Antiviral therapies and new vaccine types

Antivirals may have:

viral targets	(interfere with a particular phase of viral cycle)
host cell targets	(interfere with a cell component or process important for virus replication)

Ideal antiviral drugs

ARE: water soluble, stable in bloodstream, enter the cell easily, stable;

ARE NOT: toxic, mutagenic, carcinogenic, teratogenic, allergenic.

New antiviral therapies development rely on new tools (genomics, proteomics).

After drug discovery and preclinical testing (toxicity, efficacy, pharmacokinetics) on animals or *in vitro*, it is important to know the influence of a drug on a virus host.

Clinical testing phases:

0 – 10 people, pharmacokinetics (half-life, oral bioavailability), often skipped

I – 20-100 healthy volunteers, up to 1 year.

Is it safe for humans, what may be side effects (safety)?

II – couple of hundred of volunteers (100-300).

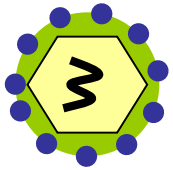
Is it effective and to what extent (efficacy, dose)?

III – comparison with the standard treatments, therapeutic effects (dose range)

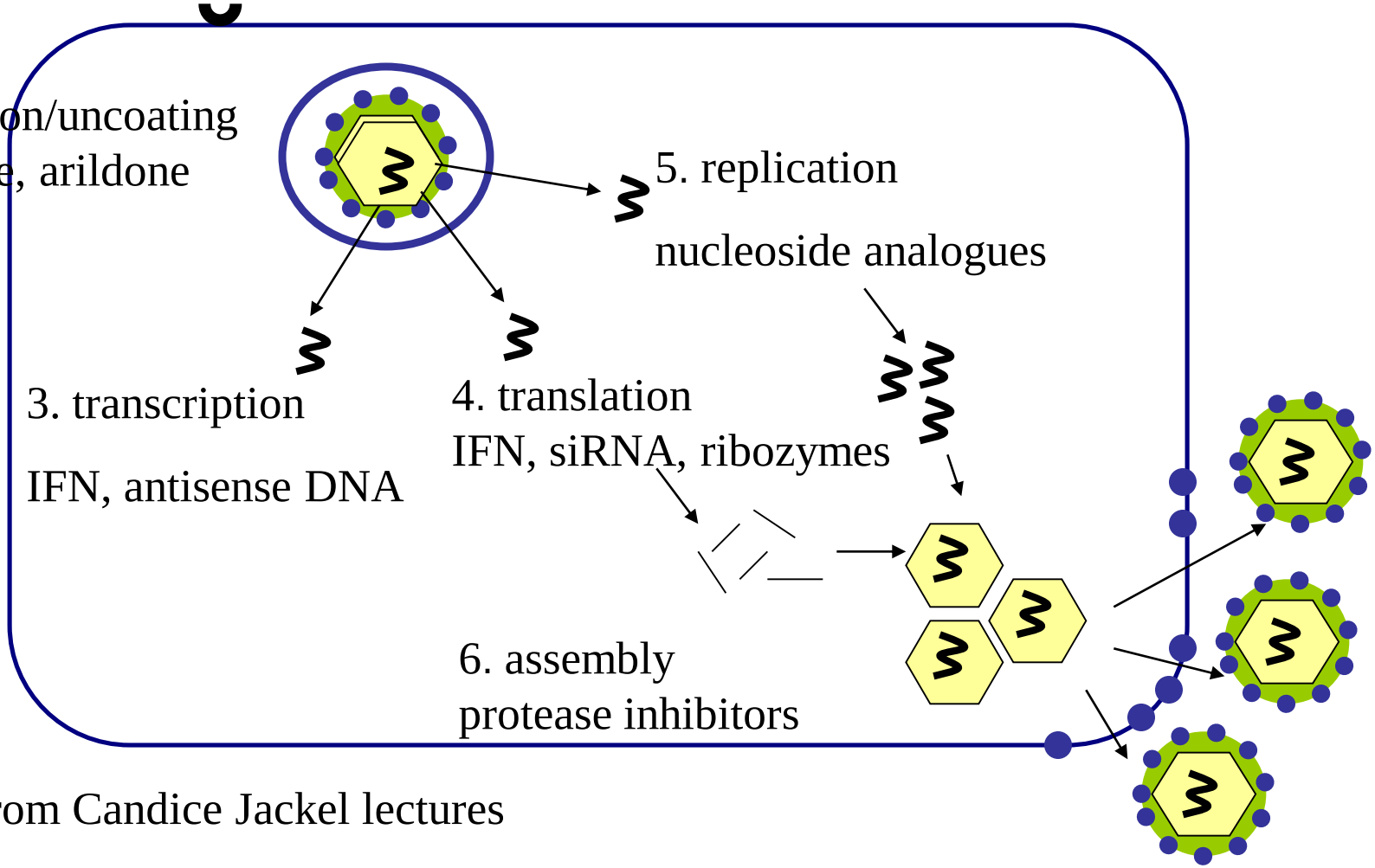
IV – whoever seeks the treatment, what are long-term effects?

Always consider the possibility of drug resistance development.

Terapies with viral targets

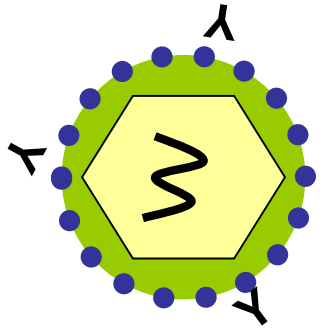


1. attachment (Abs, receptor antagonists)

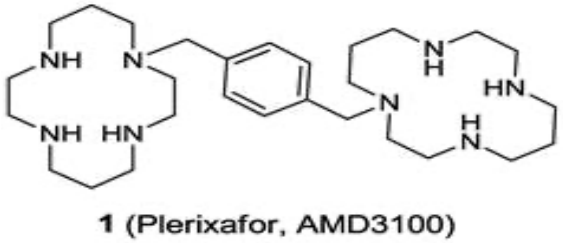


Adapted from Candice Jackel lectures

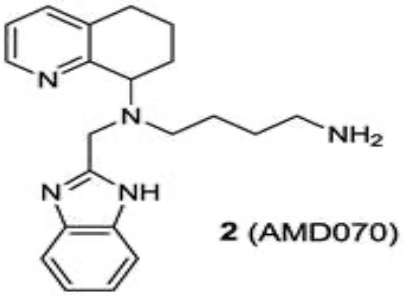
Attachment (adsorption) inhibitors



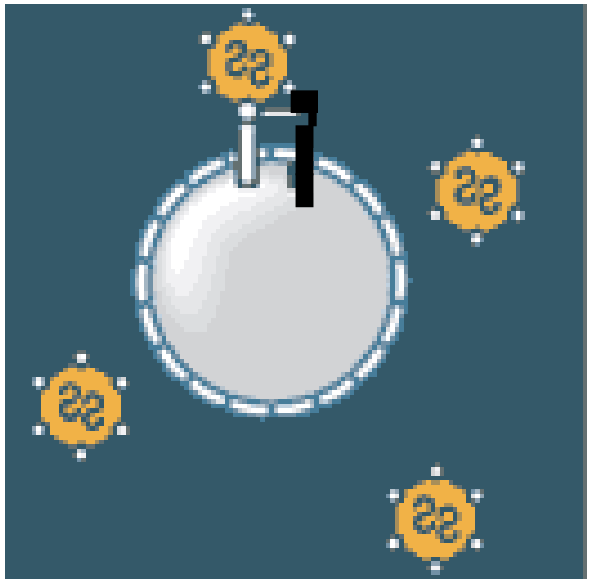
- Neutralizing antibodies
  - passive immunization
  - adoptive T-cell therapy (lymphocyte transfusion)
- Receptor antagonists
  - (glyco)peptides analogues of cell receptors
  - Inhibitors of chemokine receptors
- Others
  - galactomannan-sulphates (Dengue, YFV)

