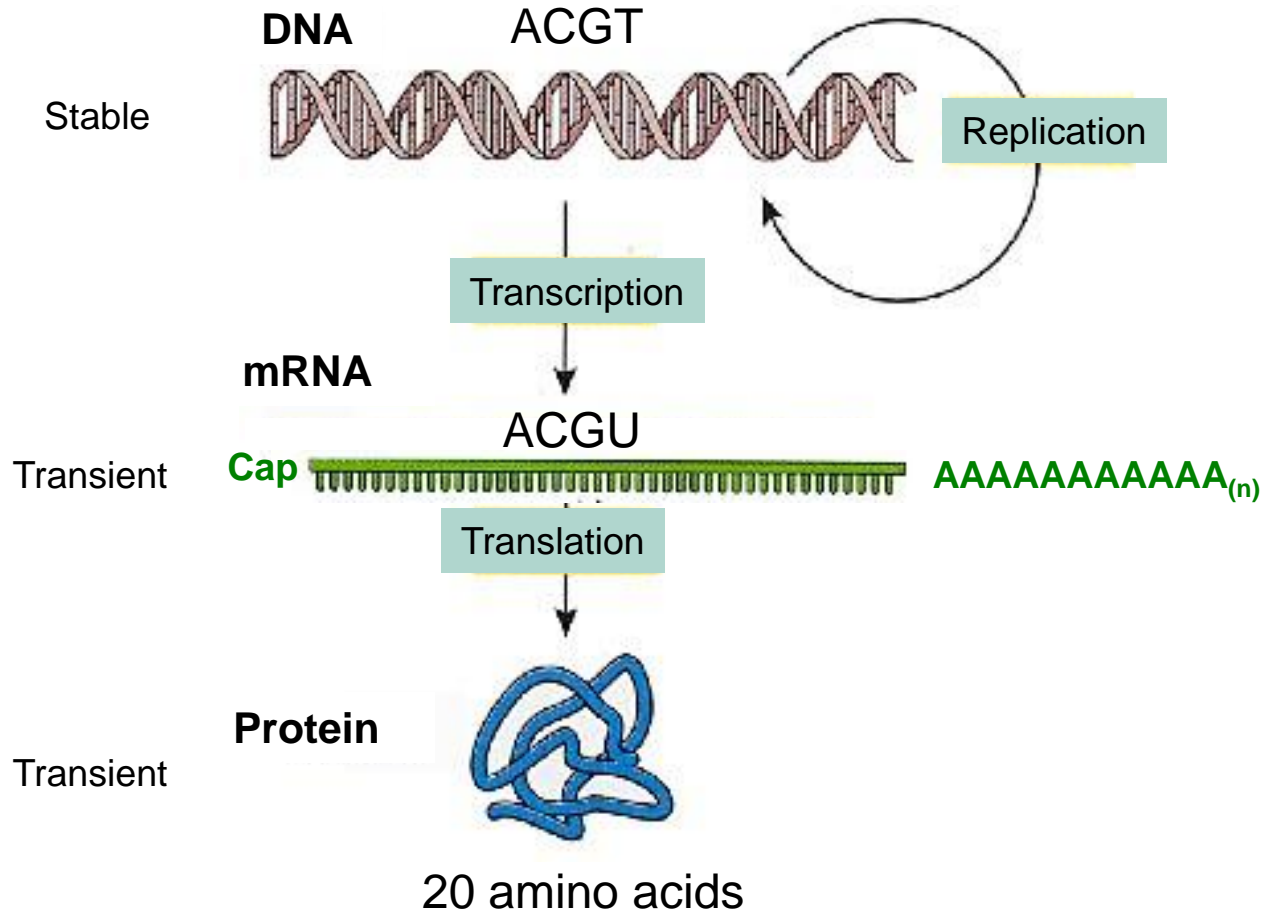


Synthetic (ivt) mRNA vaccine

In Cells

Active ingredient in vaccines





AstraZeneca
Sputnik V
Janssen

BioNTech/Pfizer
(CureVac)
Moderna

Novavax

Vaccine formats (blue: aproved against SARS-CoV-2)

