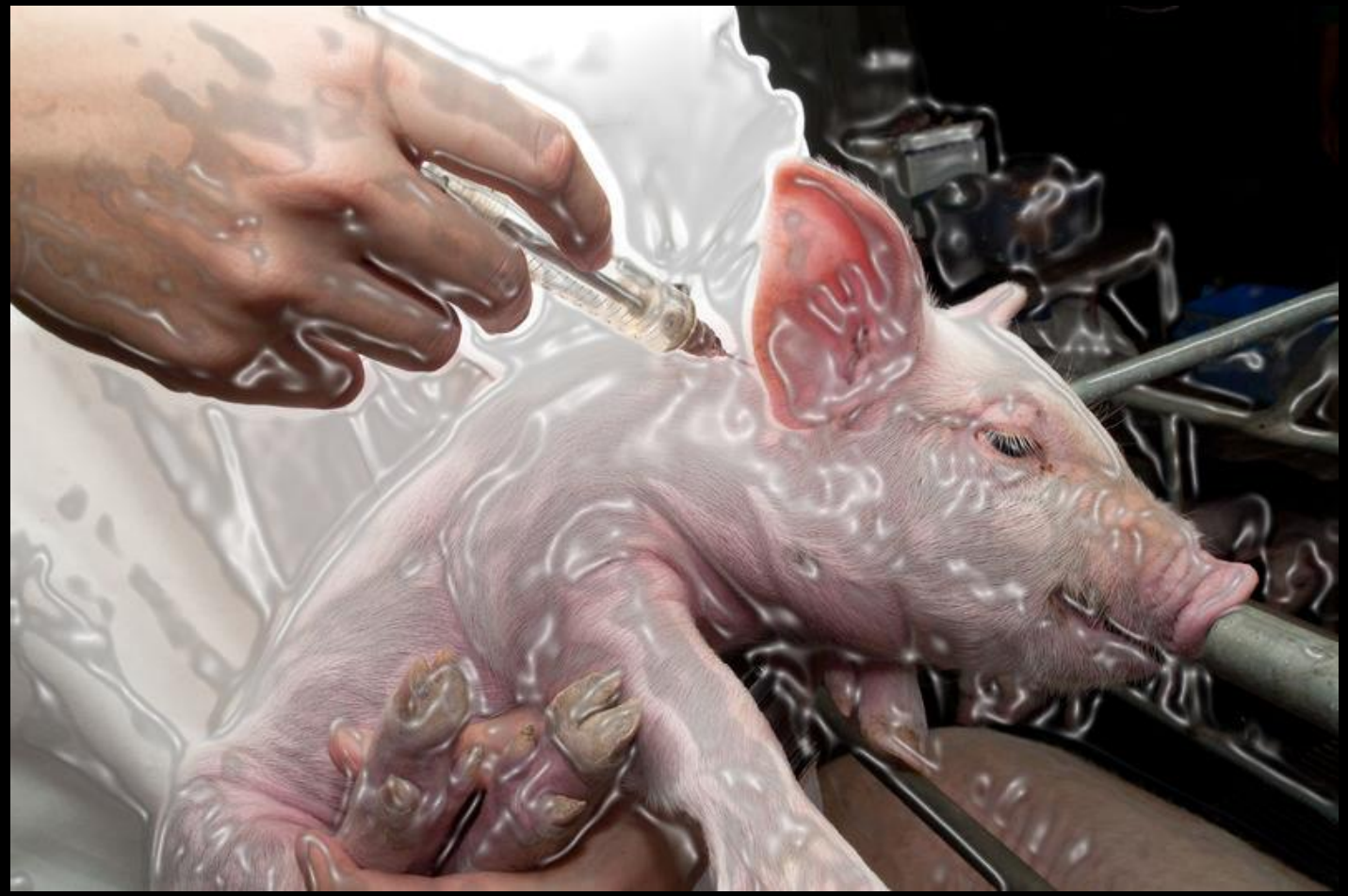
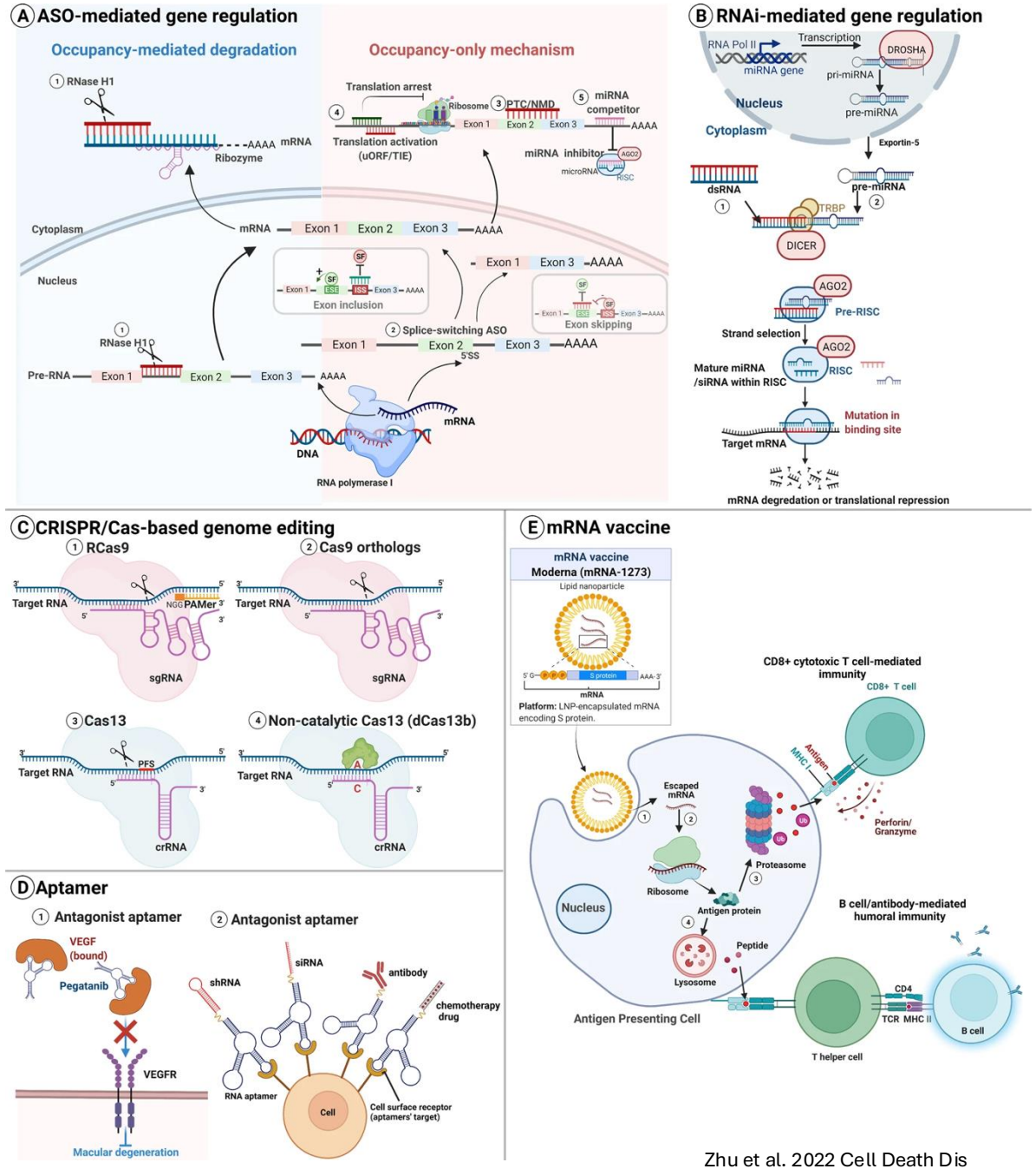
Are you comfortable with eating food that was injected with gene therapy?