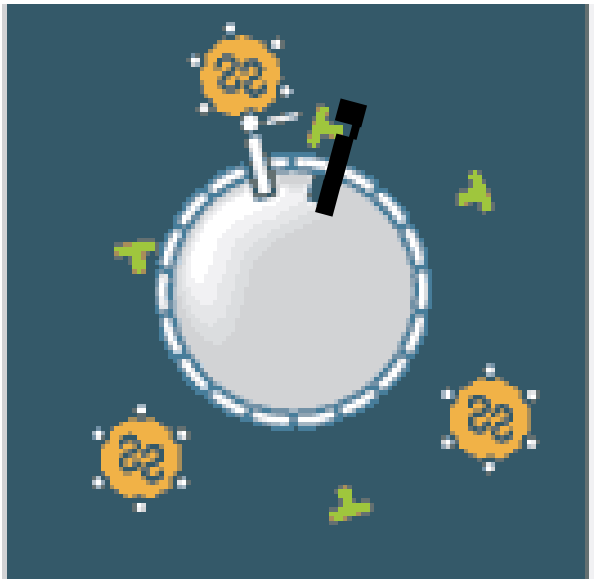


1 (Plerixafor, AMD3100)



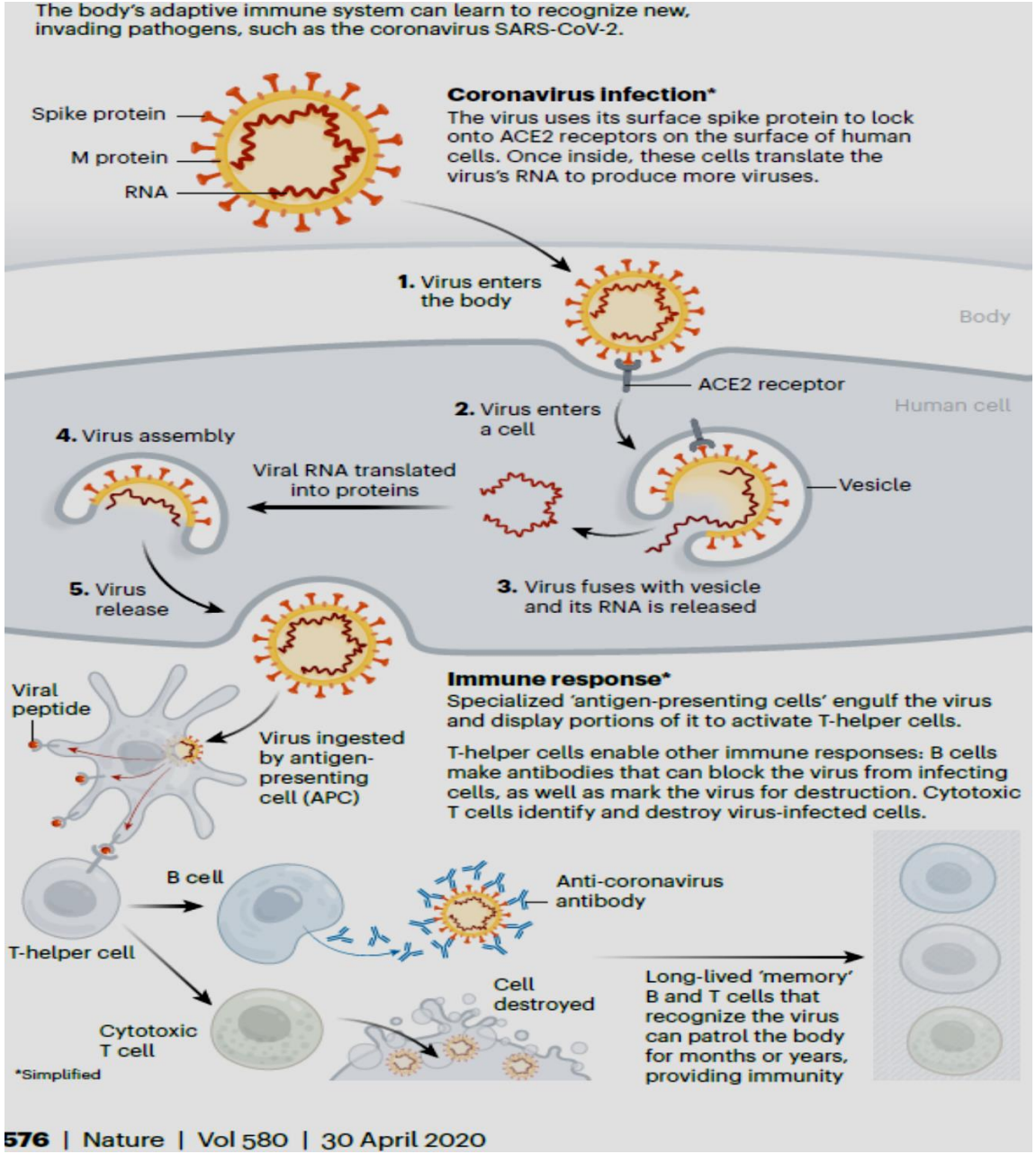
2 (AMD070)





AMD070, AMD3100 interfere with HIV adsorption and entrance into the CD4 cells– CXCR-4 is blocked. AMD070 in 2007 – phase III clinical trials, AMD3100 as Plerixafor approved by FDA in 2007.