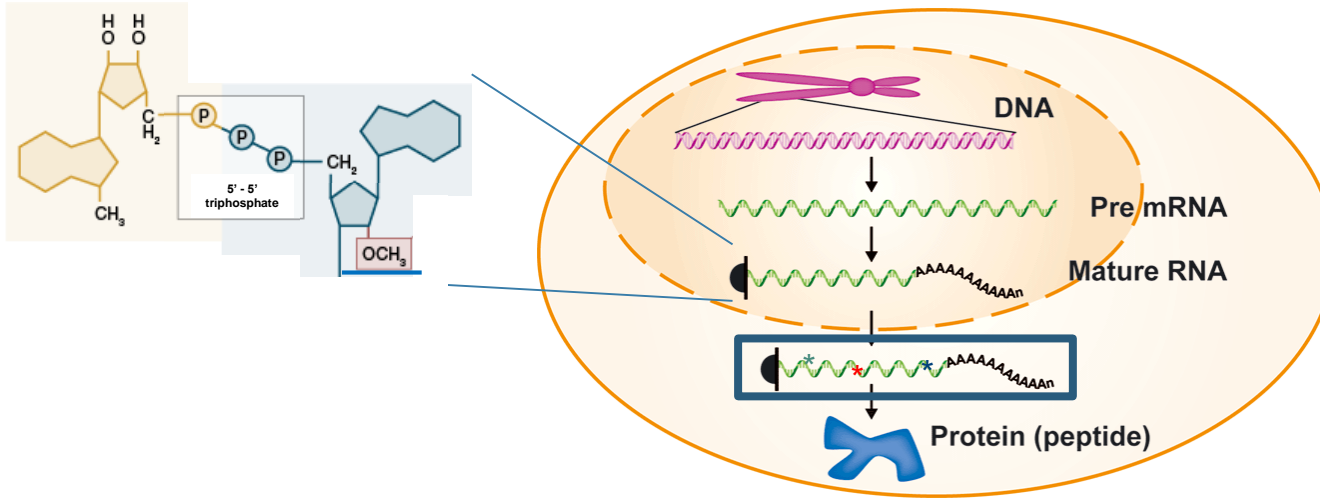
	Design	Upscaling	Re-using established GMP conditions	Theoretical Safety
Peptide	😊	😊	😞	😊
Plasmid DNA	😊	😊	😊	😞
Recombinant viral vector (adenovirus)	😊	😊	😊	😞
ivt mRNA	😊	😊	😊	😊
Proteins/ Sugars	😊	😊	😊	😊
Inactivated viruses	😊	😊	😊	😊
Attenuated viruses	😊	😊	😊	😞