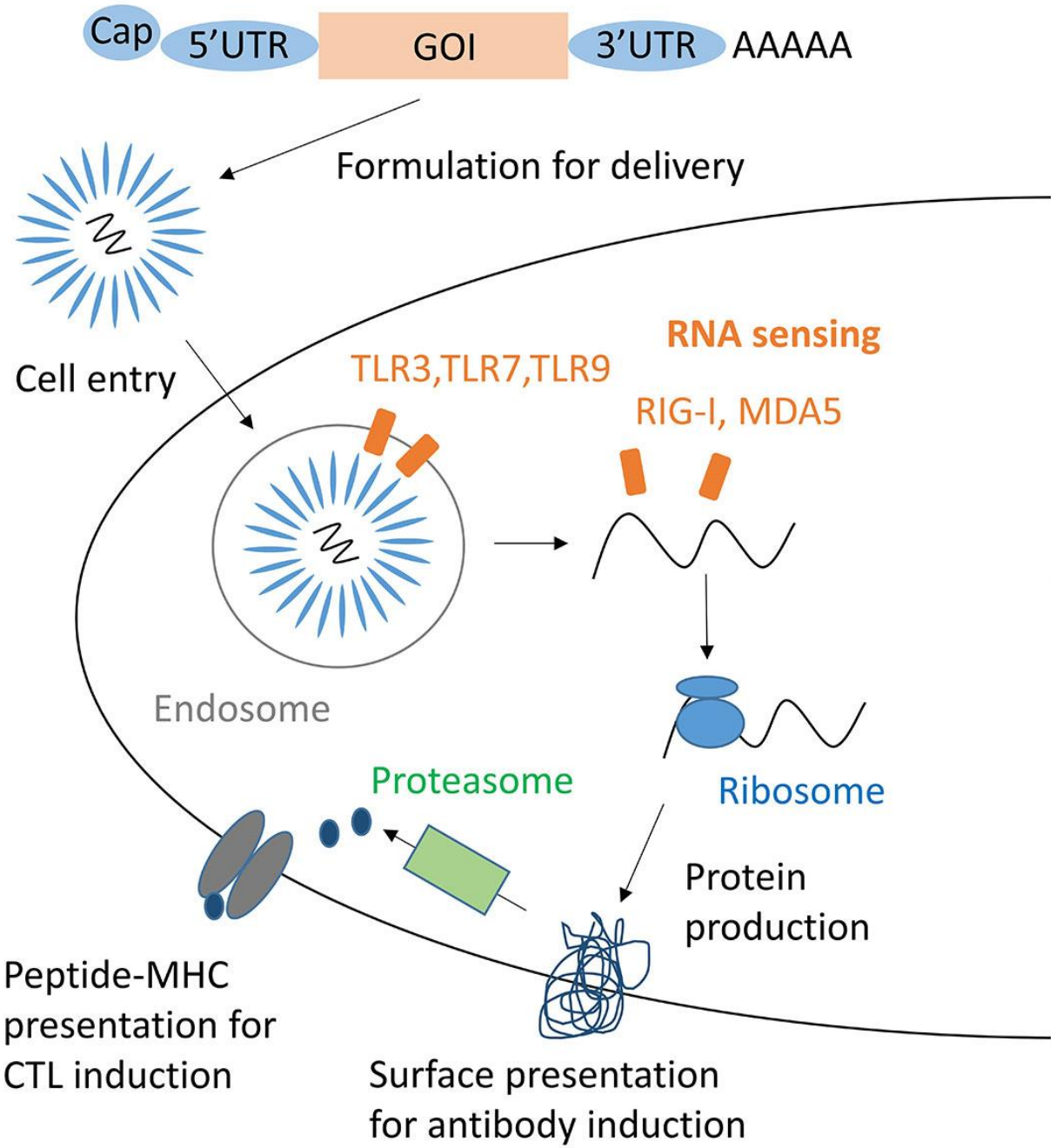


Karina Acevedo-Whitehouse, BVMS MSc PhD

- *Vaccination* based on gene therapy (*vaxgene*)



Zhu et al. 2022 Cell Death Dis



- Limited data on safety, biodistribution and pharmacokinetics
- No data on mid- and long-term effects
- No data on genotoxicity
- No data on transgenerational effects
- No data on effect of sustained antigenic stimulation
- No data on effect of accummulation of nanolipid particles from repeated booster shots

