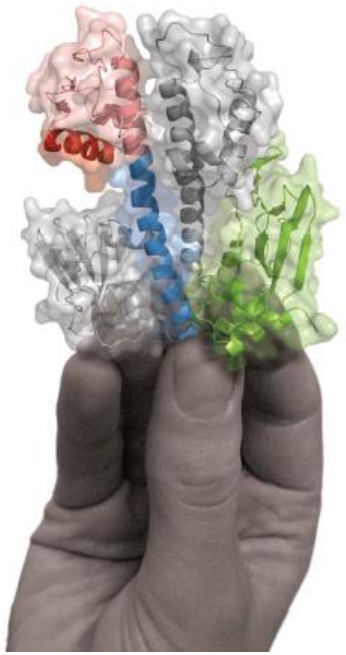
SAPIENZA
UNIVERSITÀ DI ROMA

Comunicazione Scientifica Biomedica

la Scienza a portata di mano



Comunicazione delle Scienze Biomediche

Prof.ssa Cristina Cerboni

I vaccini (parte II)

Anno Accademico 2023-2024

Alcuni tipi di vaccini per patogeni comuni

1. Vaccini attenuati (vivi, interi)
2. Vaccini inattivati (uccisi, interi)
3. Vaccini coniugati e a subunità
4. Vaccini 2.0

Il lungo elenco di vaccini disponibili per le malattie infettive nell'uomo

Vaccini disponibili per malattie infettive nell'uomo			
Malattie batteriche	**Tipi di vaccini**	**Malattie virali**	**Tipi di vaccini**
Difterite (*Corynebacterium diphtheriae*)	Tossoide	Febbre gialla	Virus attenuato
Tetano (*Clostridium tetani*)	Tossoide	Morbillo	Virus attenuato
Pertosse (*Bordetella pertussis*)	Batteri uccisi. Vaccino a subunità composto da tossoide della pertosse	Parotite	Virus attenuato
Febbre paratifoide (*Salmonella paratyphi*)	Batteri uccisi	Rosolia	Virus attenuato
Tifo epidemico (*Rickettsia prowazekii*)	Batteri uccisi	Poliomielite	Virus attenuato (Sabin) o virus ucciso (Salk)
Colera (*Vibrio cholerae*)	Batteri uccisi o estratto cellulare	Varicella	Virus attenuato
Peste (*Yersinia pestis*)	Batteri uccisi o estratto cellulare	Influenza	Virus inattivato
Tubercolosi (*Mycobacterium tuberculosis*)	Ceppo bovino attenuato di *Mycobacterium tuberculosis* (BCG)	Rabbia	Virus inattivato (umano) Virus attenuato (cani e altri animali) Virus vaccinico vivo ricombinante (animali)
Febbre tifoide (*Salmonella typhi*)	Vaccino a subunità polisaccaridiche Vaccino orale vivo attenuato	Epatite A	Vaccino a subunità (antigene dell'epatite ricombinante)
Meningite (*Neisseria meningitidis*)	Polisaccaride capsulare purificato	Epatite B	Vaccino a subunità (antigene dell'epatite ricombinante)
Polmonite batterica (*Streptococcus pneumoniae*)	Polisaccaride capsulare purificato Polisaccaride coniugato a proteina	Papillomavirus umano	Vaccino a subunità (proteine di rivestimento del virus)
Meningite (*Haemophilus influenzae*)	Polisaccaride di *H. influenzae* coniugato a proteina	Rotavirus	Virus attenuato Virus vivo ricombinante

Le vaccinazioni in Italia

- Difterite
- Tetano
- Pertosse (acellulare)
- Poliomielite
- Haemophilus influenzae di tipo b (Hib)
- Epatite B

esavalente

Morbillo + Parotite + Rosolia (+Varicella) (MPR+V)

Varicella obbligatoria per i nati dal 2017 in poi

tetravalente

- Pneumococco (eptavalente)
- Meningococco (gruppo B e C)
- Rotavirus
- Papilloma (HPV)

Vaccinazioni obbligatorie

Vaccinazioni obbligatorie dal 2017

Vaccinazioni consigliate (2018)

Altre vaccinazioni sono consigliate:

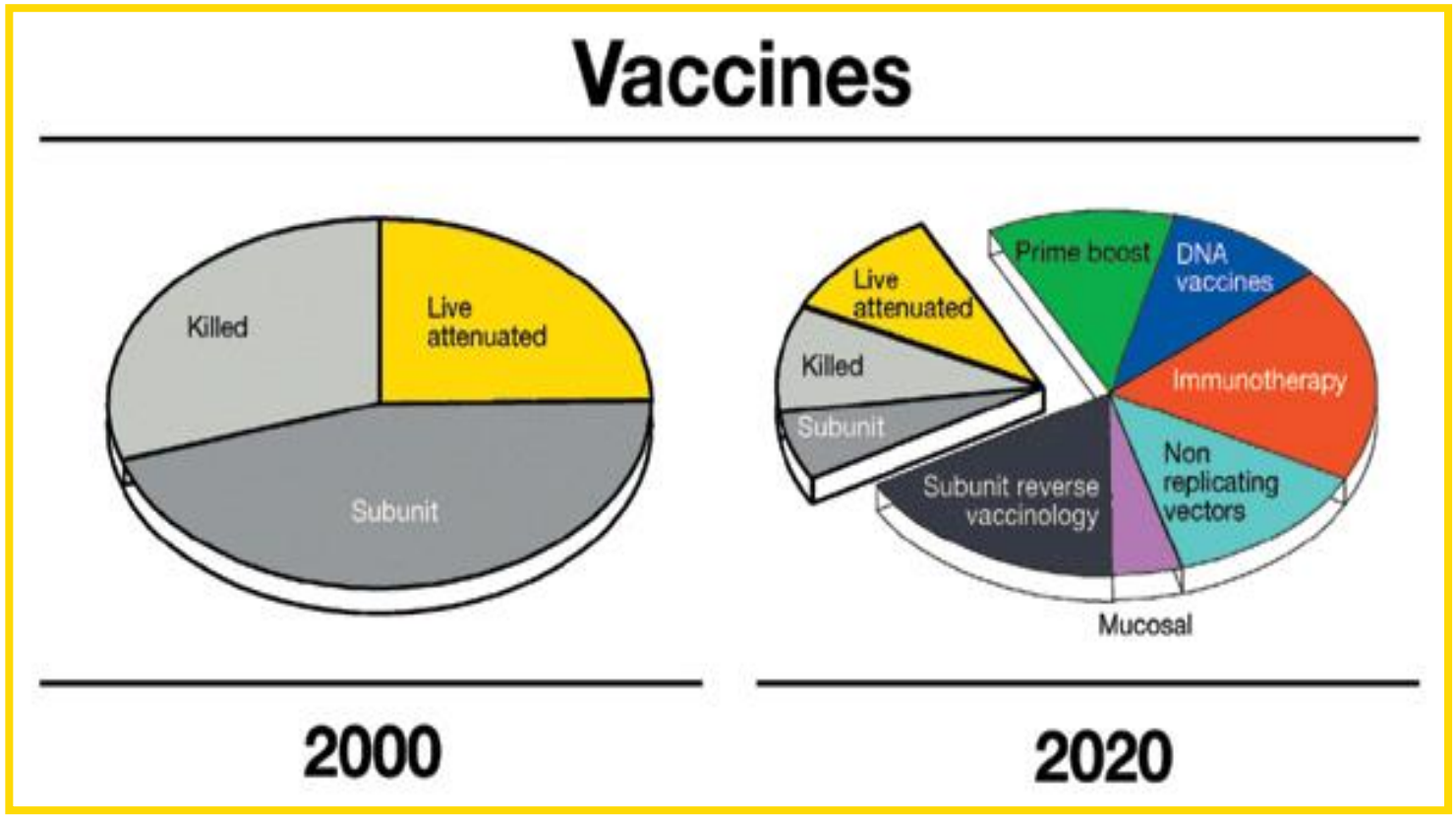
-

-

…

«I vaccini 2.0»:

- **Vaccinologia inversa**
- **Vaccini a DNA**
- **Vaccini a RNA**



Type of vaccines licensed in the year 2000 and
type of vaccines predicted to be available in the year 2020

Sviluppo dei vaccini: metodi tradizionali e… «vaccinologia inversa»!