😊 **Easy**

😊 **To be optimised / Not easy / Not garantied**

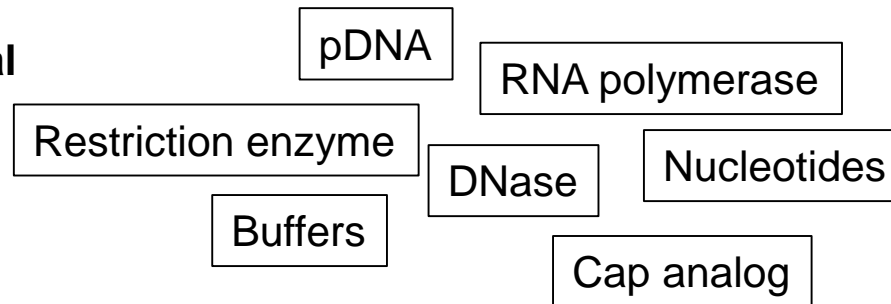
😞 **Difficult / Bad**

mRNA



Production of synthetic (ivt) mRNA

Starting material

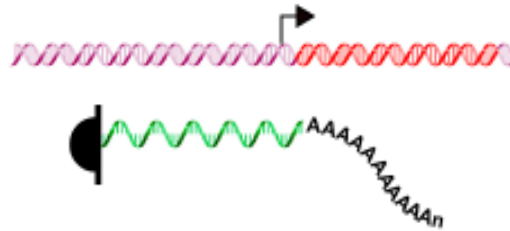


1- Linearisation



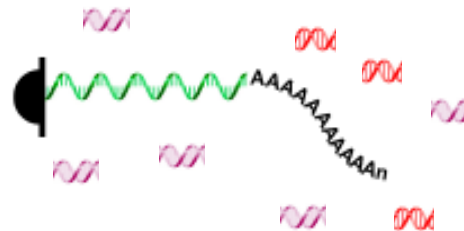
Restriction enzyme
Robust. Two hours

2- Transcription



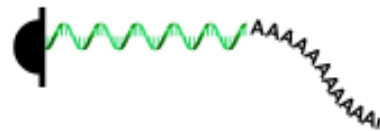
T7/SP6 RNA polymerase
Robust. Two hours

3- Degradation of DNA



DNase
Robust. Two hours

4- Purification of mRNA



LiCl precipitation:
Robust. Two hours

Facts on synthetic (ivt) mRNA vaccines

- mRNA IS VERY STABLE (in the absence of RNases!)
- RNA-liposome formulations may not be stable (aggregate, change size/form over time or by freeze/thaw)
- RNA in liposome is already an approved drug: Onpattro (Patisiran). Up to 30 mg i.v. every 3 weeks. siRNA. Treatment of polyneuropathy in people with hereditary transthyretin-mediated amyloidosis.
- ivt mRNA is vegan
- 1 million doses in 6 L and few hours (viruses require 5000 L and few days/weeks for 1 million doses)

Synthetic mRNA vaccines: Timelines

Nonreplicating synthetic mRNA vaccines:
A journey through the European (Journal
of Immunology) history. Pascolo S.
Eur J Immunol. 2023 Apr 7

Martinon et al use mRNA in liposomes to vaccinate mice against Influenza



Conry et al use naked mRNA to vaccinate mice against cancer



Hoerr et al use naked and Protamine formulated mRNA to vaccinate mice



Founding of CureVac



First injections of ivt mRNA in humans



RNA is a danger signal



1993

1995

2000

2000

2003

2004

2005

2006

2008

2010

2017

2020

PseudoU mRNA
is not immunostimulating



Manufacturing authorisation GMP
production mRNA



First publication mRNA vaccine clinical study



Founding of BioNTech



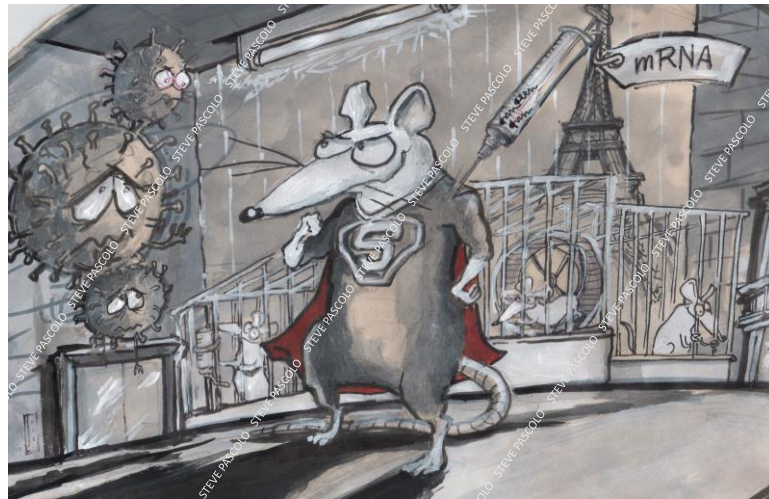
Founding of Moderna



CleanCap Trilink



Approval first synthetic mRNA vaccine
(BioNTech/Pfizer)