Antiviral adaptive immunity – basis for vaccine development

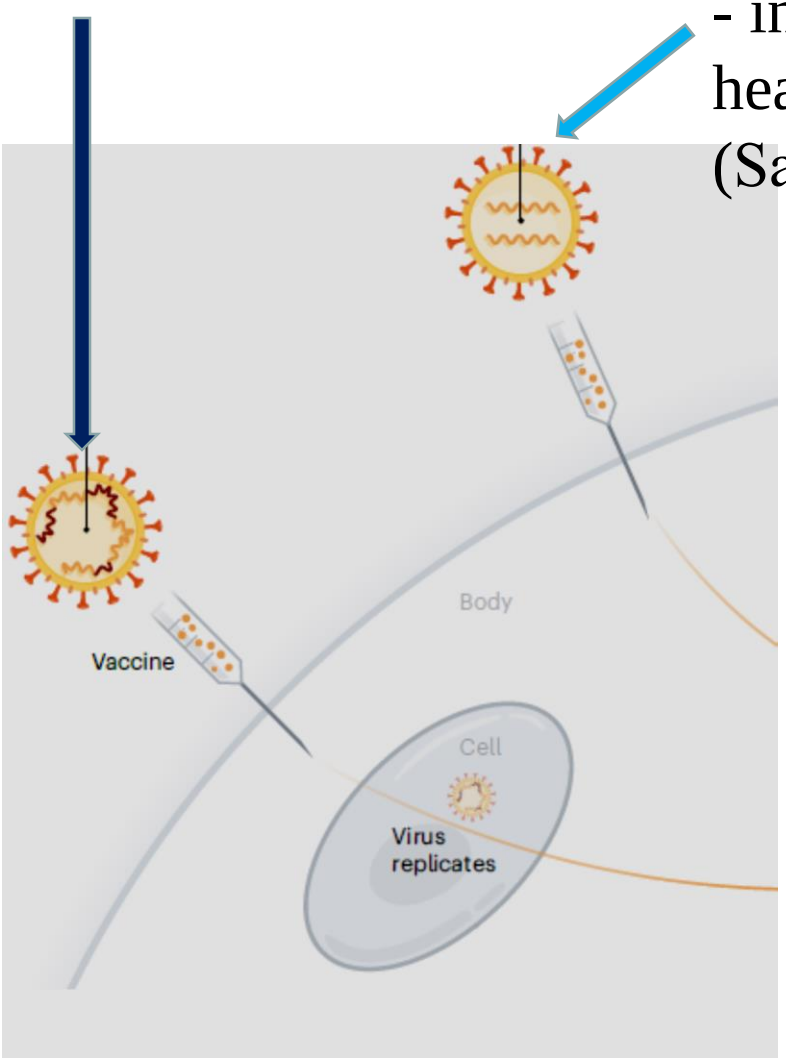


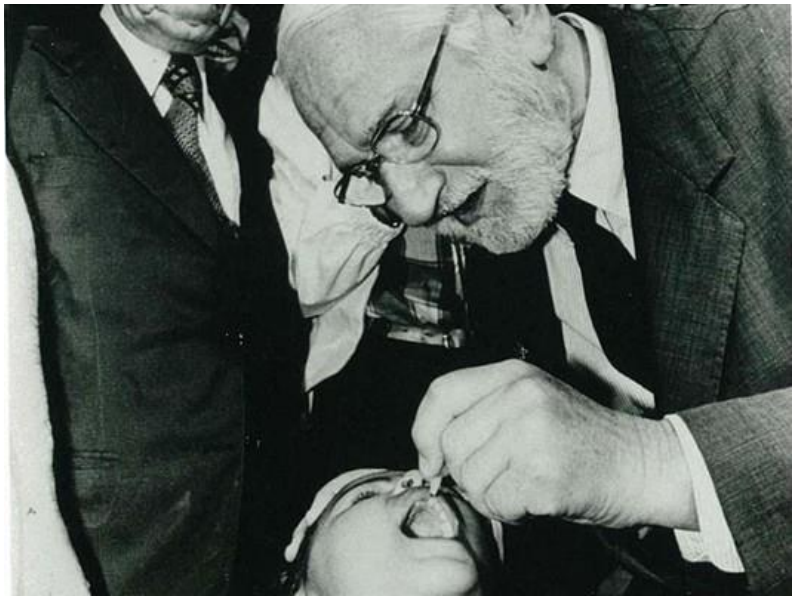
- **Types of vaccines:**

1. Complete viral particles

- attenuated, „live”) vaccines
(Sabin’s - OPV, small pox, measles, mumps…)

- inactivated viruses - irradiation, heath, chemicals (formaldehyde)
(Salk’s - IPV, hepatitis A, B, …)

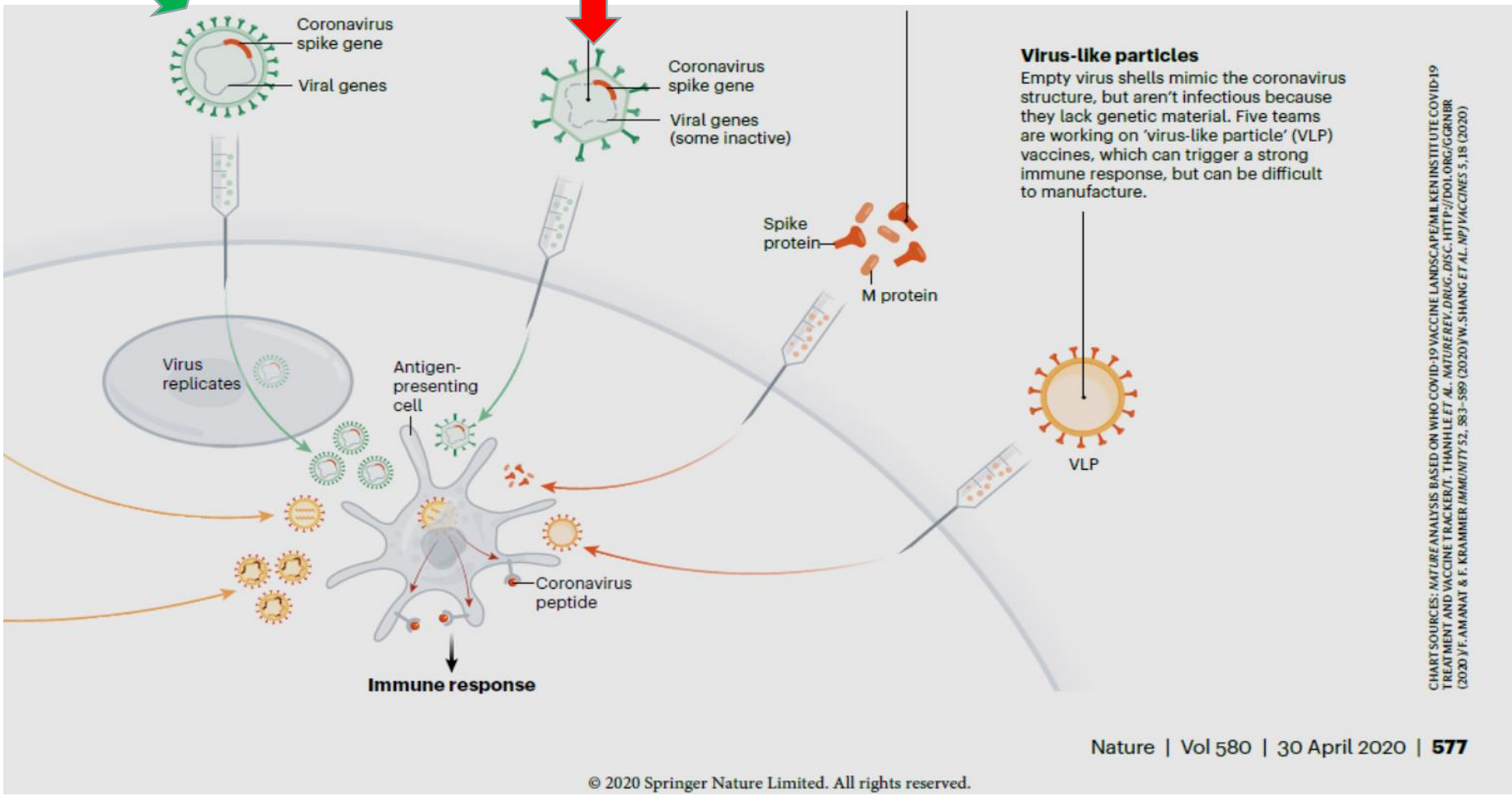




2. Vector vaccines

– recombinant viruses in vectors - replicable (Ebola vaccine - in attenuated measles virus)