Vaccini a base di peptidi sintetici

- Conoscere la sequenza aminoacidica o nucleotidica **dell'antigene**
- Identificare gli epitopi immunodominanti (bioinformatica)

Approccio sperimentale:

- Identificazione degli antigeni riconosciuti da anticorpi presenti nel siero dei pazienti
- Frammentazione della proteina nativa, con mezzi chimici o per digestione enzimatica
- Sintesi di tutti i possibili peptidi
- Analisi dell'efficacia *in vitro* e *in vivo*
- Sintesi in laboratorio dei peptidi migliori da utilizzare come vaccini

Es., meningococco tipo B, HBV, malaria, SARS-CoV-2

Sviluppo dei vaccini: metodi tradizionali e… «vaccinologia inversa»!

Vaccini a base di peptidi sintetici

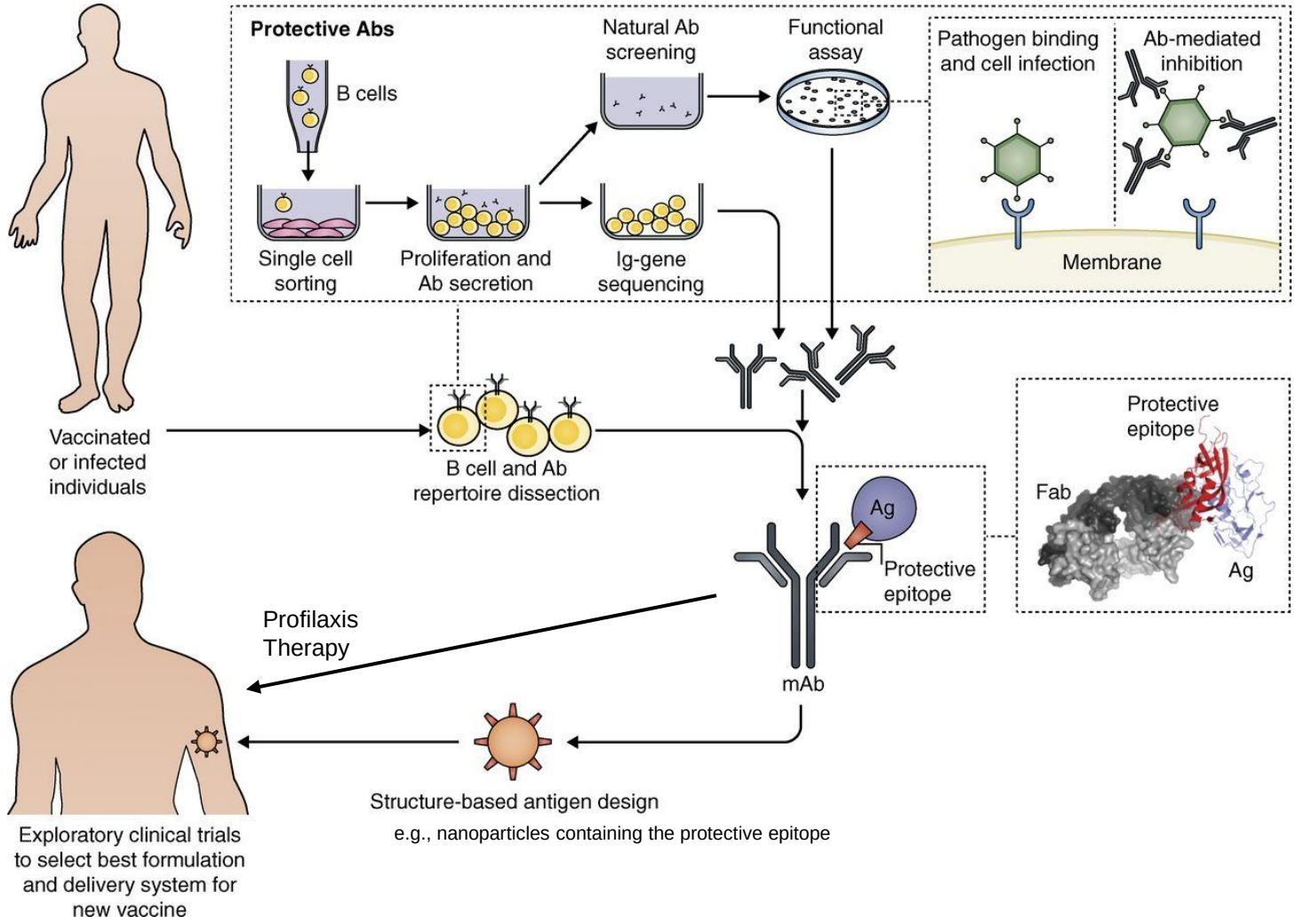
- Conoscere la sequenza genomica **del patogeno**
- Identificare gli epitopi immunodominanti (bioinformatica)

Approccio sperimentale:

- Predizione al computer per identificare geni che potrebbero codificare proteine con epitopi immunodominanti
- Tecniche del DNA ricombinante e vettori di espressione per la sintesi delle proteine
- Identificazione degli epitopi immunodominanti
- Analisi dell'efficacia *in vitro* e *in vivo*