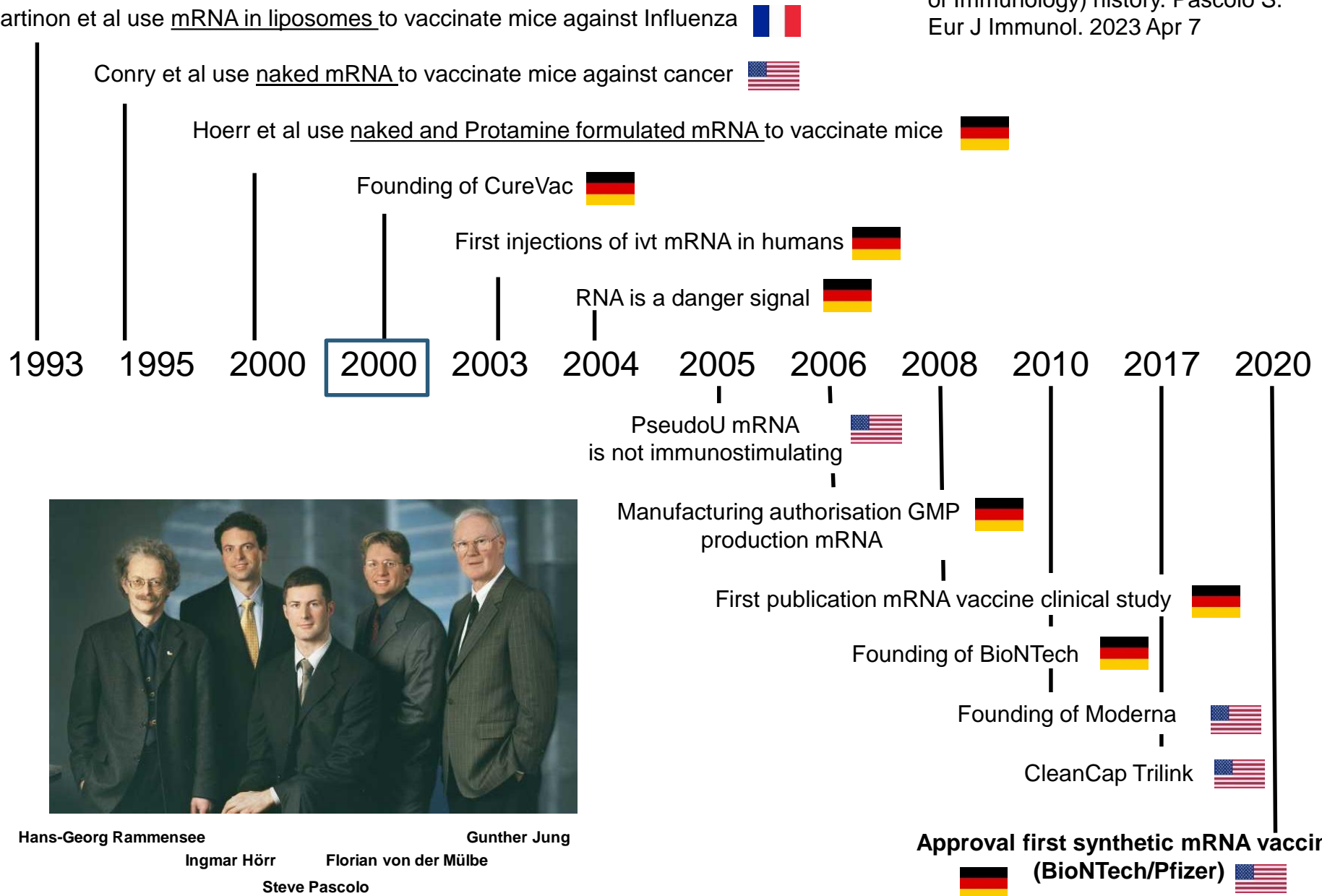


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Martinon....Meulien. European Journal of Immunology 1993 "Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA"

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Founding of BioNTech



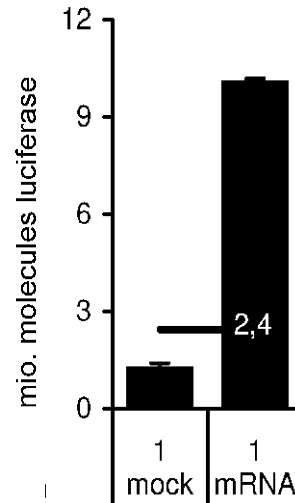
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CleanCap Trilink