- ~2,000,000,000 have received at least one dose of this technology worlwide

- **Is this technology used on livestock?**

Sandbrink & Shattock 2020 Front Immunol

Autoinflammation
Autoimmunity

DNA damage
Genomic and chromosomal instability

Cellular degeneration

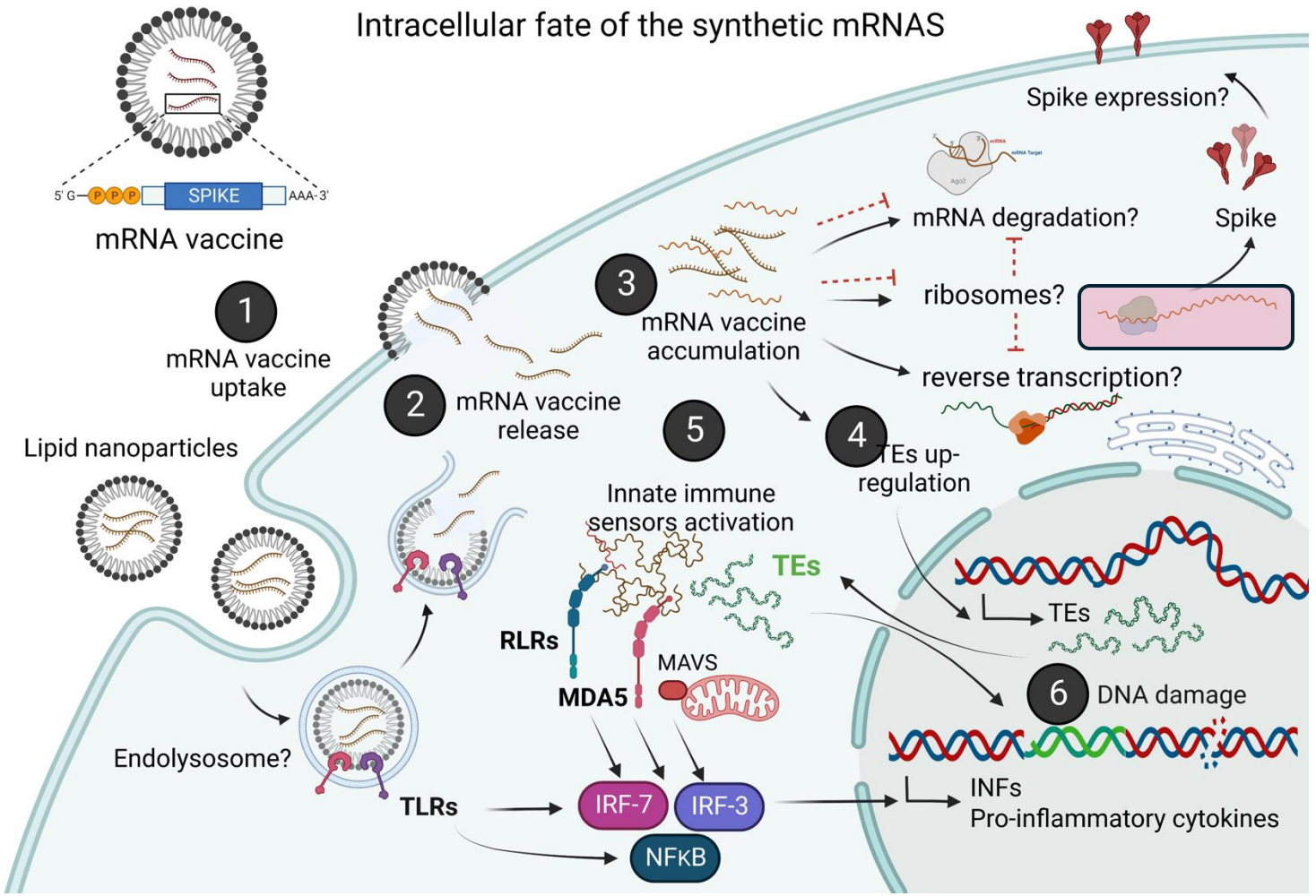
Ribosomal frameshift mutations

Mulroney et al. 2023 Nature

mRNA vaccines may make unintended proteins, but there's no evidence of harm

Alterations that help messenger RNA persist in living cells can trip up protein synthesis

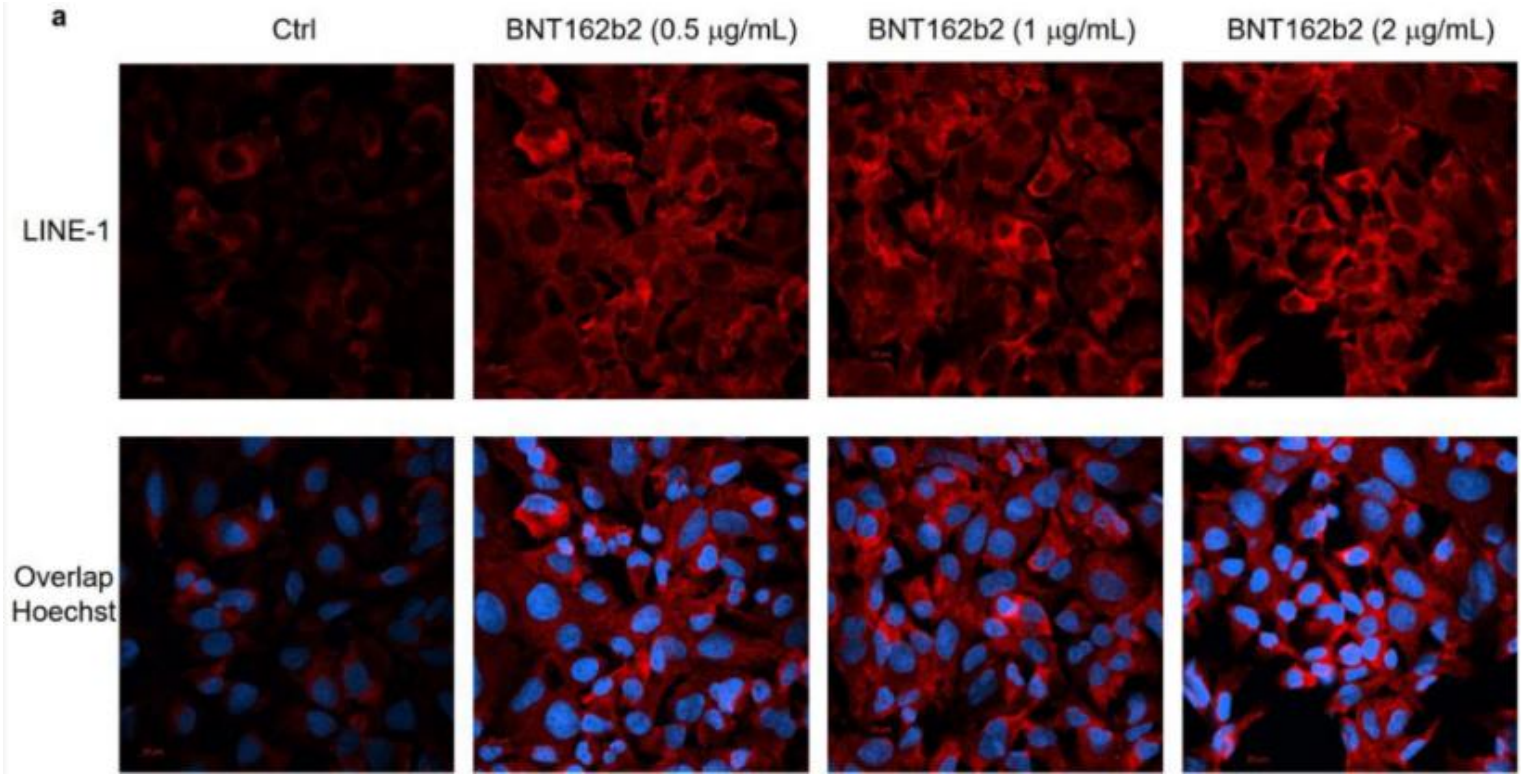
6 DEC 2023 · 11:00 AM ET · BY GRETCHEN VOGEL



Acevedo-Whitehouse & Bruno 2023 Med Hypoth

Viable for at least 186 days
Production of vaxgene-Spike for months

Brogna et al., 2023, Krauson et al., 2023, Mörz et al., 2023, Samaniego-Castruita et al., 2023, Baumeier et al., 2022, Fertig et al., 2022, Magen et al., 2022, Röltgen et al., 2022