Es., meningococco tipo B, HBV, malaria, SARS-CoV-2

Interplay of B cell technology and structural biology in vaccine design

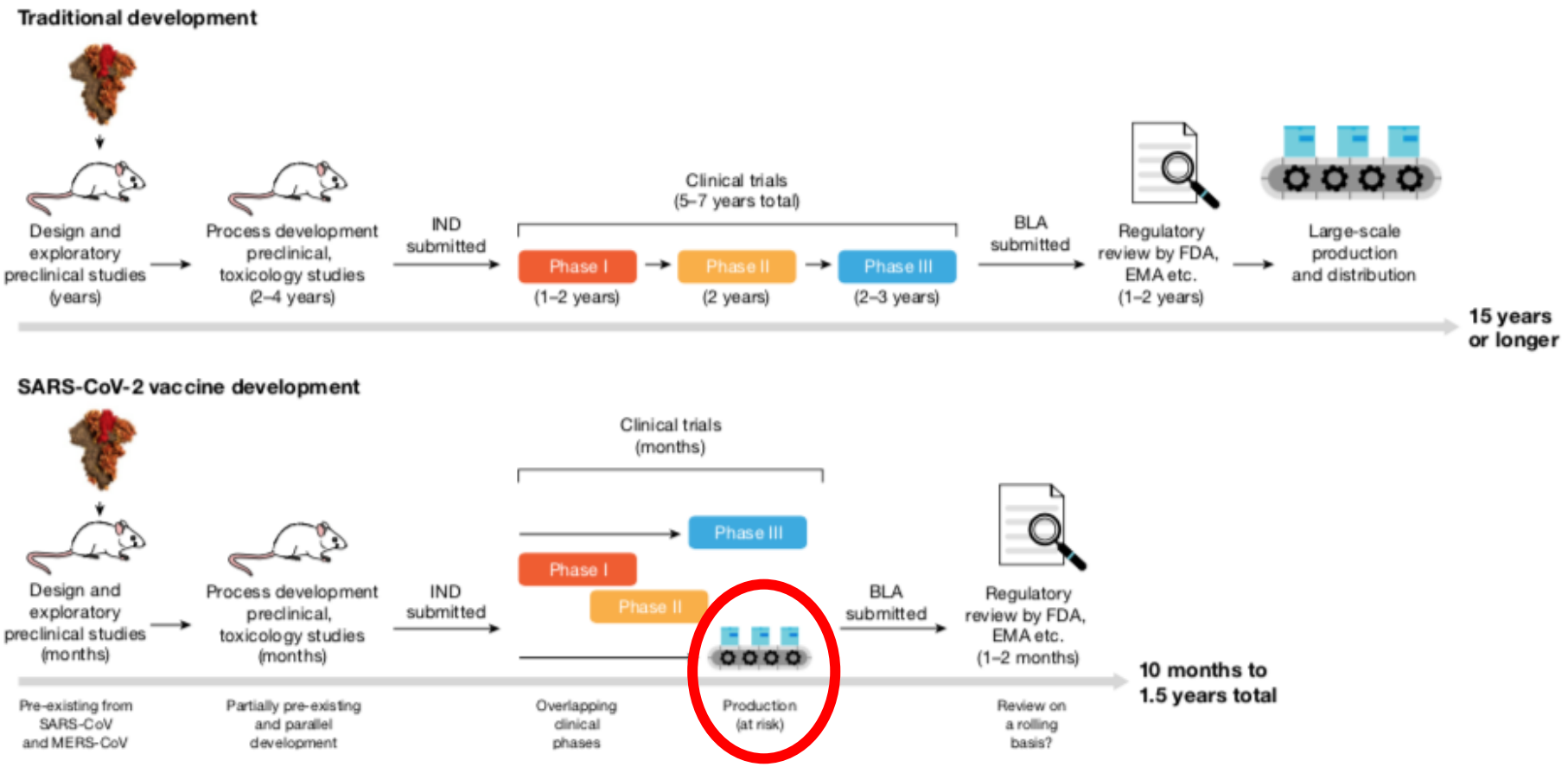


Selection of best antibodies for:
Specificity
Affinity
Neutralization

Questa metodica è definita **vaccinologia analitica o inversa** (metodo agnostico): si isolano anticorpi senza sapere contro quale antigene sono stati generati. Questa metodologia è utilizzata attualmente come strategia per la preparazione di vaccini contro la malaria.

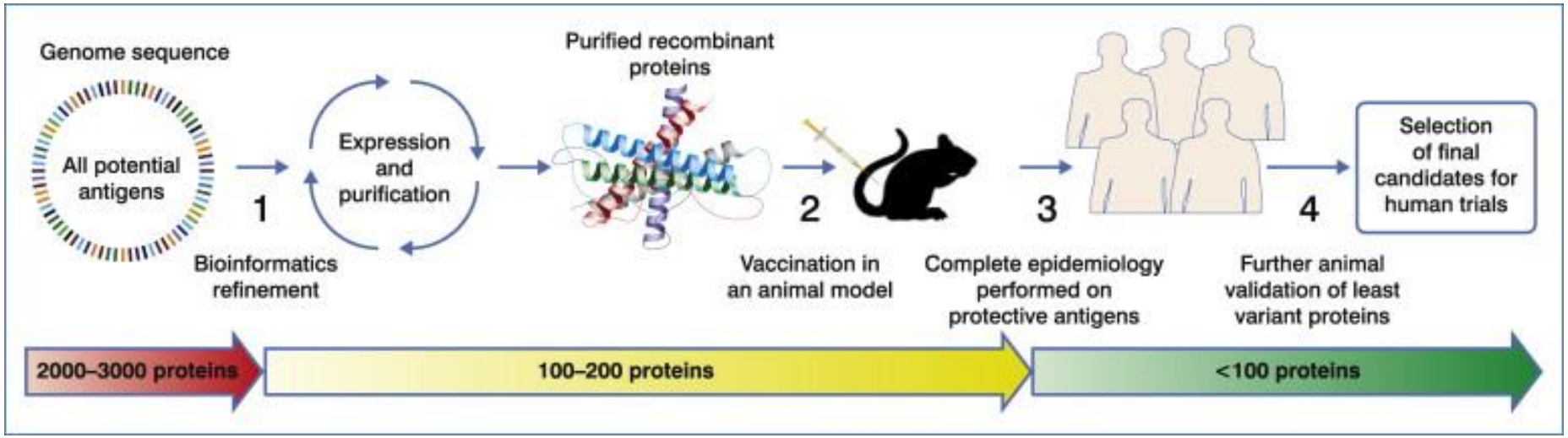
Rino Rappuoli et al. J Exp Med 2016 JEM

Traditional and accelerated vaccine-development pipelines



IND=Investigational new drug
BLA= Biological licence application

Reverse vaccinology



1-2 years vs. 5-15 years

Review

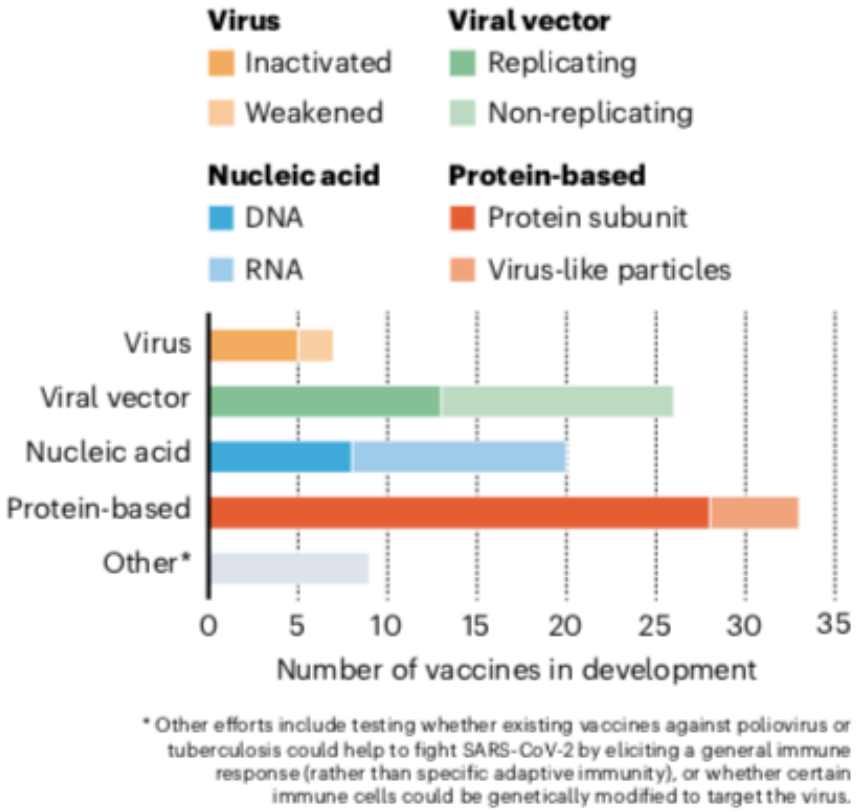
SARS-CoV-2 vaccines in development

https://doi.org/10.1038/s41586-020-2798-3
Received: 23 August 2020
Accepted: 17 September 2020
Published online: 23 September 2020
Check for updates