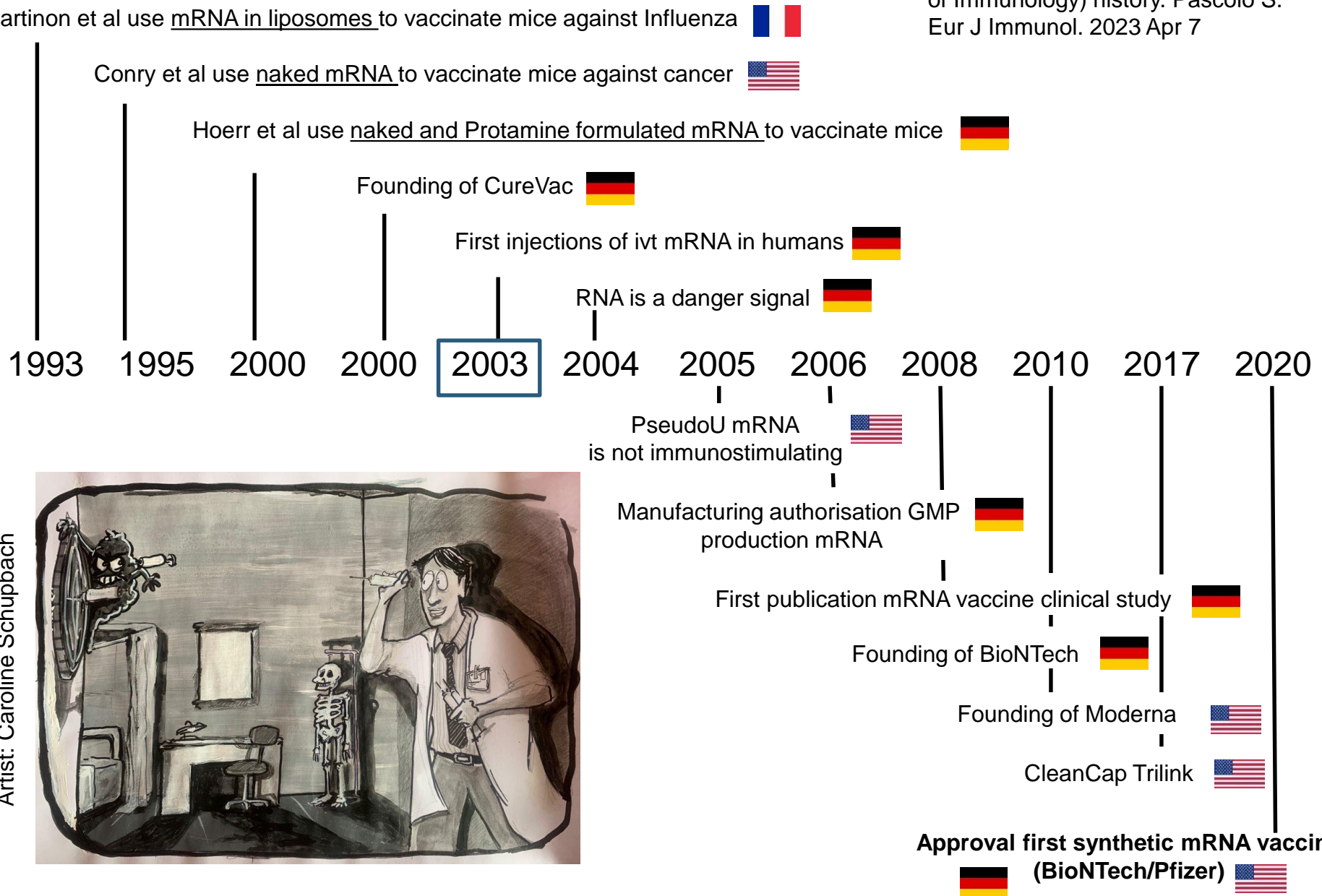
Approval first synthetic mRNA vaccine
(BioNTech/Pfizer)



Probst....Pascolo. Gene Therapy 2007 "Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent"

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Manufacturing authorisation GMP
production mRNA



First publication mRNA vaccine clinical study



Founding of BioNTech



Ozlem Tureci CMO
Ugur Sahin CEO/CSO

Founding of Moderna



CleanCap Trilink



Approval first synthetic mRNA vaccine
(BioNTech/Pfizer)



Artist: Caroline Schubbach



Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine.

Polack (...) Türeci (...) Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group.

N Engl J Med. 2020 Dec 31;383(27):2603-2615.