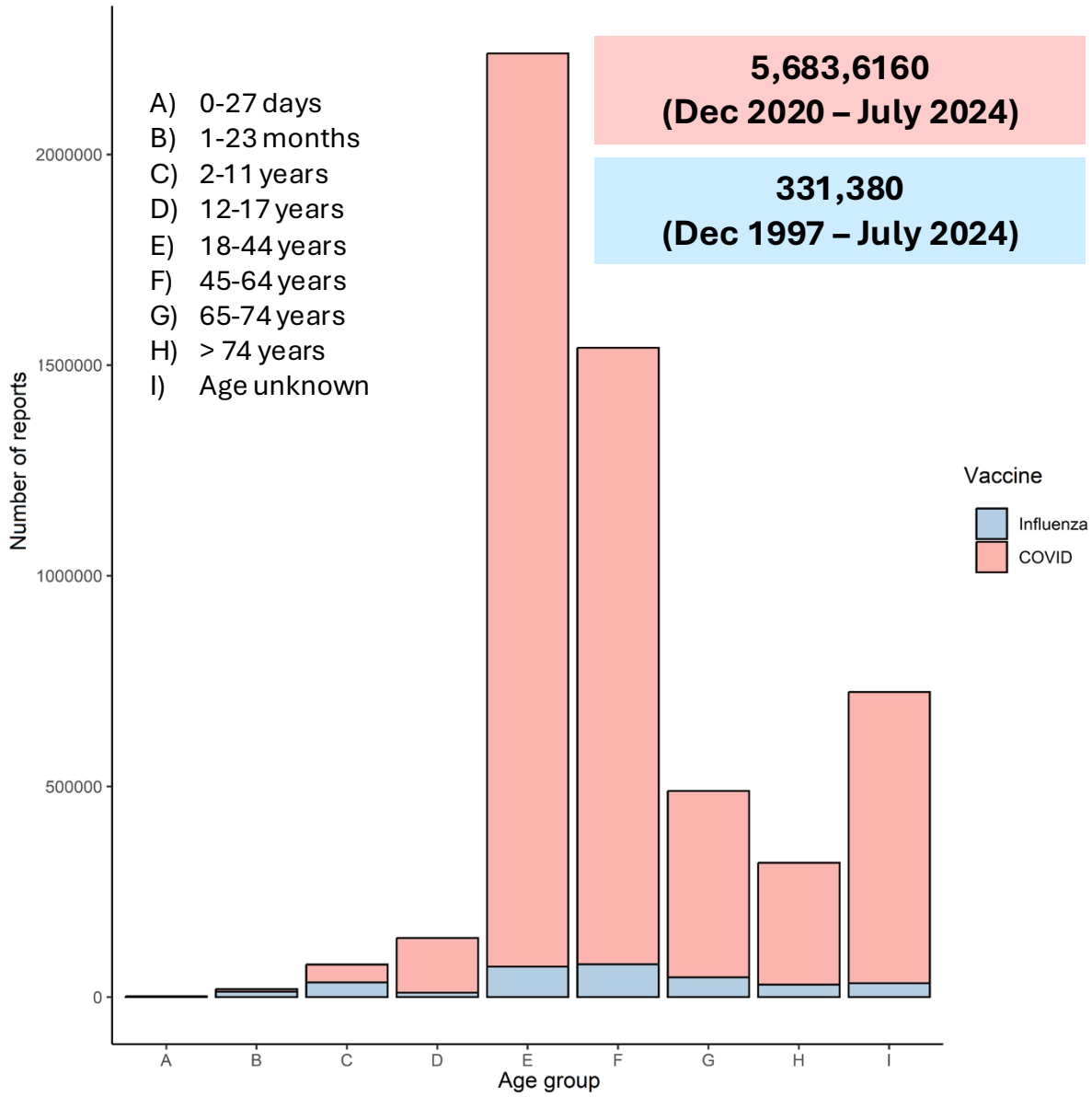


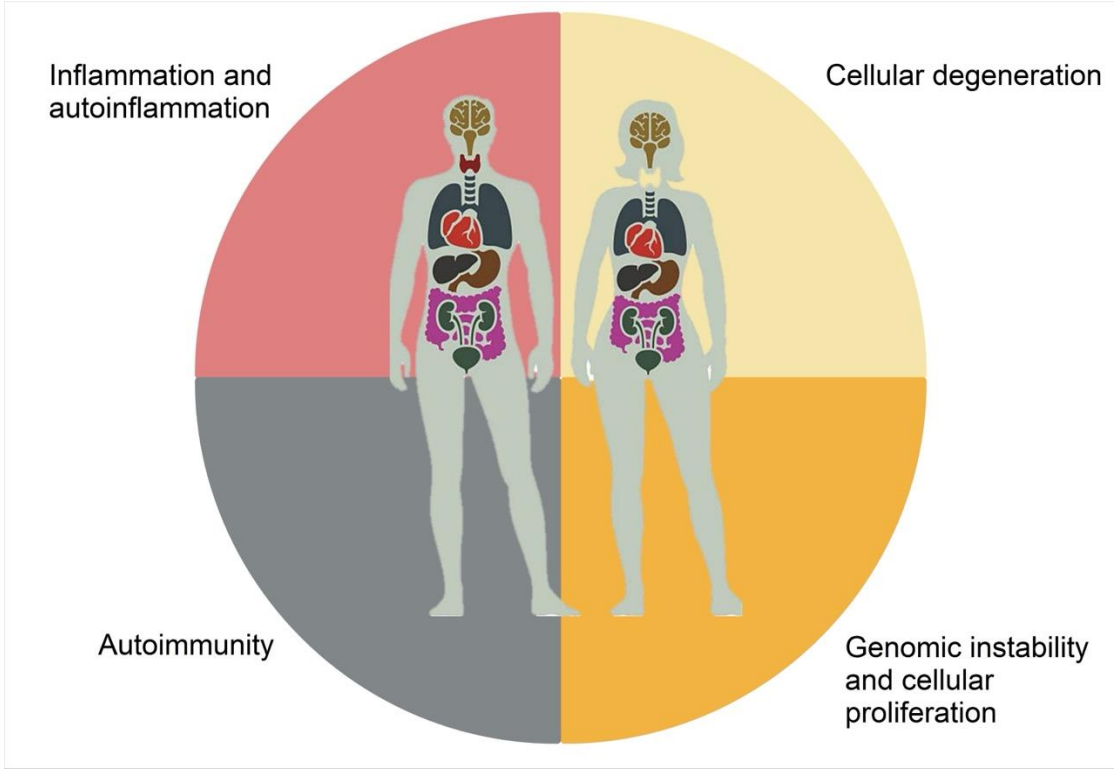
Aldén et al. 2022 Curr Issues Mol Biol

Based on *in vitro* assay using hepatic cancer cell line:
LINE-1 activation, reverse-transcription of vaxgene mRNA, nuclear entry

Genomic integration of the sequence? – perpetuation in transfected cells
Frameshift mutations?

EMA/707383/2020. Committee for Medicinal Products for Human Use (CHMP)


A) 0-27 days
B) 1-23 months
C) 2-11 years
D) 12-17 years
E) 18-44 years
F) 45-64 years
G) 65-74 years
H) > 74 years
I) Age unknown
5,683,6160
(Dec 2020 – July 2024)
331,380
(Dec 1997 – July 2024)
Vaccine
Influenza
COVID
Number of reports
2000000
1500000
1000000
500000
0
A
B
C
D
E
F
G
H
I
Age group


Inflammation and autoinflammation
Cellular degeneration
Autoimmunity
Genomic instability and cellular proliferation

Wait… it is only getting worse!
Self amplifying RNA “vaccines”

Self-replicating Semliki Forest virus RNA as recombinant vaccine

X. Zhou*†, P. Berglund*, G. Rhodes‡, S.E. Parker‡, M. Jondal† and P. Liljeström*∞

Recombinant RNA based on the Semliki Forest virus (SFV) replicon was used to express the nucleoprotein of influenza virus in mice. Two strategies were employed to deliver the RNA. In the first, recombinant RNA was packaged into infectious suicide SFV particles which were used directly for immunization. The second approach involved injection of in vitro-*synthesized RNA directly into the quadriceps muscle. Both approaches resulted in the generation of humoral responses with high antibody titres. Immunization with suicide particles showed that a strong, class I-restricted cytotoxic T-cell response can be obtained using only 100 infectious units. We conclude that the self-replicative recombinant SFV RNA may be quite useful as a nucleic acid vaccine.*