Florian Krammer[1✉]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in late 2019 in China and is the causative agent of the coronavirus disease 2019 (COVID-19) pandemic. To mitigate the effects of the virus on public health, the economy and society, a vaccine is urgently needed. Here I review the development of vaccines against SARS-CoV-2. Development was initiated when the genetic sequence of the virus became available in early January 2020, and has moved at an unprecedented speed: a phase I trial started in March 2020 and there are currently more than 180 vaccines at various stages of development. Data from phase I and phase II trials are already available for several vaccine candidates, and many have moved into phase III trials. The data available so far suggest that effective and safe vaccines might become available within months, rather than years.

An array of vaccines to fight Sars-CoV-2!



Callaway E, Nature 2020

Feature

THE RACE FOR CORONAVIRUS VACCINES

By Ewen Callaway; design by Nik Spencer.

More than 90 vaccines are being developed against SARS-CoV-2 by research teams in companies and universities across the world. Researchers are trialling different technologies, some of which haven't been used in a licensed vaccine before. At least six groups have already begun injecting formulations into volunteers in safety trials; others have started testing in animals. *Nature*'s graphical guide explains each vaccine design.

Types of vaccine in development

More than **180 vaccine candidates** against SARS-CoV-2. The World Health Organization (**WHO)** maintains a working document that includes most of the vaccines in development and is available at ***https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines.***

Overview of the SARS-CoV-2 vaccine development landscape

VIRAL-VECTOR VACCINES

Around 25 groups say they are working on viral-vector vaccines. A virus such as measles or adenovirus is genetically engineered so that it can produce coronavirus proteins in the body. These viruses are weakened so they cannot cause disease. There are two types: those that can still replicate within cells and those that cannot because key genes have been disabled.

PROTEIN-BASED VACCINES

Many researchers want to inject coronavirus proteins directly into the body. Fragments of proteins or protein shells that mimic the coronavirus's outer coat can also be used.

Replicating viral vector (such as weakened measles)

The newly approved Ebola vaccine is an example of a viral-vector vaccine that replicates within cells. Such vaccines tend to be safe and provoke a strong immune response. Existing immunity to the vector could blunt the vaccine's effectiveness, however.

Non-replicating viral vector (such as adenovirus)

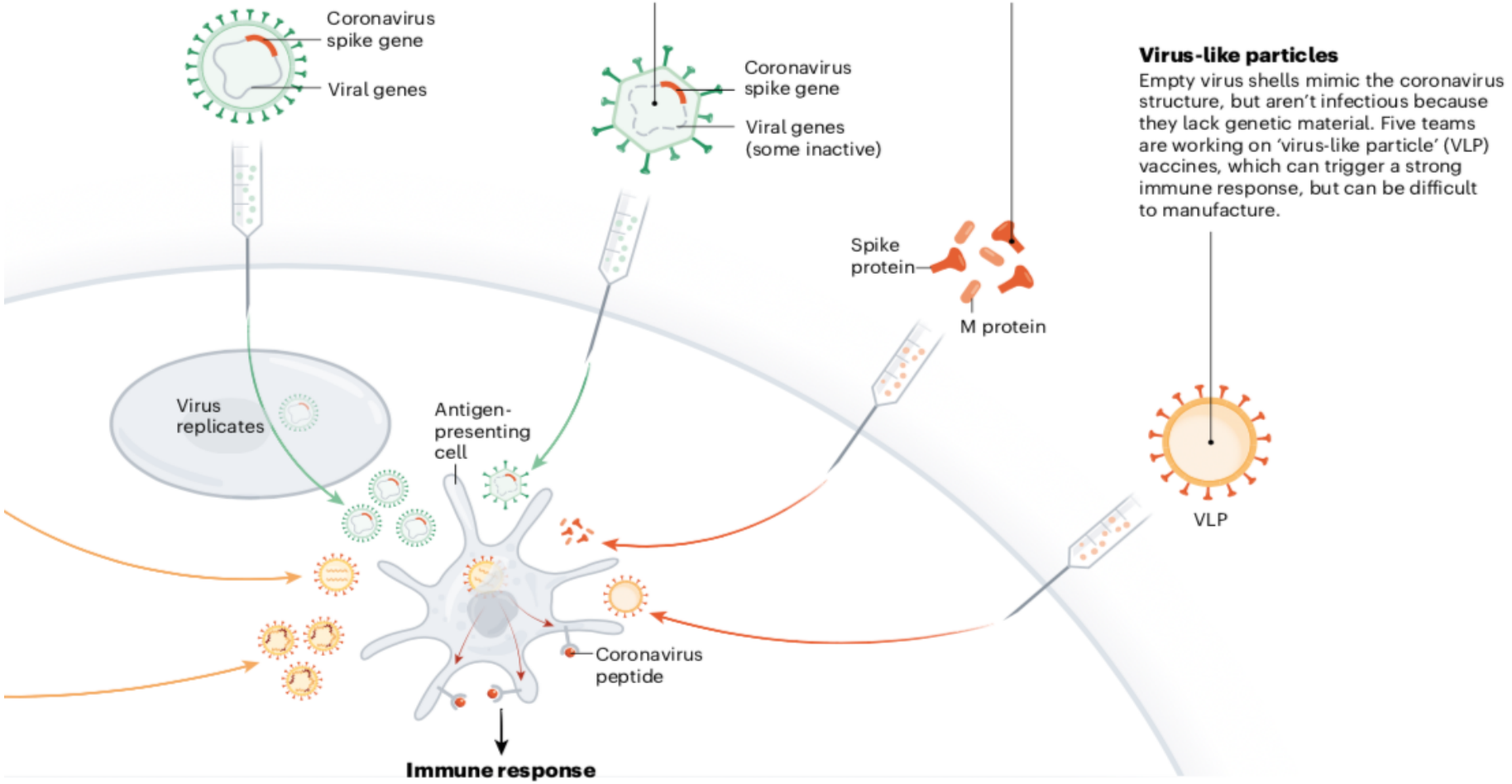
No licensed vaccines use this method, but they have a long history in gene therapy. Booster shots can be needed to induce long-lasting immunity. US-based drug giant Johnson & Johnson is working on this approach.

Protein subunits

Twenty-eight teams are working on vaccines with viral protein subunits — most of them are focusing on the virus's spike protein or a key part of it called the receptor binding domain. Similar vaccines against the SARS virus protected monkeys against infection but haven't been tested in people. To work, these vaccines might require adjuvants — immune-stimulating molecules delivered alongside the vaccine — as well as multiple doses.