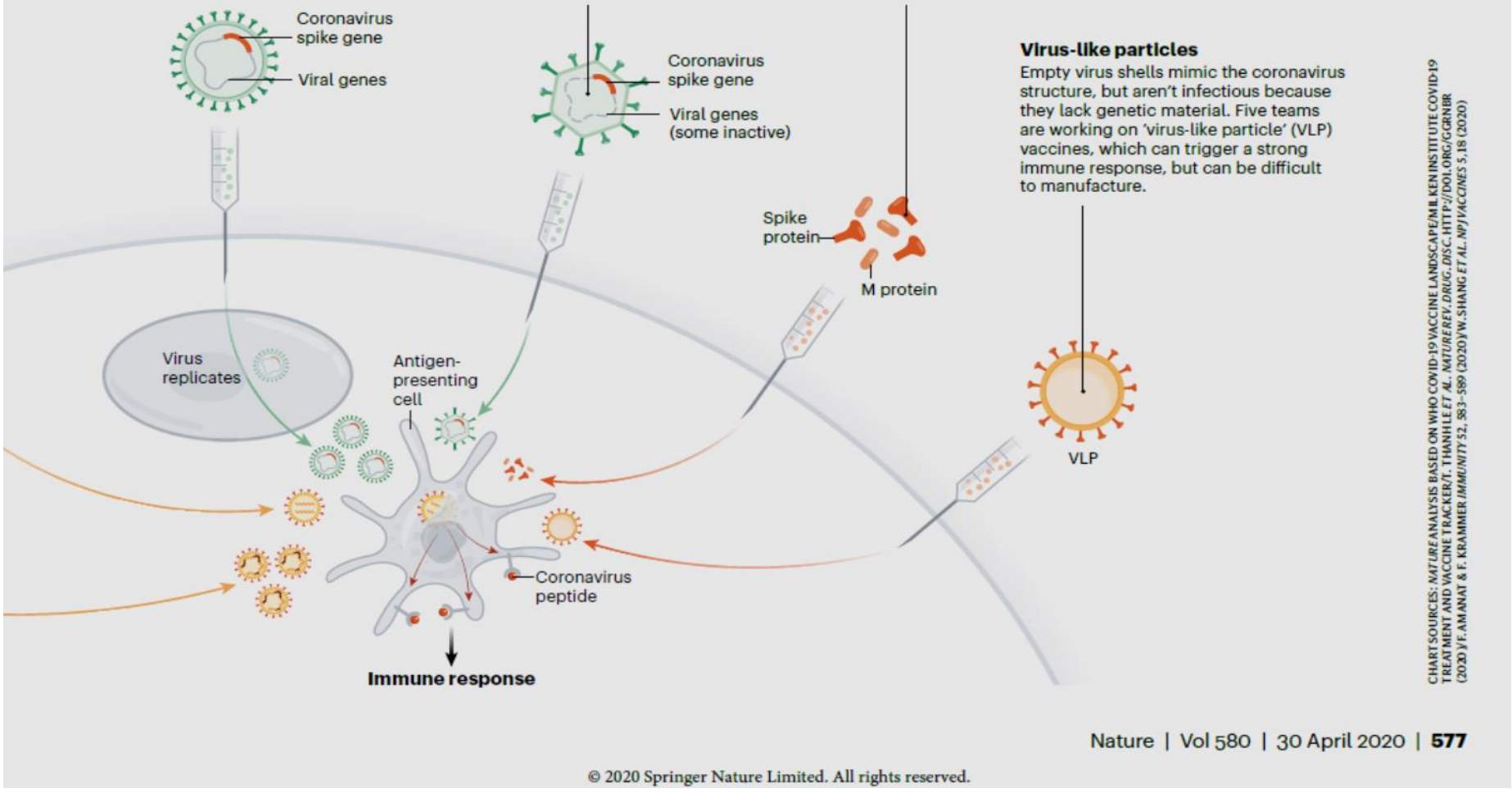
or not replicable (adenovirus vectors in humans).



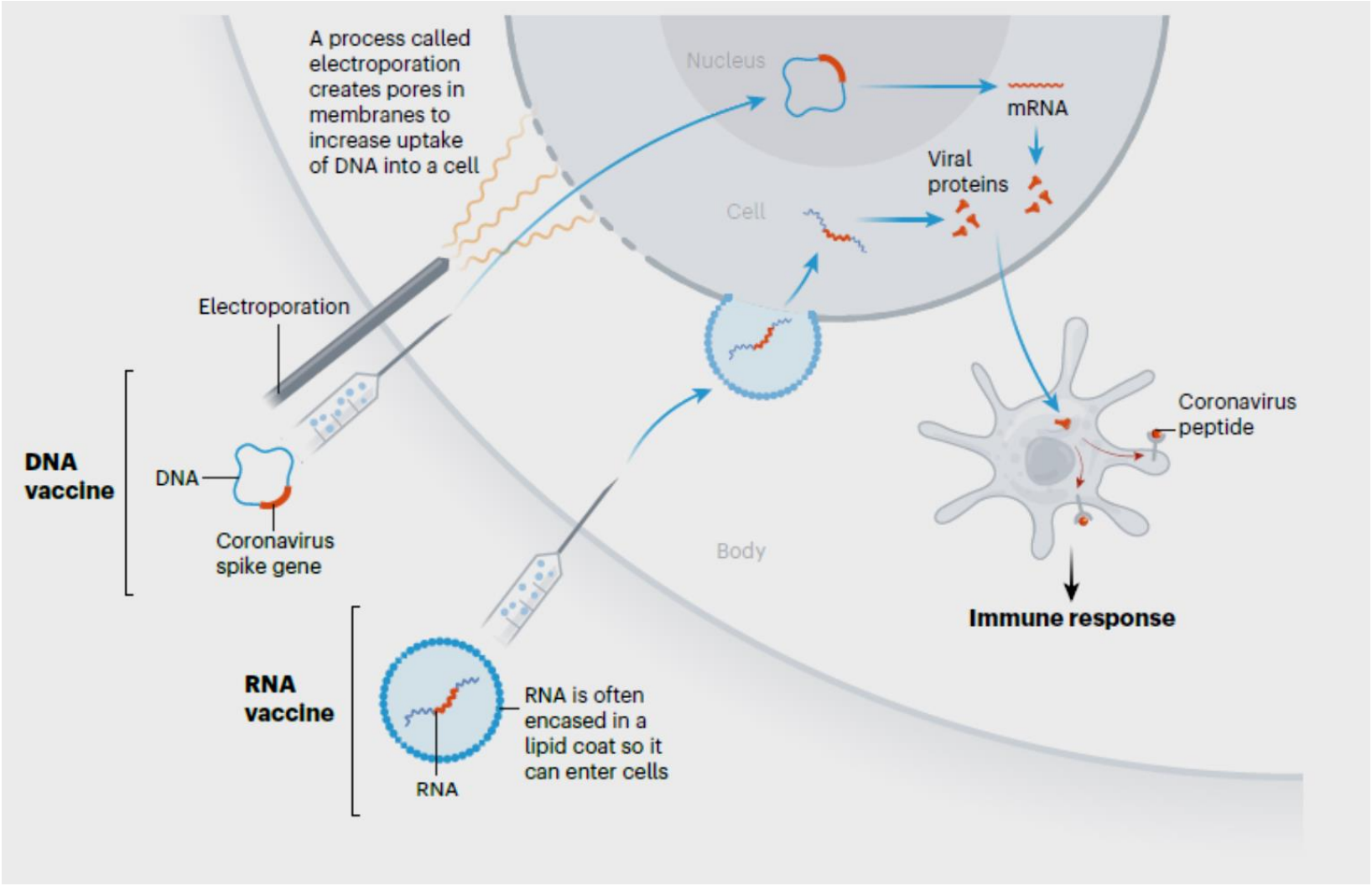
3. Virus proteins as vaccines – protein subunits (S, M) envelope proteins or glycoproteins (purufied or recombinant)

4. VLPs (HPV vaccine).

Protein vaccines are stable, easy storage and transport, not virulent, good as polyvaccines but higher doses are effective, costly manufacturing, quick immune response is not induced (IFN), antibody production is induced late.