Synthetic mRNA vaccines against pathogens (clinical studies)

moderna

Phase I	Phase II	Phase III
		COVID-19
		Flu
		Respiratory syncytial virus
Metapneumovirus		
Parainfluenza virus type 3		
		Cytomegalovirus
Epstein-Barr virus		
	Zika	
HIV		
VZV		

Phase I	Phase II	Phase III
		COVID-19
		Flu
	VZV	
HSV-2		
Tuberculosis		
Malaria		

BIONTECH

Synthetic mRNA vaccines against cancers (clinical studies)

BIONTECH

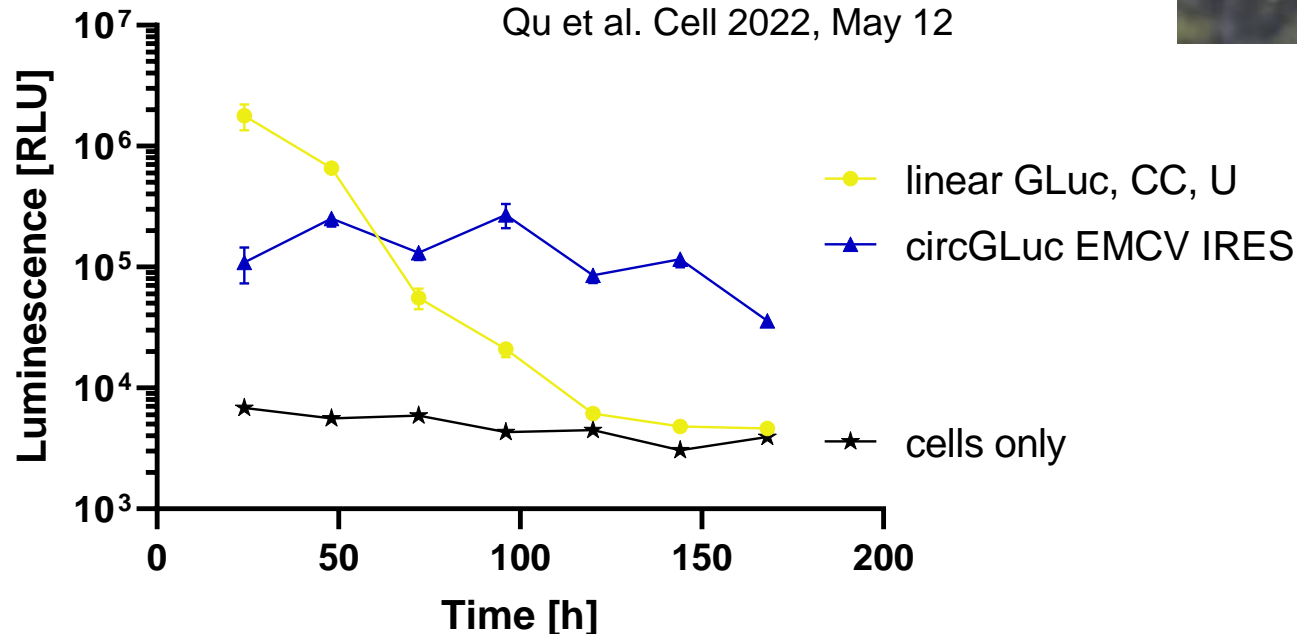
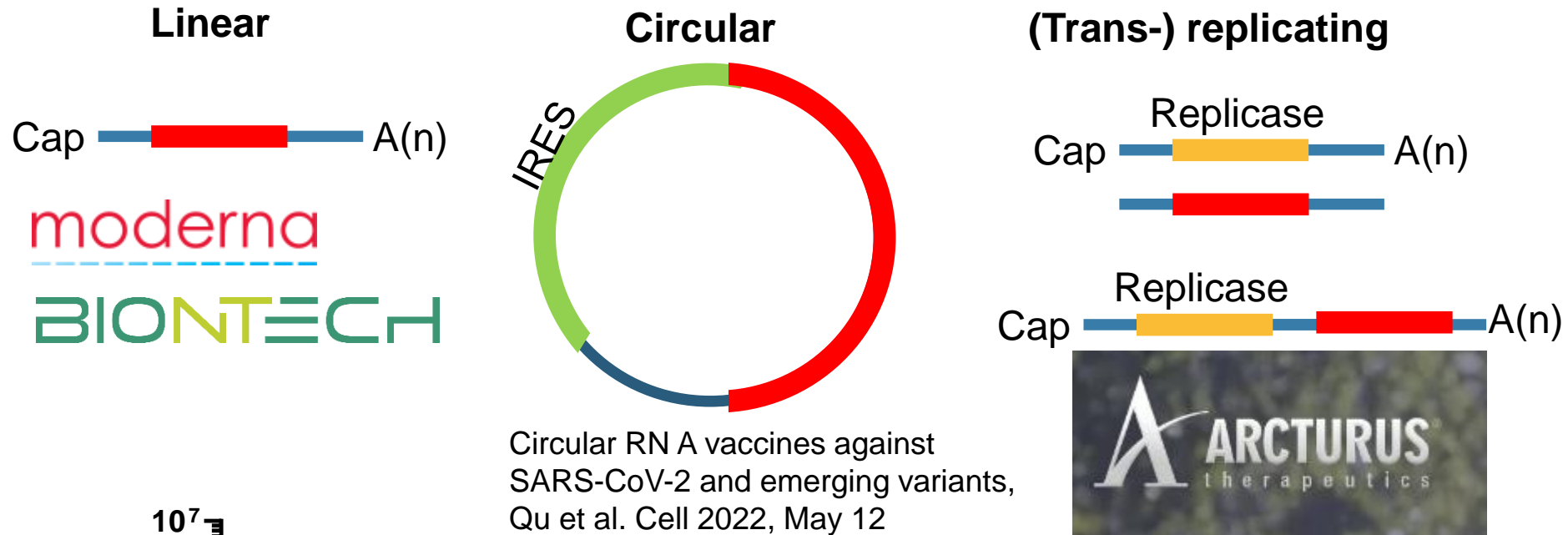
Phase I	Phase II	Phase III
	Melanoma (TAA)	
Prostate (TAA)		
	Head & neck (TAA)	
Ovary (TAA)		
	Melanoma (TSA)	
	Colorectal (TSA)	
Solid cancers (TSA)		