Virus-like particles

Empty virus shells mimic the coronavirus structure, but aren't infectious because they lack genetic material. Five teams are working on 'virus-like particle' (VLP) vaccines, which can trigger a strong immune response, but can be difficult to manufacture.



Other anti Sars-Cov2 vaccines in phase III clinical trials (*November 28, 2020*)

Inactivated SARS-CoV-2 (virus inattivato, Sinovac e Butantan Institute)

Inactivated Novel Coronavirus Pneumonia (COVID-19) vaccine (virus inattivato, Hennan Provincial Center for Disease Control and Prevention e Sinopharm)

BBIBP-CorV (virus inattivato, Beijing Institute of Biological Products Co., LTD e Laboratorio Elea Phoenix S.A.)

Ad26.COV2.S (vettore vaccinale, Janssen Vaccines & Prevention (Johnson & Johnson))
- Ad5-nCoV (vettore vaccinale, CanSino Biologics)

Gam-COVID-Vac (vettore vaccinale, Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation)

Inactivated Novel Coronavirus Pneumonia (COVID-19) vaccine (Vero cells) (virus inattivato, Wuhan Provincial Center for Disease Control and Prevention)

NVX-CoV2373 (proteine ricombinanti, Novavax)

CoVLP (proteine ricombinanti, Medicago)

BBV152 - COVAXIN (virus inattivato, Bharat Biotech International Ltd)

Table 1 | Overview of NHP results

Company (ref.)	Vaccine candidate (type)	Dose range (route)	Neut. titre after prime	Neut. titre after boost	T cell response	Challenge dose (route)	URT protection	LRT protection	Species
Sinovac[34]	PiCoVacc (inactivated virion + aluminium hydroxide)	3–6 μg (i.m.)	None[a]	1:10 range[a] after first boost; 1:50 range[a] after second boost	ND	10^6 $TCID_{50}$ (i.t.)	Partial[b]	Partial (low dose)[b] Complete (high dose)	Rhesus macaques
Beijing Institute of Biological Products[33]	BBIBP-CorV (inactivated virion + aluminium hydroxide)	4–8 μg (i.m.)	1:100 range[a]	1:200 range[a]	ND	10^6 $TCID_{50}$ (i.t.)	Partial[b]	Complete[b]	Cynomolgus macaques
AstraZeneca[49]	ChAdOxnCoV-19 (non-replicating AdV)	2.4×10^{10} VP; 1× or 2× (i.m.)	1:5–1:40 range[a]	1:10–1:160 range[a]	Yes	2.6×10^6 $TCID_{50}$ (i.t., oral, i.n., ocular)	None (1×)[c] None (2×)[c]	Partial (1×)[c] Complete (2×)[c]	Rhesus macaques
Janssen[41]	Ad26COVS1 (non-replicating AdV)	1×10^{11} VP (i.m.)	1:100 range[d]	NA	Low	10^5 $TCID_{50}$ (i.n, i.t.)	Complete in S.PP group[c]	Complete in S.PP group[c]	Rhesus macaques
Moderna[57]	mRNA-1273 (mRNA via LNPs)	2×10–100 μg (i.m.)	ND[e]	1:501–1:3,481 range[d]	Yes, CD4, T_{FH}	7.6×10^5 $TCID_{50}$ (i.n., i.t.)	None (10 μg)[c] Partial (100 μg)[c]	Partial (10 μg)[c] Complete (100 μg)[c]	Rhesus macaques
Novavax[79]	NVX CoV2373 (spike protein + Matrix-M)	2× 2.5–25 μg	Not reported	17,920–23,040 range[a]	ND	10^4 plaque-forming units (i.n., i.t.)	Partial (low dose)[c] Complete (higher doses)[c]	Complete[c]	Cynomolgus macaques

[a]Based on microneutralization assay with CPE as readout.

[b]Based on viral genome RNA copy number.

[c]Based on subgenomic RNA copy number.

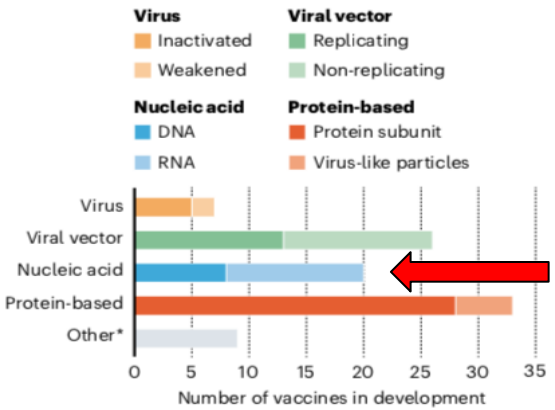
[d]Based on microneutralization assay with a SARS-CoV-2 reporter virus; 50% reduction in relative light units as readout.

[e]Not assessed using authentic SARS-CoV-2.

Neut., neutralizing antibody; NA, not applicable; ND, not determined; i.m., intramuscular; i.n., intranasal; i.t., intratracheal; T_{FH}, T follicular helper cells.

AN ARRAY OF VACCINES

All vaccines aim to expose the body to an antigen that won't cause disease, but will provoke an immune response that can block or kill the virus if a person becomes infected. There are at least eight types being tried against the coronavirus, and they rely on different viruses or viral parts.