5. DNA and RNA vaccines – safe (no infectious material… S-protein gene), good humoral and cell immunity induced.
First approved RNA vaccine for humans for SARS-CoV-2 2020/21.



DNA vaccines

(Nat. Rev. Genet., 9: 776-788, 2008.

1990s - DNA (plasmid) injected subcutaneously (cutaneous, intramuscular exposure) can elicit humoral and cellular immunity, (inducing especially cytotoxic T lymphocytes, CTLs).

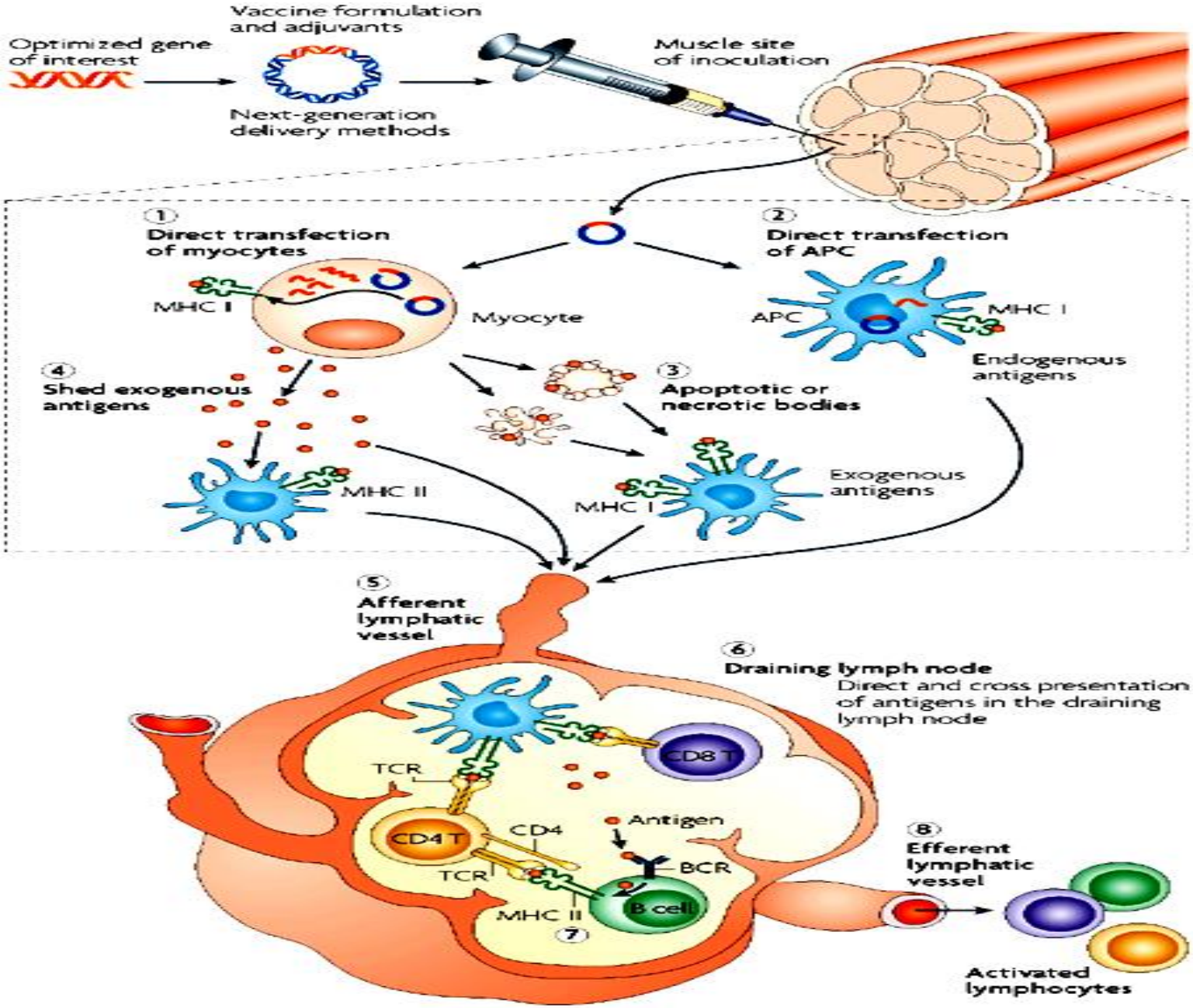
In veterinary medicine 4 DNA vaccines approved for:

canine melanoma, 2007, USA,

growth hormone releasing hormone (swine and food animals), 2007, Australia,

West Nile virus (horses), 2005, USA,

Infectious hematopoietic necrosis virus (salmon), 2005, Canada.



Nature Reviews | Genetics

They may protect against:

viral,

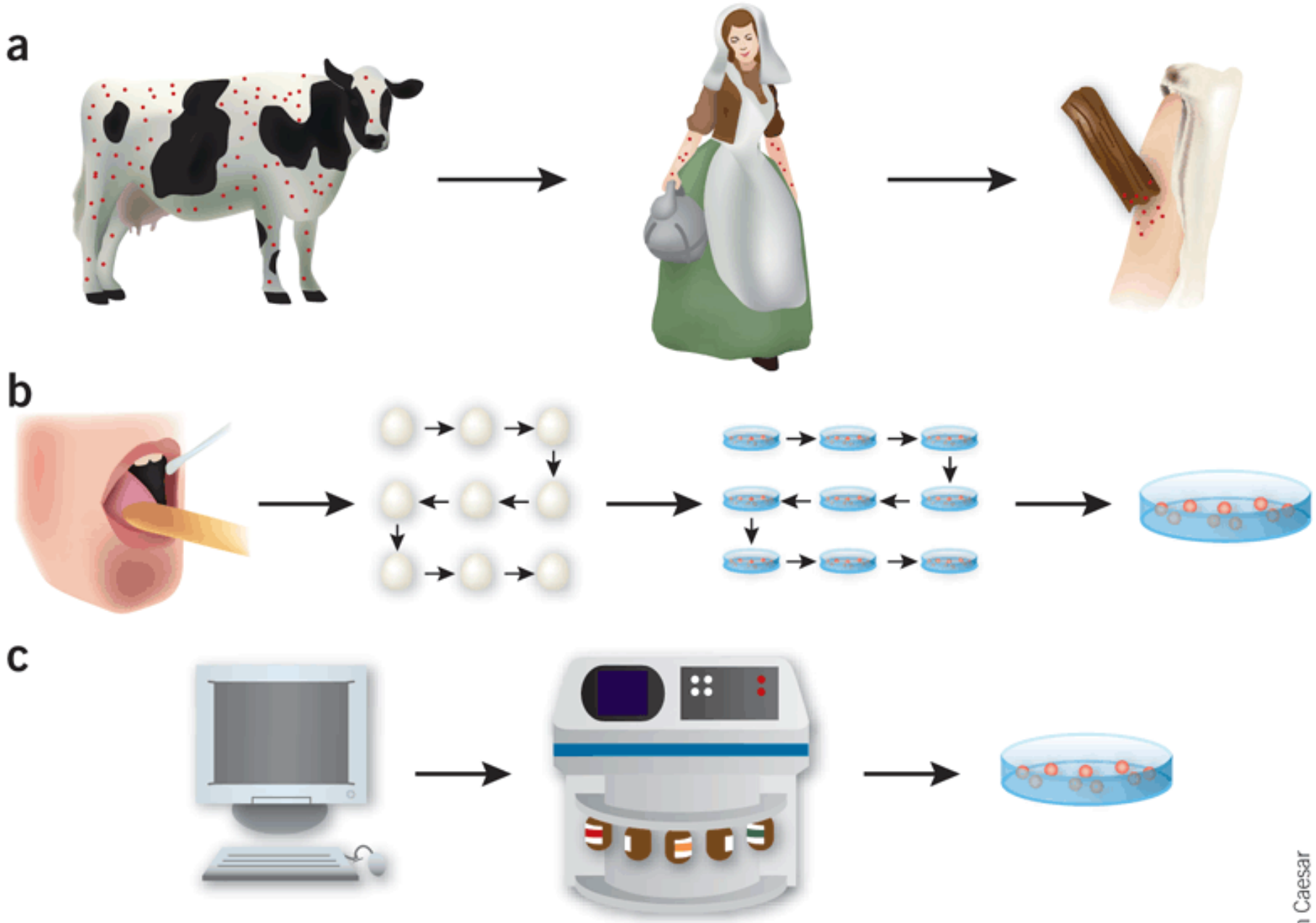
bacterial,

parasitic infections,

cancer, ischemia, allergies, autoimmune diseases – depending on the antigen type inserted into a plasmid.

Safe! No downsides of attenuated live or inactivated virus vaccines but have some others (Table in the paper).

They can be used for pre-vaccination (classical vaccines are applied afterwards).



Nat. Biotech. 26(9): 1000-1001, 2008.

New viral vaccines

New vaccine types developed from information on:

protein subunits (data from structural studies invaluable)

nucleic acid sequence databases (for DNA and recombinant vaccines)

cell-signaling microarrays (the choice of the most suitable adjuvant).

Live attenuated viruses are the most efficient vaccines –

single dose, long-lasting protection, cost-effective (high virus titer, minimal manipulation), no adjuvant needed.

Attenuation in biological systems (passaging), one virion replication (b), or from a related virus (a) are obsolete methods.

Attenuation through manipulation of *codon pair bias*.

Science 320: 1784-1787, 2008.

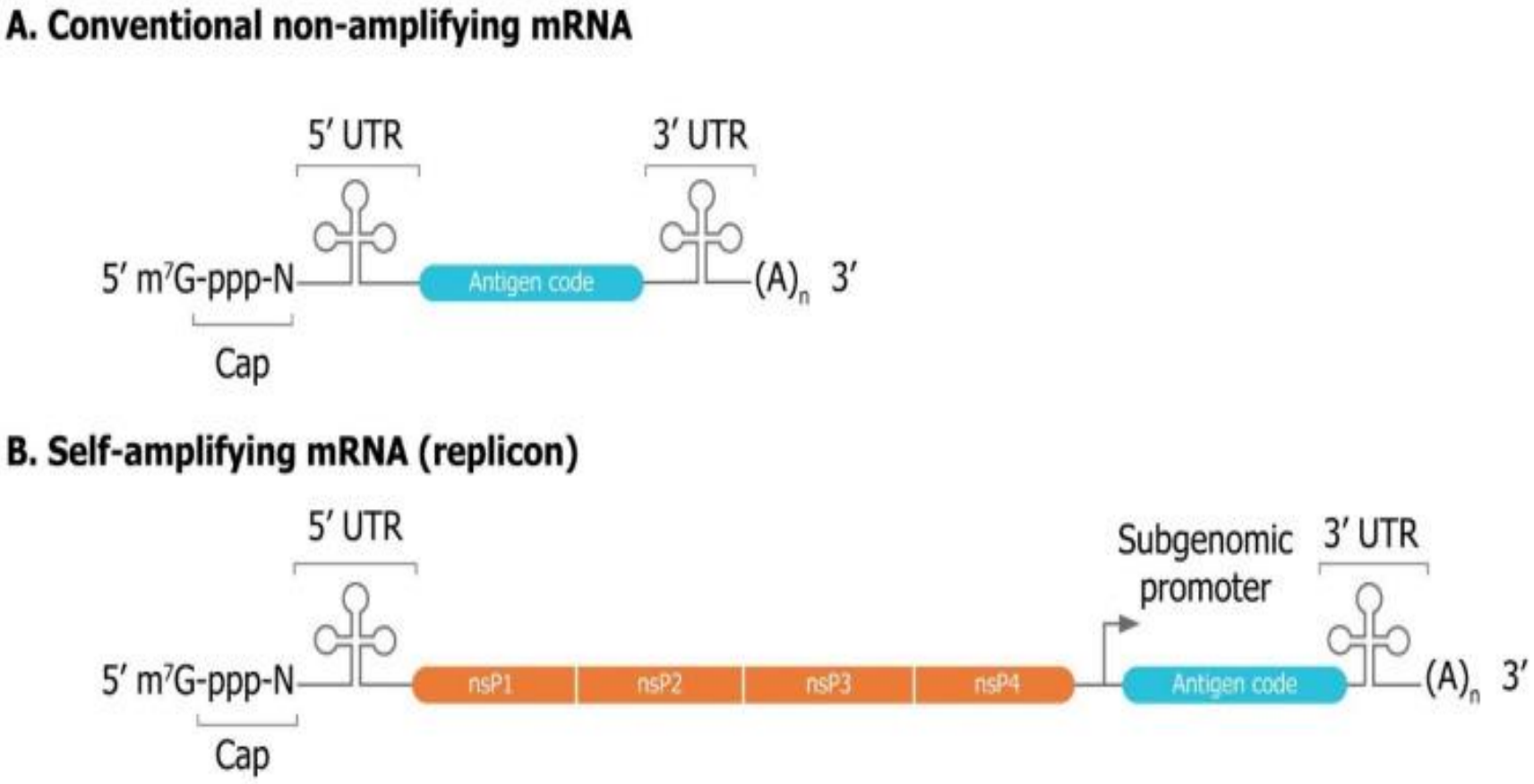
Poliovirus, preferentially uses some codons for translation (and anticodon pairs on tRNA).

Poliovirus capsid protein P1 codon manipulation can reduce virus translation and replication efficiency.