Keywords: Semliki Forest virus; suicide particles; immunization of naked RNA

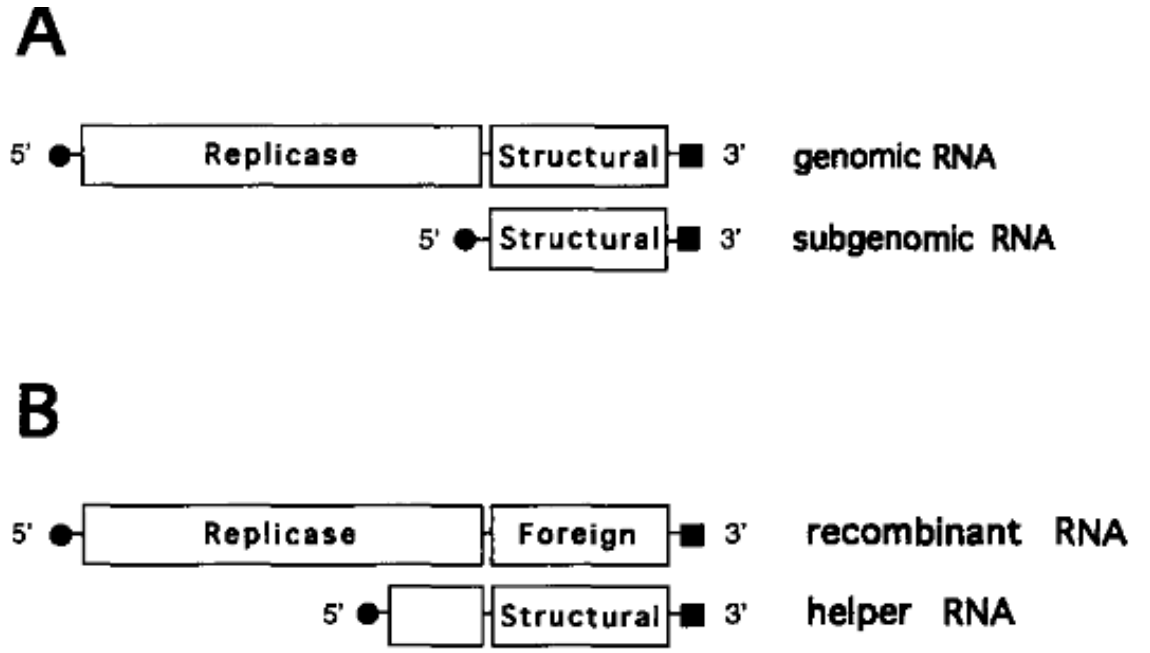
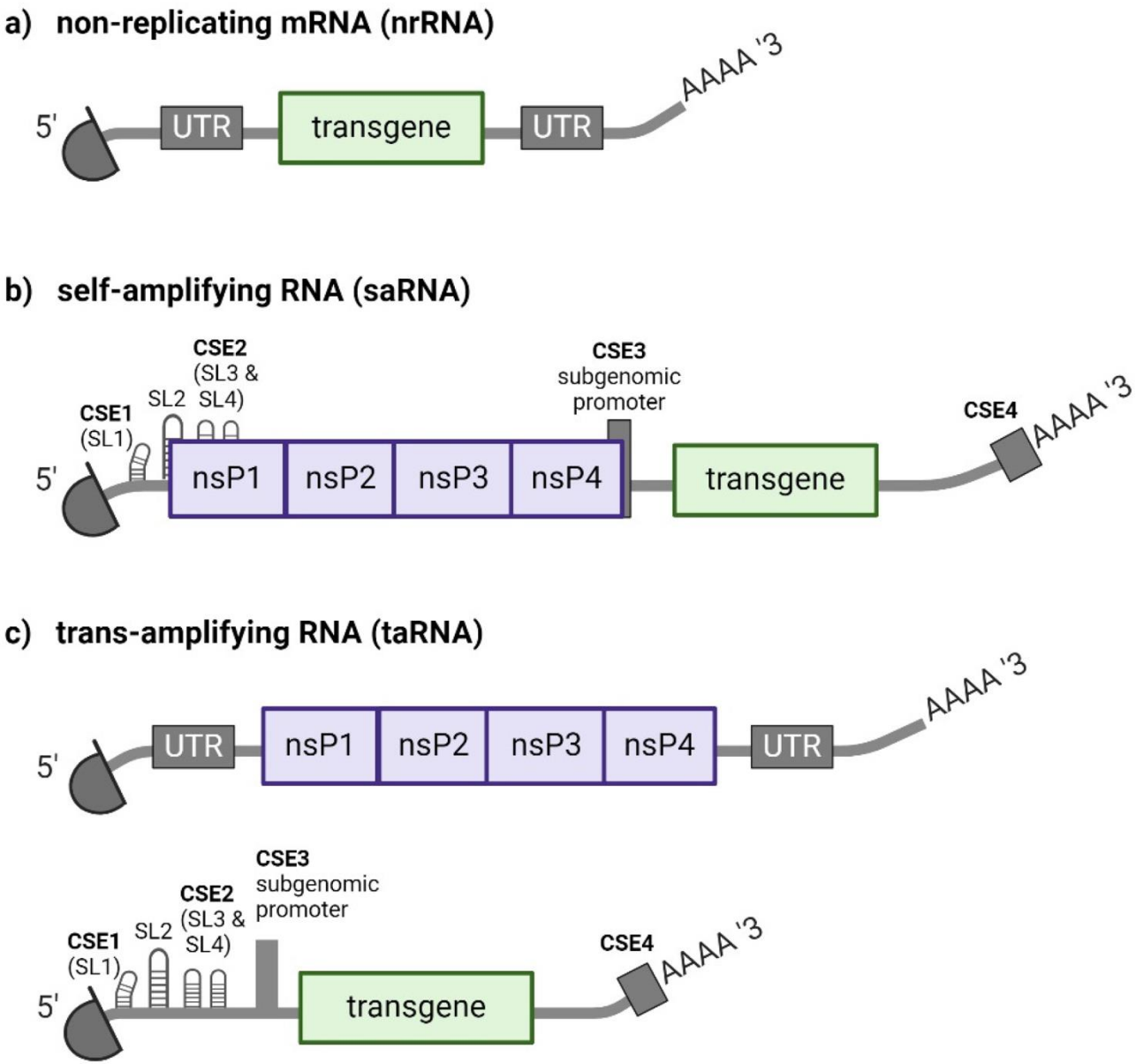