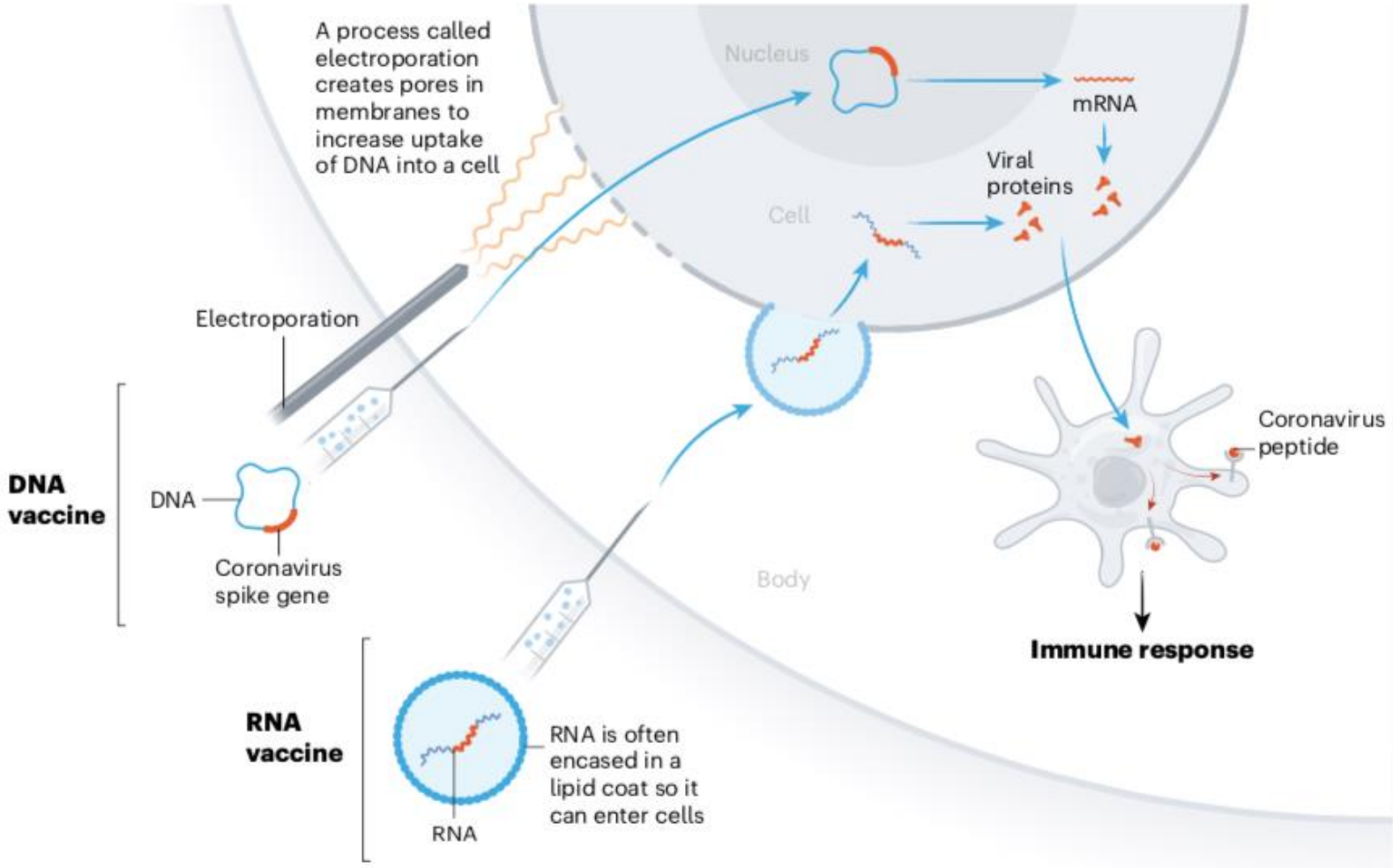


* Other efforts include testing whether existing vaccines against poliovirus or tuberculosis could help to fight SARS-CoV-2 by eliciting a general immune response (rather than specific adaptive immunity), or whether certain immune cells could be genetically modified to target the virus.

NUCLEIC-ACID VACCINES

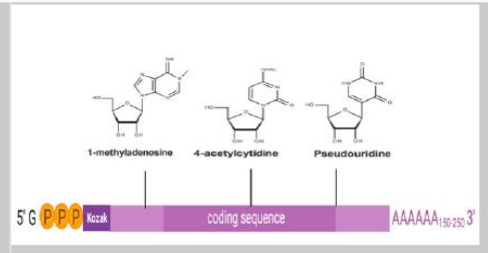
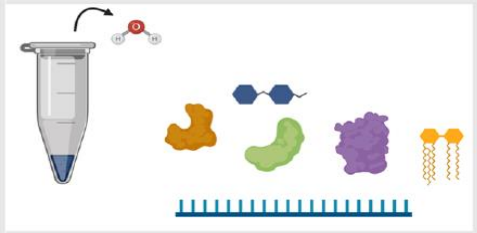
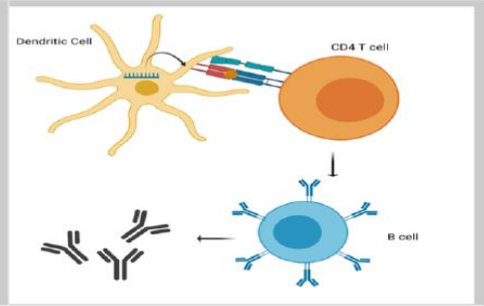
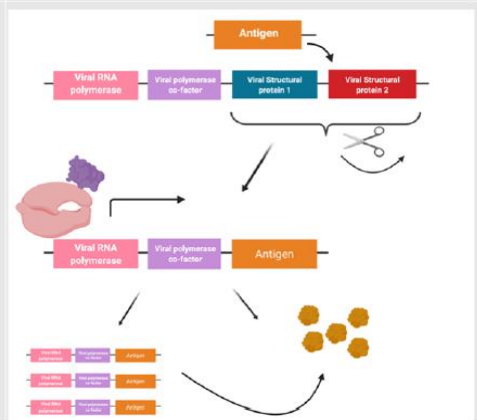
At least 20 teams are aiming to use genetic instructions (in the form of DNA or RNA) for a coronavirus protein that prompts an immune response. The nucleic acid is inserted into human cells, which then churn out copies of the virus protein; most of these vaccines encode the virus's spike protein.

RNA- and DNA-based vaccines are safe and easy to develop: to produce them involves making genetic material only, not the virus. But they are unproven: no licensed vaccines use this technology.