SAVE approach (synthetic attenuated virus engineering):

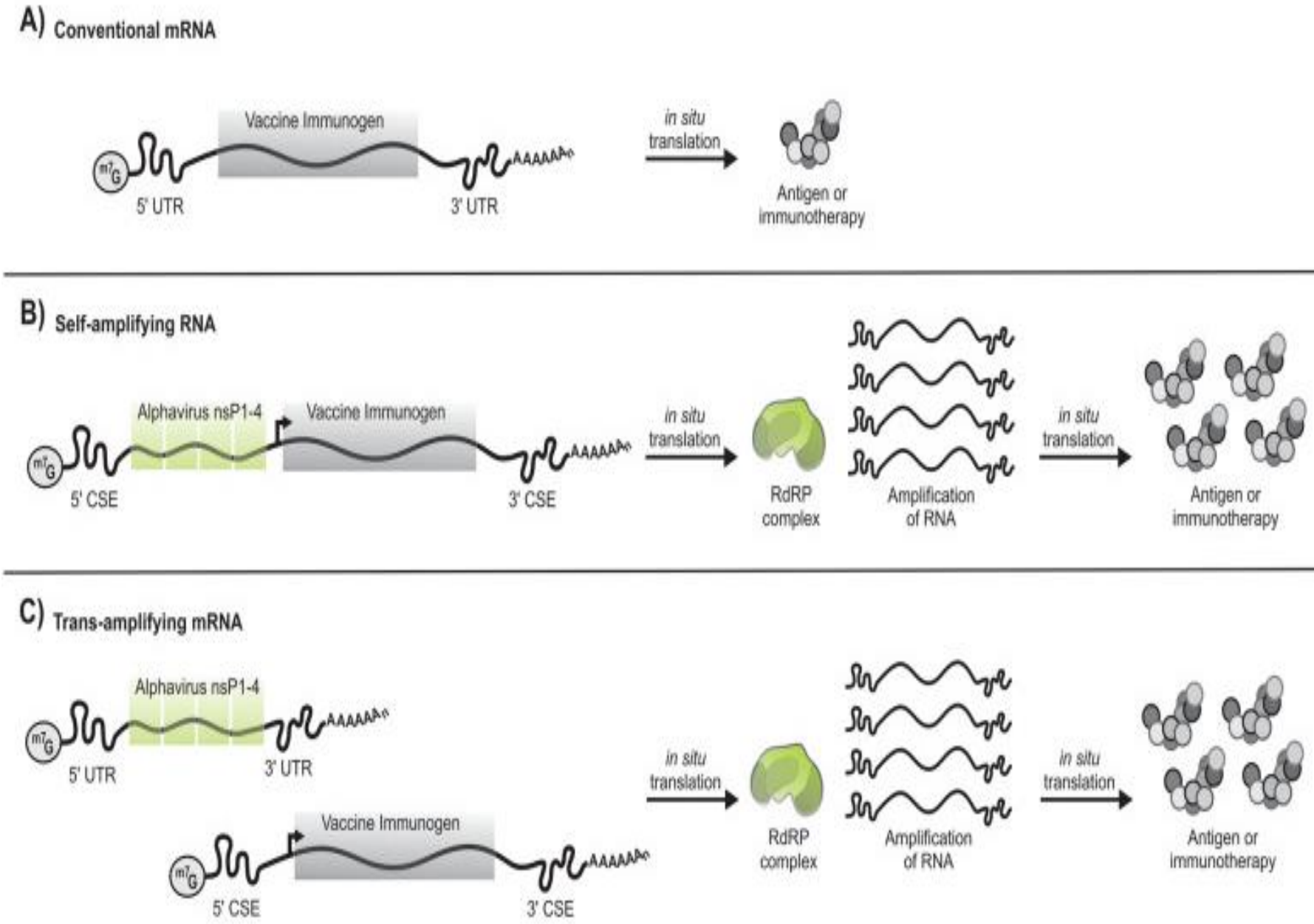
Synonymous mutations are introduced into DNA, (preferential viral codons are changed), that gene is recombined with wild-type genome segments to generate attenuated viruses (reduction in translation, replication, selection for attenuated neurotropism).

New self amplyfiying RNA vaccines - **saRNA**



RNA replicon has nsP1-P4 – alfavirus non-structural proteins (eg. VEEV, for RdRP), subgenomic promotor, and usual terminal structures elements of mRNA .

doi: 10.3390/vaccines9020097



https://doi.org/10.1038/s41434-020-00204-y

CSE= conserved sequence element

First self-amplifying mRNA vaccine approved. Nat Biotechnol 42, 4 (2024). https://doi.org/10.1038/s41587-023-02101-2

News in brief

First self-amplifying mRNA vaccine approved

Regulators in Japan have given a green light to a COVID-19 vaccine that uses self-amplifying messenger RNA (sa-mRNA), the first vaccine of this kind to get full approval.

The ARCT-154 vaccine was co-developed by Arcturus Therapeutics and CSL. The innovative sa-mRNA vaccine enables host cells in the body to make copies of the mRNA which encodes viral replicase genes in addition to immunogenic genes. ARCT-154 uses mRNAs encoding replicase components of the Venezuela equine encephalitis virus and the spike glycoprotein of the SARS-CoV-2 D614G variant enclosed in a proprietary lipid nanoparticle. The self-replicative activity of sa-mRNA vaccines enables them to be used at lower concentrations than conventional mRNA vaccines to achieve similar or better antigen expression, meaning they could be safer and manufactured at large scale.

The approval by Japan's Ministry of Health is based on various phase 3 study results, including a COVID-19 booster trial with 5 μg ARCT-154 that resulted in higher immune responses than with 30 μg Comirnaty (Pfizer/BioNTech). In previously vaccinated adults, 70% of those boosted with ARCT-154 developed antibodies against Omicron BA.4/5, compared with 58% of those boosted with Comirnaty. And antibody titres were higher at 28 days in people who received ARCT-154. Both vaccines were equally well tolerated.

Arcturus and CSL have also filed ARCT-154 for European regulatory approval. The companies have additional sa-mRNA vaccine candidates against other SARS-Cov-2 variants in clinical testing, as have companies such as ImmunityBio and Gritstone Bio. Another sa-RNA vaccine called Gemcovac-19, manufactured by Gennova Biopharmaceuticals, was approved in India in June 2022, but for emergency use only. Vaccine candidates using sa-mRNA are also in clinical trials for influenza.

Casgevy is targeted at patients' $CD34^+$ hematopoietic stem cells, which are isolated from the bone marrow. The CRISPR editing components are introduced in the lab by electroporation as a ribonucleoprotein complex, comprising a synthetic guide RNA and a *Streptococcus pyogenes* Cas9 endonuclease. Administering Casgevy is complicated by the impact of the disease on hematopoiesis in patients. They need to undergo blood transfusions for two months before cell mobilization and then two rounds of mobilization and apheresis. (In β-thalassemia, in contrast, no pre-treatment transfusions are needed, and a single round of mobilization and apheresis usually suffices.) To make room for the edited cells, patients also need to undergo busulfan-based myeloablative preconditioning, which is highly toxic.