Phase I	Phase II	Phase III
	Solid tumors (TSA: individualised)	
Lung, pancreas, colorectal (TSA: KRAS)		

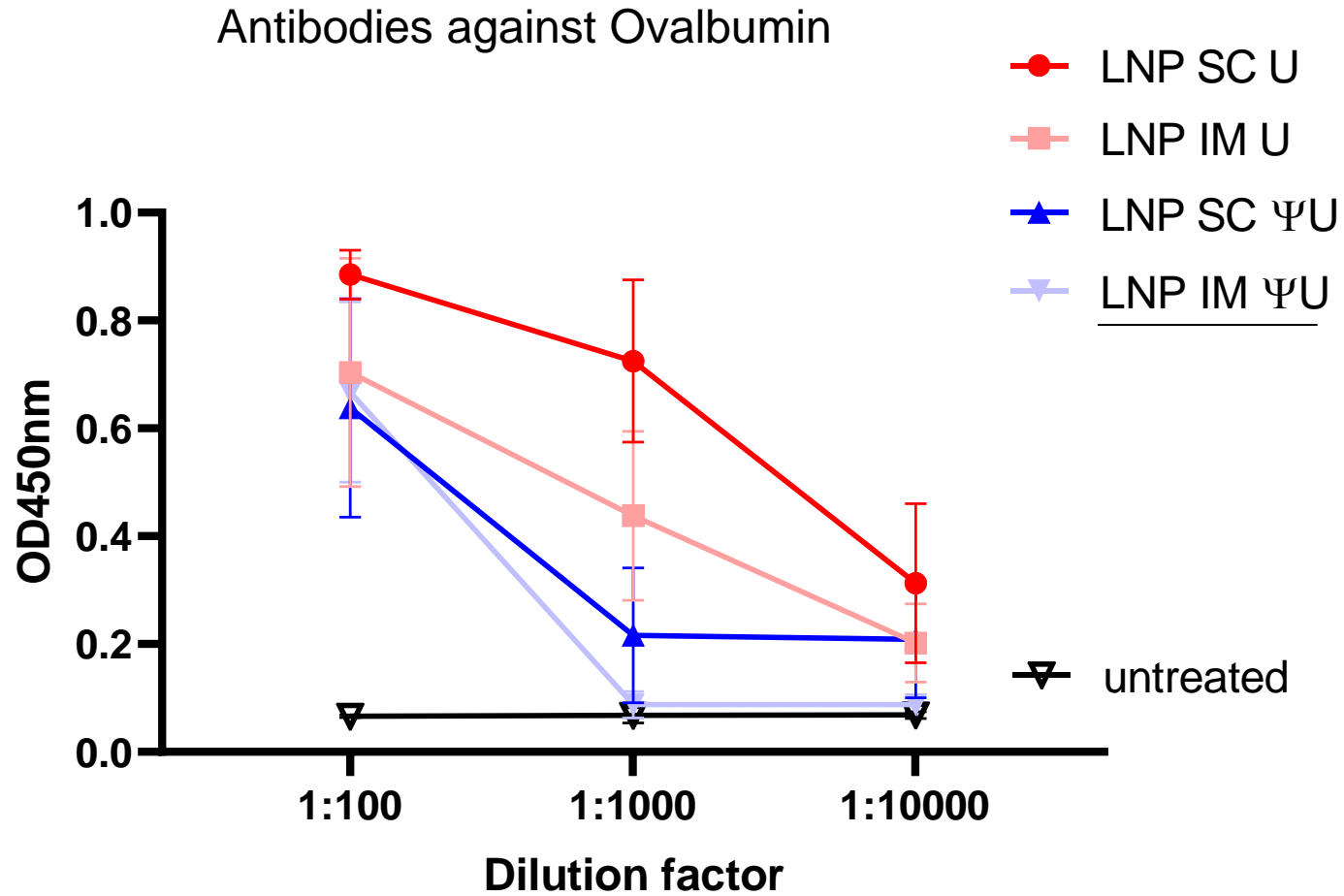
moderna

TAA: Tumor Associated Antigens (shared)
TSA: Tumor Specific Antigens (e.g.mutations)

Synthetic mRNA vaccines: Potential improvements



Synthetic mRNA vaccines: Potential improvements



Potential of synthetic mRNA in medicine

Potential of synthetic mRNA in medicine

Activation of the immune system: Modified or non modified mRNA

- Vaccines against (all) viruses
- Vaccines against cancer (shared tumor antigens or individualised vaccines against mutations)
- Vaccines against allergies

No activation of the immune system: Modified mRNA or non-modified circular mRNA

- Tolerizing the adaptive immune system (vaccines against autoimmune diseases)