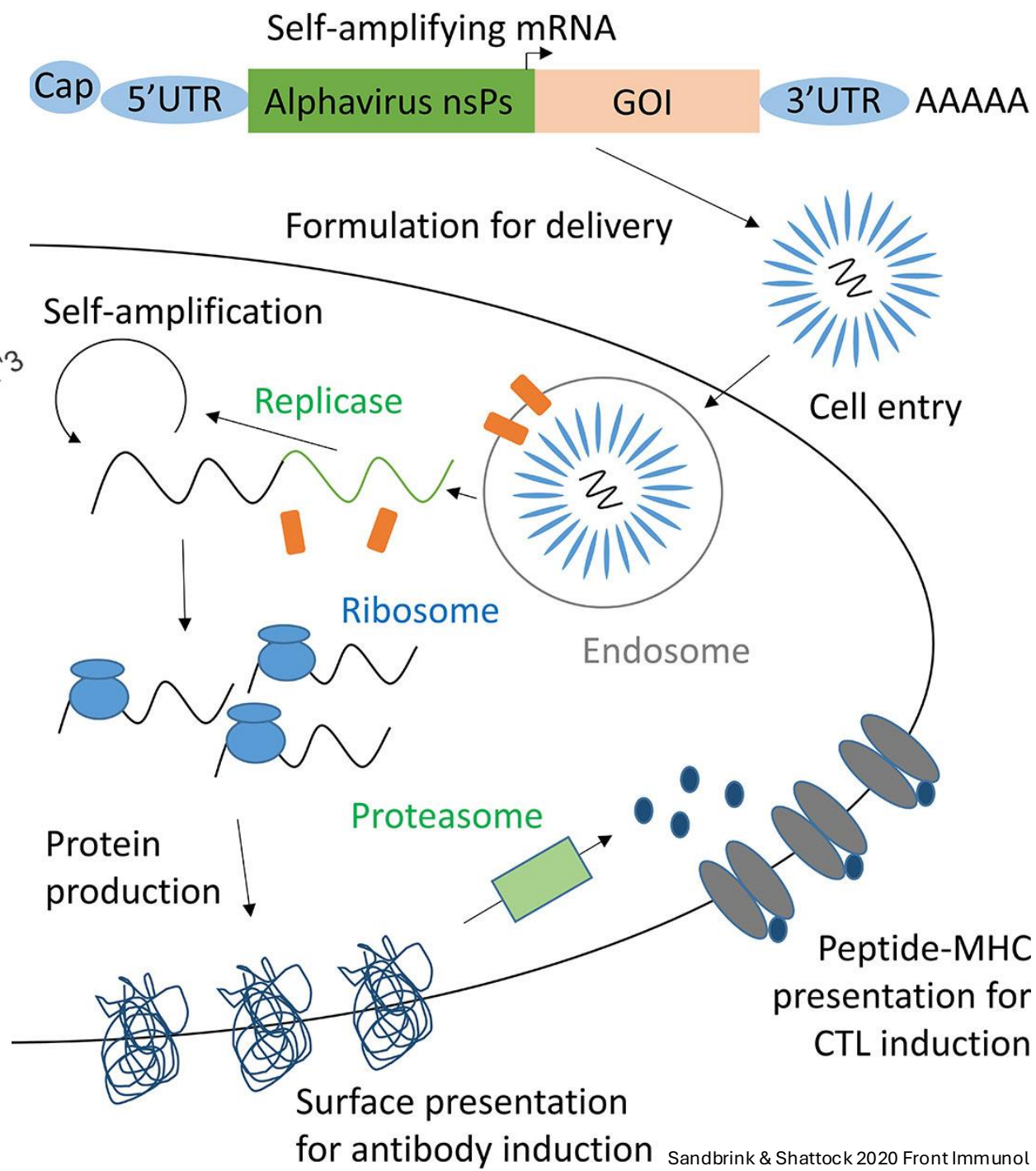
Figure 1 (A) RNAs of the SFV replicon (see text for further details). (B) RNAs of the SFV expression system. In the recombinant RNA, the SFV stuctural genes have been replaced by the foreign antigen-encoding gene. The helper RNA is used to package recombinant RNA into SFV particles (see *Figure 2*)

Mancini et al. 2015 Prog Mol Biol Trans Sci

Alphavirus RNAdRNA: mutation rate is 10^{-6} to $^{10-4}$ substitutions per nucleotide per replication Lu et al. 2022 Front Microbiol



Yildiz et al. 2024 Viruses



Sandbrink & Shattock 2020 Front Immunol

viruses

MDPI

Review

mRNA Vaccine Development for Emerging Animal and Zoonotic Diseases

Ting Le [1,†], Chao Sun [1,†], Jitao Chang [1,*], Guijie Zhang [2,*] and Xin Yin [1,*]

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[2] Departments of Animal Science, School of Agriculture, Ningxia University, Yinchuan 750021, China
* Correspondence: changjitao@caas.cn (J.C.); guijiezhang@nxu.edu.cn (G.Z.); yinxin@caas.cn (X.Y.)
† These authors contributed equally to this work.

Abstract: In the prevention and treatment of infectious diseases, mRNA vaccines hold great promise because of their low risk of insertional mutagenesis, high potency, accelerated development cycles, and potential for low-cost manufacture. In past years, several mRNA vaccines have entered clinical trials and have shown promise for offering solutions to combat emerging and re-emerging infectious diseases such as rabies, Zika, and influenza. Recently, the successful application of mRNA vaccines against COVID-19 has further validated the platform and opened the floodgates to mRNA vaccine's potential in infectious disease prevention, especially in the veterinary field. In this review, we describe our current understanding of the mRNA vaccines and the technologies used for mRNA vaccine development. We also provide an overview of mRNA vaccines developed for animal infectious diseases and discuss directions and challenges for the future applications of this promising vaccine platform in the veterinary field.

Keywords: mRNA vaccine; infectious disease; zoonoses; immune response; viruses

Citation: Le, T.; Sun, C.; Chang, J.; Zhang, G.; Yin, X. mRNA Vaccine Development for Emerging Animal and Zoonotic Diseases. *Viruses* **2022**, *14*, 401. https://doi.org/10.3390/v14020401

Academic Editors: Dapeng Li, Ahmed O. Hassan and Jingyou Yu

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Accepted: 14 February 2022
Published: 15 February 2022

1. Introduction

Vaccination has made a tremendous contribution to human and veterinary medicine [1]. Two major viral diseases, smallpox and rinderpest, have been eradicated worldwide with the help of vaccination [2,3]. During the COVID-19 pandemic, different types of vaccines have been developed and authorized for human use [4–10]. Among them, COVID-19 mRNA vaccines have shown notable effectiveness in disease prevention and have attracted public attention. This is the first time ever a mRNA vaccine has been licensed for human use. The emergence of mRNA vaccines has fundamentally revolutionized vaccine development [8,11–14].

In general, the mRNA encoding the immunogens is encapsulated in a lipid shell. Once delivered into the cytoplasm of target cells, the host cell machinery directs the translation of the antigen proteins that induce effective and long-lasting immune responses (Figure 1).

Foot and mouth disease – (2010) trials in mice

Rabies

First approved for swine IAV in 2012 (USDA)

Table 1. Selected RNA virus vector vaccine references.

Virus family	Subfamily	Genus	Virus (strain or modification)	Gene of interest
Rhabdoviridae		Lyssavirus	RABV	HCV E2
				Botulinum neurotoxin
				SARS-CoV N or S
				HIV-1 gp160
				HIV-1 Gag
				IL-2 and IL-4
				IFN-β
				SHIV Env and SIV Gag
				SIV Gag-Pol
		Vesiculovirus	VSV	HIV-1 gp120
				IAV(H1N1) HA or NA
				IAV(H5N1) HA
				HBV MS
				ANDV GPC
				HCV C/E1/E2
				RSV G or F
				Yersinia pestis LcrV
				Various filovirus GP
				CD4
				CD4 and CXCR4
				CHIKV E1/E2
				SIV Gag and Env
				HIV-1 Gag and Env
			VSV for oncolytic virotherapy	Various genes
		Novirhabdovirus	VHSV	WNV E
Paramyxoviridae	Pneumovirinae	Pneumovirus	hRSV (subgroup A)	hRSV G (subgroup B)
			bRSV	hRSV F and/or G
Flaviviridae		Flavivirus	YFV (17D)	JEV prME
				DEN1–4 prME
				WNV prME
			DEN4 (814669 and derivatives)	TBEV prME
				LGT prME
				SLEV prME
				WNV prME
				DEN2 prME
			DEN2 (PDK-53)	DEN1, 3, 4 prME
			KUN	EBOV GP
				HIV-1 Gag
			JEV (SA14-14-2)	WNV prME
				DENV prME
			MVEV (IRES attenuated)	IFN-β
			WNV (SCFV)	DENV prME
				TBEV prME
		Pestivirus	BVDV (CP7)	CSFV E2
				CSFV E1/E2
				BDV E2
			BVDV (SD1)	GFP
			BVDV (NADL)	Heterologous Erns
			CSFV	BVDV Erns or E2
				JEV E(truncated)
Orthomyxoviridae		Influenzavirus	Influenza A	Circumsporozoite (CS) protein of *P. yoelii*
				CS protein of *P. falciparum*
				HIV gp41
				HIV gp41
				HIV GAG