Vertex has stated that it aims to recruit about 50 treatment centers in the United States, which would cover the patients it deems eligible for treatment — those aged 12 years or over with severe disease and recurrent attacks. The personnel overhead is substantial, and current treatment centers will take time to gear up. Kanter says her organization, at peak, will be able to administer Casgevy or Bluebird Bio's lentiviral gene therapy lovotibeglogene autotemcel, if approved, to about 12 patients per year. But it will first need to hire several more people. Similar limitations apply on a broader scale. Kanter, who is also president of the National Alliance of Sickle Cell Centers, cites a survey it conducted among 51 members, 38 of which said they planned to offer gene editing and gene therapies to patients. But half of them will need another year to get ready.

Other existing therapies may prevail. For sickle cell disease, hematopoietic stem cell transplant (HSCT) is already an important treatment option. Allogeneic HSCT from matched sibling donors can be curative, but only a small minority of patients has a perfectly matched sibling who is also free from the condition. Haploidentical HSCT followed by cyclophosphamide therapy, which requires only a partial match between donor and recipient human leukocyte antigen loci, is an option that could reach a much wider population. What's more, it does not require myeloablative therapy. At a cost of about $400,000, it is also substantially cheaper than genetic approaches. Given the pricing of other gene therapies, Casgevy could cost as much $2 million per patient.

But any conversation with patients about their treatment options must be fully transparent, says Michael Rutledge DeBaun, founder and director of the Vanderbilt-Meharry Center for Excellence in Sickle Cell Disease at Vanderbilt University Medical Center. That includes weighing the pros and cons of undergoing high-risk treatment now or waiting for better alternatives. "Let's be clear about what we're offering and what we're not offering to our patients," he says. Children and adults with a poor near-term prognosis are the best candidates for new therapies. "I reject the notion for children that we should cure them before they have a bad outcome," he says. He also thinks there will be further options, beyond this approval. "I'm cautiously optimistic that in the future — not today — we will have a range of therapeutic options that will have better results," he says.

Most people with sickle cell disease do not have any such choices, however. The condition is most prevalent in sub-Saharan Africa, and most patients live in low-income countries. DeBaun has extensive experience working in Nigeria, as well as the United States. The most urgent issue in Nigeria is ensuring that all patients have access to hydroxyurea, an inexpensive drug that boosts fetal hemoglobin production and reduces the frequency of crises. It has been a mainstay of therapy in wealthier countries for several decades, but is still not widely available in Nigeria or in other African countries. Sadly for most patients, the arrival of revolutionary new gene and gene editing therapies will not make any real difference to the enormous burdens the disease imposes on them.

Cormac Sheridan
Dublin, Ireland

Many antiviral drugs and therapies are currently in different phases of testing (TGEV- swine transmissible gastroenteritis virus plant vaccine, measles H in a mouse model).

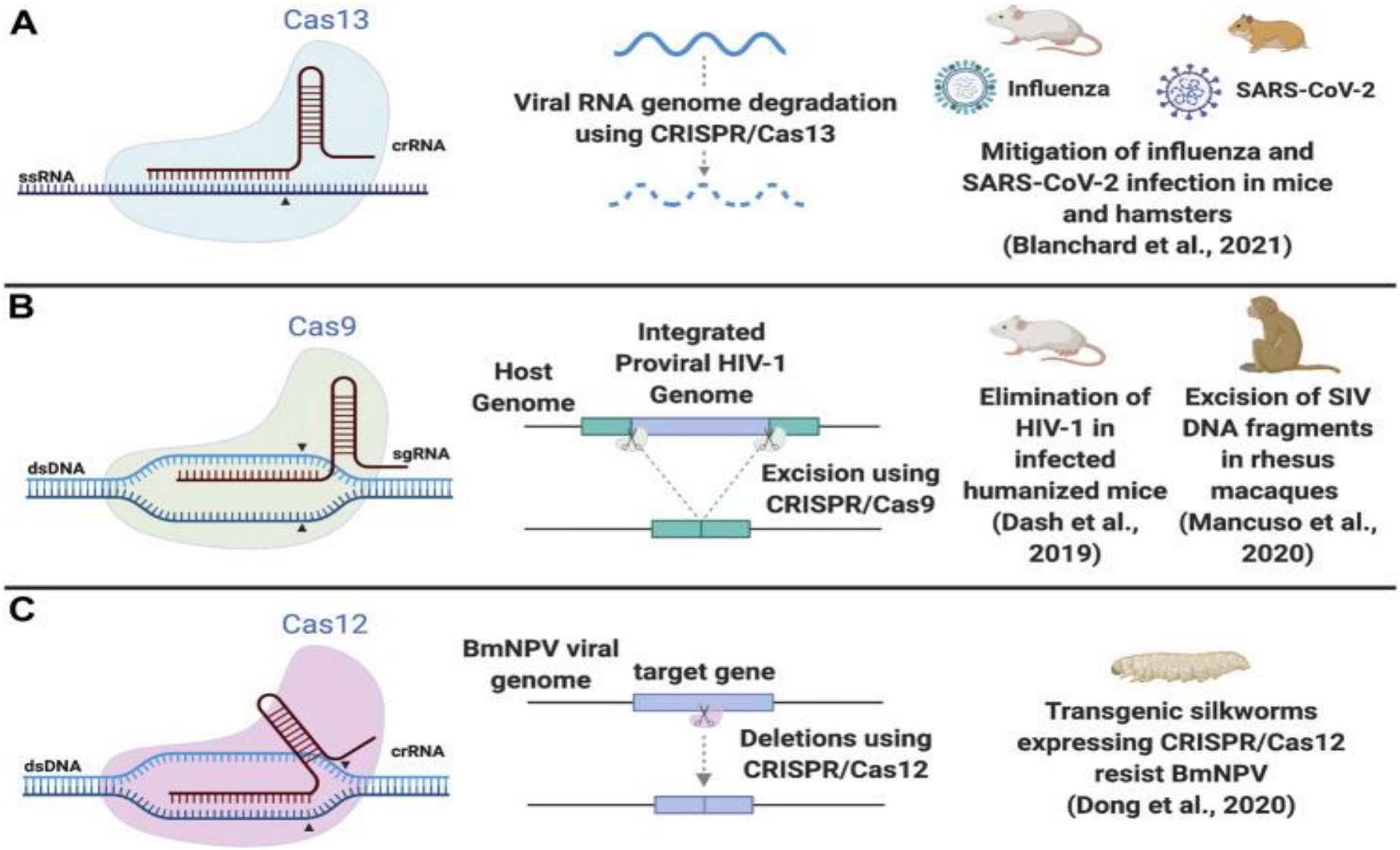
Generation of resistant virus strains is possible.

Combined therapy is often necessary:

Highly active anti-retroviral therapies (HAART)

zidovudine (AZT) , lamivudine (3TC) and protease inhibitor (Indinavir).

CRISPR-Cas applications in antiviral therapied in development…but applied already in virus detection.



Baddeley HJE, Isalan M. The Application of CRISPR/Cas Systems for Antiviral Therapy. Front Genome Ed. 2021 Oct 13;3:745559. doi: 10.3389/fgeed.2021.745559

Genomics and proteomics discoveries drive new antivirals and vaccines development…“defective viral genomes” (DVGs) in Chikungunya, Zika virus therapies is in development (2022.),

| Virology | Minireview | 3 April 2024

Defective viral genomes: advances in understanding their generation, function, and impact on infection outcomes

Authors: Justin W. Brennan, Yan Sun | AUTHORS INFO & AFFILIATIONS

DOI: https://doi.org/10.1128/mbio.00692-24 • Check for updates

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