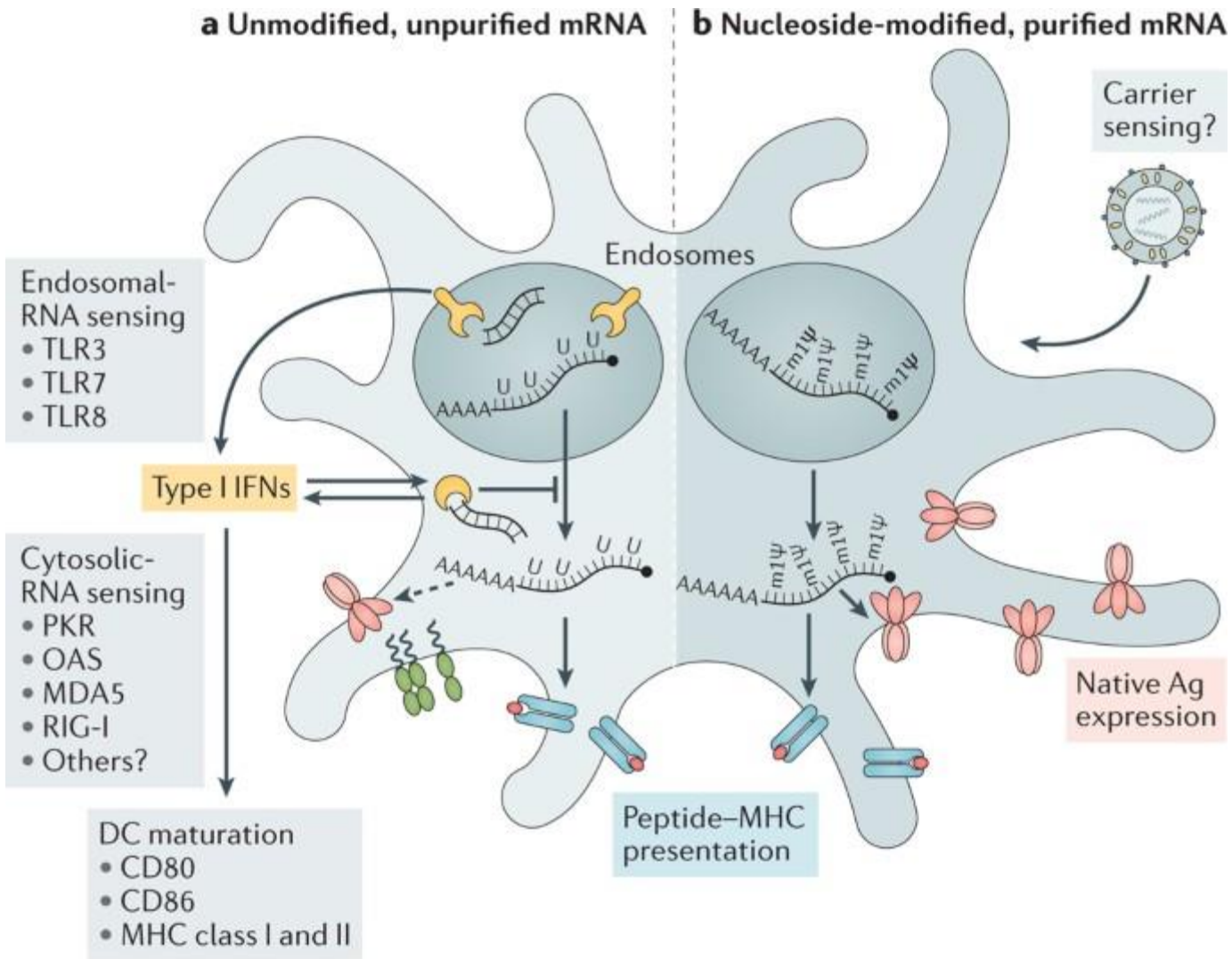
RNA vaccines

RNA Vaccines

Method Name	Method Diagram	Description of Method	Results
RNA Engineering		Adding 5' cap and Kozak sequences and 3' poly-A sequence to mimic structure of endogenous mRNA to avoid detection by innate immune sensors, and adding modified nucleosides to increase RNA stability	Widely used in vaccine design
Thermostable RNA		Freeze-drying RNA and incubation with various biomolecules increase RNA stability even at high temperature	Widely used in vaccine design
Dendritic Cell Vaccines		RNA is transfected into dendritic cells in-vitro, which can then activate CD4 T cells and stimulates antibody production in vivo.	Most explored for use as cancer vaccines- several in clinical trials.
Self Replicating RNA		The structural proteins in the genome of an RNA virus are replaced with the coding sequence for the antigen- the viral polymerase can keep producing RNA which amplifies and maintains the amount of the antigenic protein.	Pre-clinical testing has shown effectiveness in inducing protection.

Frontiers in Immunology

September 2020



Nature Reviews | Drug Discovery

Sicurezza ed efficacia

Efficacia:

-Vaccino Pfizer 95%
-Vaccino Moderna 94%
-Vaccino Astrazeneca 82%
-Vaccino Johnson&Johnson 67%

AIFA,Ottobre 2021

Table 3. Real world vaccine effectiveness data

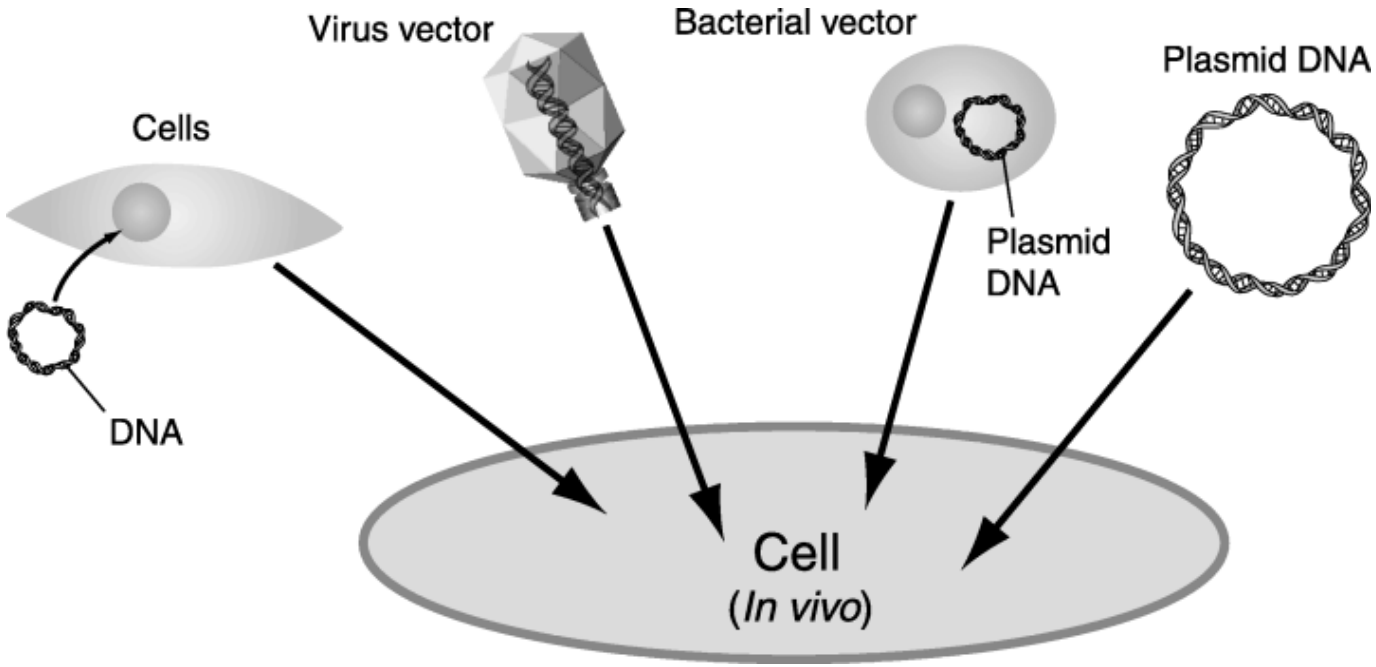
Sponsor	Real-world vaccine effectiveness against prototype SARS-CoV-2 strain	Real-world vaccine effectiveness against SARS-CoV-2 variants							
		Alpha (B.1.1.7)		Beta (B.1.351)		Gamma (P.1)		Delta (B.1.617.2)	
		I/SI	SD	I/SI	SD	I/SI	SD	I/SI	SD
Pfizer/BioNTech	64%–99%	65%–100%	84%–100%	75%–88%	95%–100%	79%–88%	95%–100%	79%–88%	96%
Moderna	68%–99%	79%–100%	90%–96%	88%–96%	96%–100%	79%–88%	100%	–	–
AstraZeneca	–	66%–100%	86%–92%	–	–	–	–	60%–61%	92%
Janssen	77%	–	–	–	–	–	–	–	–

(I/SI= infection/symptomatic infection; SD= severe disease)