- Expression of therapeutic proteins: erythropoietine, antibodies, etc
- Regeneration: blood vessels, retina, skin, muscles, neurones...
- Reprogramming cells: iPSC, CAR-T cells against cancer
- Modifying genomes (mRNA coding special proteins: e.g. CAS9)

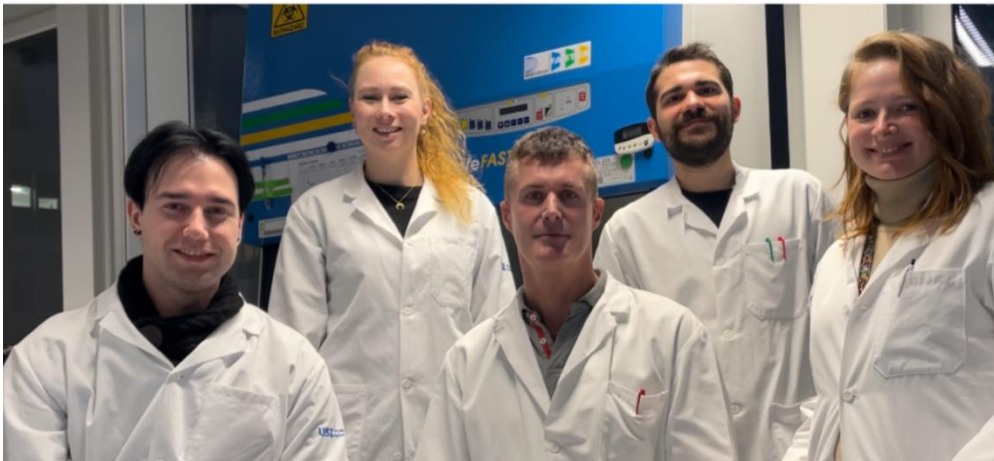


Synthetic Messenger RNA-Based Vaccines: from Scorn to Hype.
Pascolo S. Viruses. 2021 Feb 9;13(2):270.

Vaccines against COVID-19: Priority to mRNA-Based Formulations.
Pascolo S. Cells. 2021 Oct 11;10(10):2716.

Nonreplicating synthetic mRNA vaccines:
A journey through the European (Journal of Immunology) history.
Pascolo S. Eur J Immunol. 2023 Apr 7

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UZH URPP «Translational Cancer Research», UZH Stiftung für wissenschaftliche Forschung,
USZ Dermatology



Artist Caroline Schüpbach