Mogler & Kamrud 2015 Exp Rev Vaccines

https://www.merck-animal-health-usa.com/species/swine/sequivity

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MERCK Animal Health

ABOUT US SPECIES PRODUCTS RESOURCES RESPONSIBILITY

SEQUIVITY® Overview Products Sequivity® Resources FIND A REP

Combat current and future swine diseases with SEQUIVITY from Merck Animal Health. A **revolutionary swine vaccine platform**, SEQUIVITY harnesses RNA particle technology to create customized prescription vaccines against strains of influenza A virus in swine, porcine circovirus (PCV), rotavirus and beyond. It's supported by a sophisticated dashboard filled with comprehensive data and insights all to help you stay on top.

KEY BENEFITS

TARGETED
SEQUIVITY only targets swine pathogen gene sequences of interest.

SAFE
Doesn't replicate or cause disease, delivering pathogen information to the immune system safely. There's no need to transfer or handle live material like autogenous, killed or modified live vaccines.

DYNAMIC
Targets existing and evolving swine pathogens, including diseases not covered by conventional swine vaccines.

FLEXIBLE
Allows for the creation of multivalent formulations by blending RNA particles to target multiple swine pathogens in one shot.

POWERFUL
SEQUIVITY® technology now combines with the power of our Microsol Diluvac Forte® (MDF) adjuvant to give veterinarians the upper hand in the fight against swine diseases.

Feedback

From pathogen collection through creation, SEQUIVITY follows a precise step-by-step process to create safe, flexible, targeted prescription vaccines in only 12 to 16 weeks.

Collect pathogens.

A sample is collected by a veterinarian and sent to a diagnostic lab.

Sequence genes.

The gene of interest (GOI) is sequenced and sent electronically to SEQUIVITY analysts.

Insert into RNA particles.

GOI is synthesized and inserted into the RNA production platform.

Create a safe, flexible, targeted vaccine.

After incubation, RNA particles released from the production cells are harvested, purified and formulated into a final vaccine that is delivere to the farm.

See the future.

Through large-scale information collection, the revolutionary SEQUIVITY Dashboard allows for smarter analysis of the

- We have no idea if it is safe
  - Animal health (individual level)
  - Animal health (population level)
  - Animal health (ecosystem level)
  - Human healh

Safe and Effective in Improving the Health and Well-Being of Animals

Vaccines developed with RNA technology do not pose any risks to the food supply when animals are vaccinated. **All vaccines undergo rigorous safety studies**. In fact, the use of RNA Particle vaccine technology has proven to be an effective tool in improving the health and well-being of animals. **Millions of doses of the SEQUIVITY vaccine platform have safely been used by veterinarians** in swine herds for more than ten years since the USDA first issued the license in 2012. It is also being used in other countries such as Canada, Chile, Mexico, and the Philippines.

Vaccine 41 (2023) 6941–6951

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Quadrivalent neuraminidase RNA particle vaccine protects pigs against homologous and heterologous strains of swine influenza virus infection

Pravina Kitikoon [a,*], Susan M. Knetter [a], Mark A. Mogler [b], Chandra L. Morgan [a], Allison Hoehn [a], Supraja Puttamreddy [b], Erin L. Strait [a,1], Ruud P.A.M. Segers [c]

[a] Merck Animal Health, De Soto, KS 66018, United States
[b] Merck Animal Health, Ames, IA 50010, United States
[c] MSD Animal Health, 5830 AA Boxmeer, The Netherlands

Vaccine 40 (2022) 5569–5578

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Bivalent hemagglutinin and neuraminidase influenza replicon particle vaccines protect pigs against influenza a virus without causing vaccine associated enhanced respiratory disease

Meghan Wymore Brand [a], Tavis K. Anderson [a], Pravina Kitikoon [b], J. Brian Kimble [a], Nicholas Otis [a], Phillip C. Gauger [c], Carine K. Souza [a], Bryan Kaplan [a], Mark Mogler [d], Erin Strait [b,1], Amy L. Vincent Baker [a,*]

[a] Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA 50010, United States
[b] Merck Animal Health, De Soto, KS 66018, United States
[c] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50010, United States
[d] Merck Animal Health, Ames, IA 50010, United States

As with COVID, they are defining “efficaciousness” mainly as the generation of specific antibodies and brief challenge trials.

Safety studies are brief and incomplete.

No studies have evaluated potential impacts for humans consuming their meat or byproduct.

308 pigs – randomized into treatment and placebo*

Observed for clinical signs of disease for 15 days after each dose; respiratory signs for 5 days

At necropsy: only respiratory examination

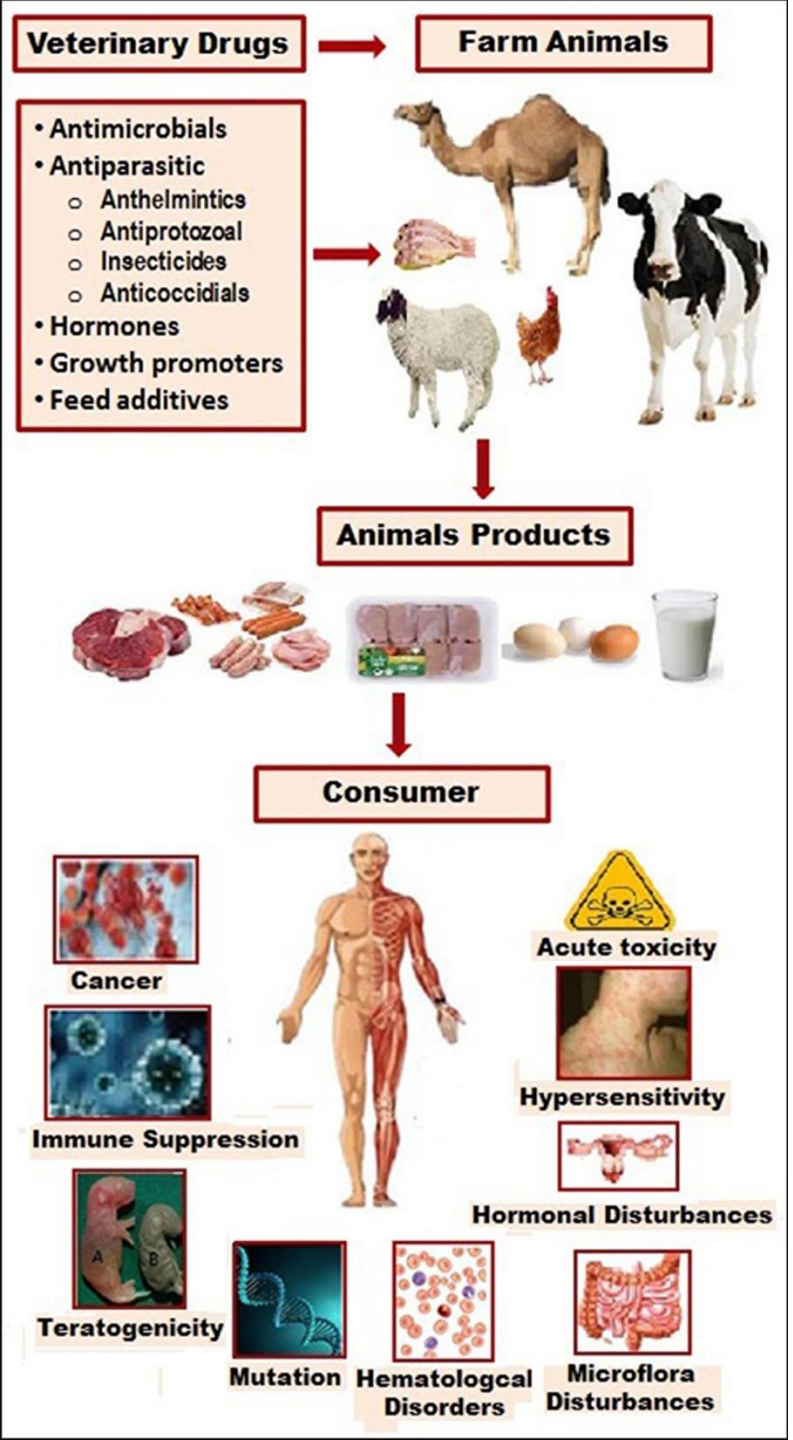
ARTICLE OPEN

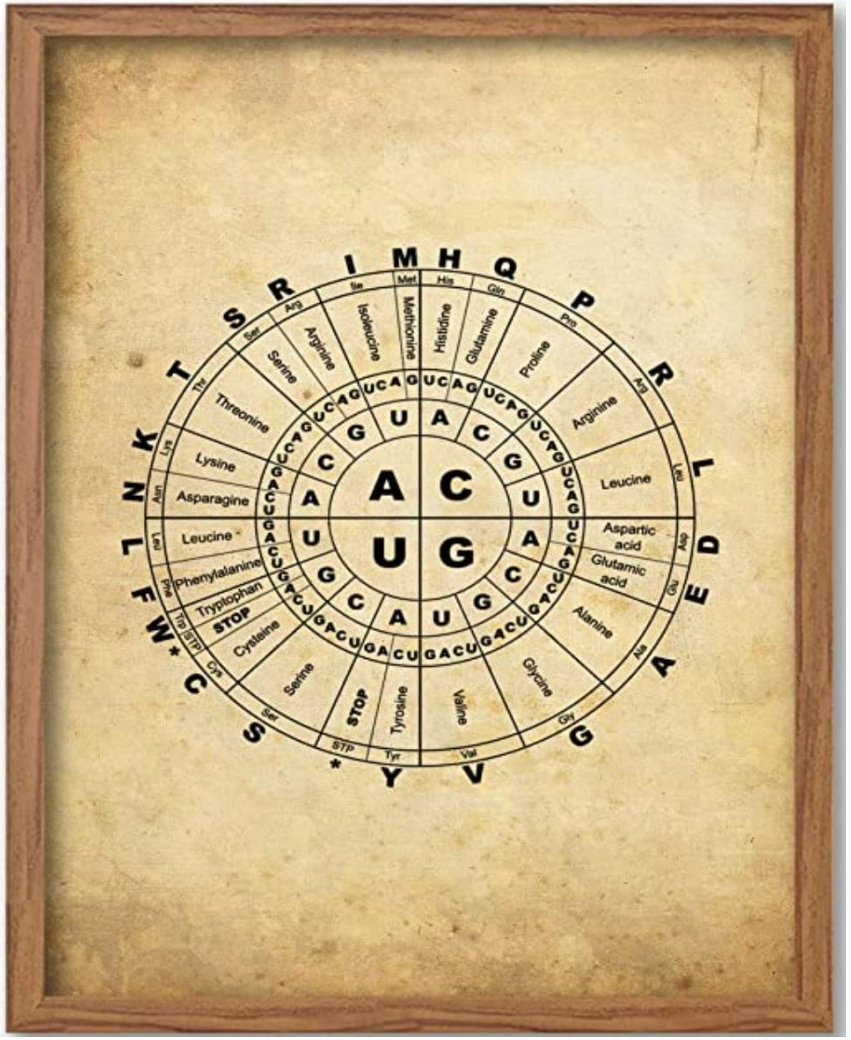
An alphavirus replicon-based vaccine expressing a stabilized Spike antigen induces protective immunity and prevents transmission of SARS-CoV-2 between cats

Martijn A. Langereis [1✉], Irina C. Albulescu [2], Judith Stammen-Vogelzangs [1], Morindy Lambregts [1], Ken Stachura [3], Suzan Miller [3], Angela M. Bosco-Lauth [4], Airn E. Hartwig [4], Stephanie M. Porter [4], Michelle Allen [5], Mark Mogler [6], Frank J. M. van Kuppeveld [2], Berend-Jan Bosch [2], Paul Vermeij [1], Ad de Groof [1], Richard A. Bowen [4], Randy Davis [3], Zach Xu [3] and Ian Tarpey [7]

Early in the SARS-CoV-2 pandemic concerns were raised regarding infection of new animal hosts and the effect on viral epidemiology. Infection of other animals could be detrimental by causing clinical disease, allowing further mutations, and bares the risk for the establishment of a non-human reservoir. Cats were the first reported animals susceptible to natural and experimental infection with SARS-CoV-2. Given the concerns these findings raised, and the close contact between humans and cats, we aimed to develop a vaccine candidate that could reduce SARS-CoV-2 infection and in addition to prevent spread among cats. Here we report that a Replicon Particle (RP) vaccine based on Venezuelan equine encephalitis virus, known to be safe and efficacious in a variety of animal species, could induce neutralizing antibody responses in guinea pigs and cats. The design of the SARS-CoV-2 spike immunogen was critical in developing a strong neutralizing antibody response. Vaccination of cats was able to induce high neutralizing antibody responses, effective also against the SARS-CoV-2 B.1.1.7 variant. Interestingly, in contrast to control animals, the infectious virus could not be detected in oropharyngeal or nasal swabs of vaccinated cats after SARS-CoV-2 challenge. Correspondingly, the challenged control cats spread the virus to in-contact cats whereas the vaccinated cats did not transmit the virus. The results show that the RP vaccine induces protective immunity preventing SARS-CoV-2 infection and transmission. These data suggest that this RP vaccine could be a multi-species vaccine useful to prevent infection and spread to and between animals should that approach be required.

npj Vaccines (2021)6:122; https://doi.org/10.1038/s41541-021-00390-9





Codon optimization

Very high and sustained production of antigenic protein (20-30% of cell protein production)

Autoimmunity*/Autoinflammation

Immune suppressive states

Cancer

Prion-like behaviour*

What on earth are we doing?

Atta et al. 2022 Environ Sci Poll Res

Only two things are infinite: the universe and human stupidity, and I am not sure about the former.

Albert Einstein

Only two things are infinite: hope and pharma's unscrupulous ambition, and I am definitely sure about the former.

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AKASHA
sembrando conciencia

Telegram: https://t.me/akashacomunidad