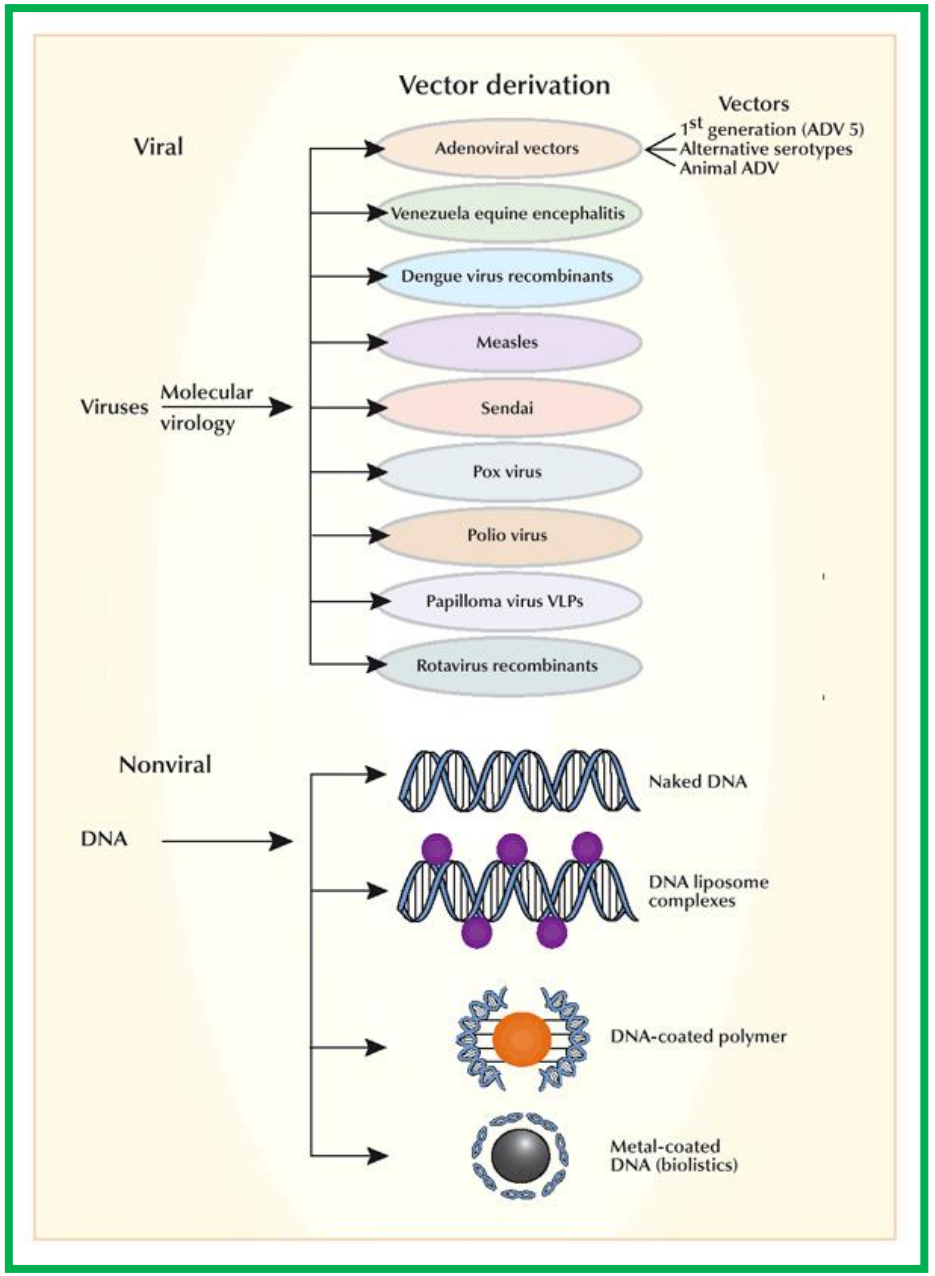
Bok, Mascola, Immunity 2021

Alcuni esempi di vaccini a DNA

Vaccini a DNA



Alcuni esempi di vaccini a DNA

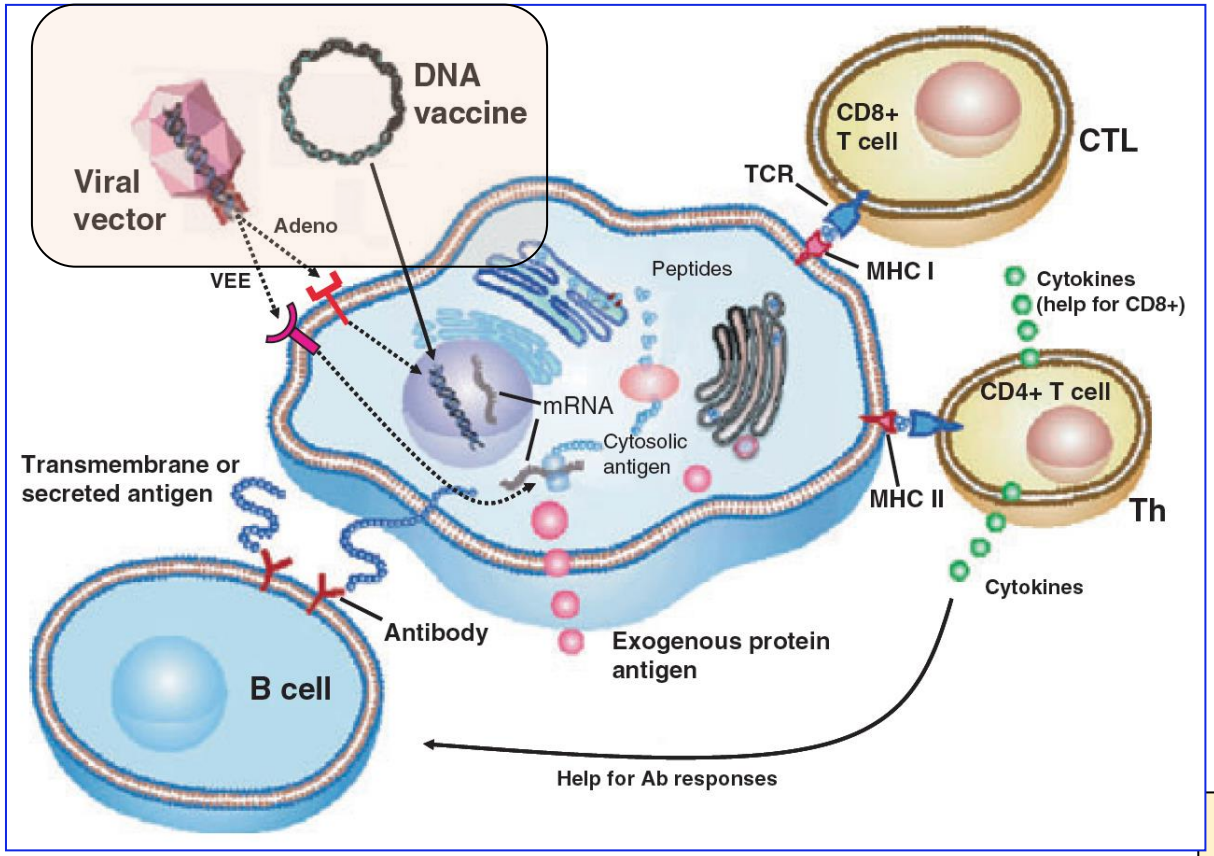


Vettori ricombinanti o vaccini a DNA

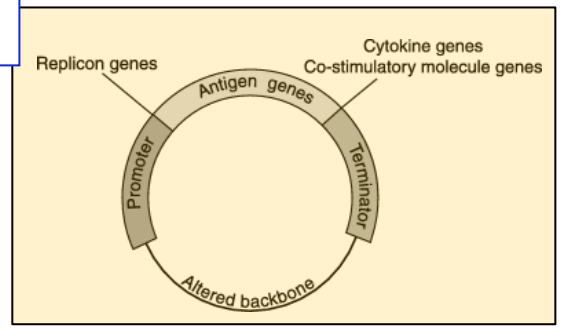
Alcuni esempi di vaccini a DNA

Vaccini a DNA

Meccanismo di generazione di CTLs, Th, anticorpi



I vaccini a DNA possono indurre sia risposte cellulo-mediate che la produzione di anticorpi



University of Oxford e Astra Zeneca

Vaccine type:
Non replicating adenovirus expressing the spike protein

Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial

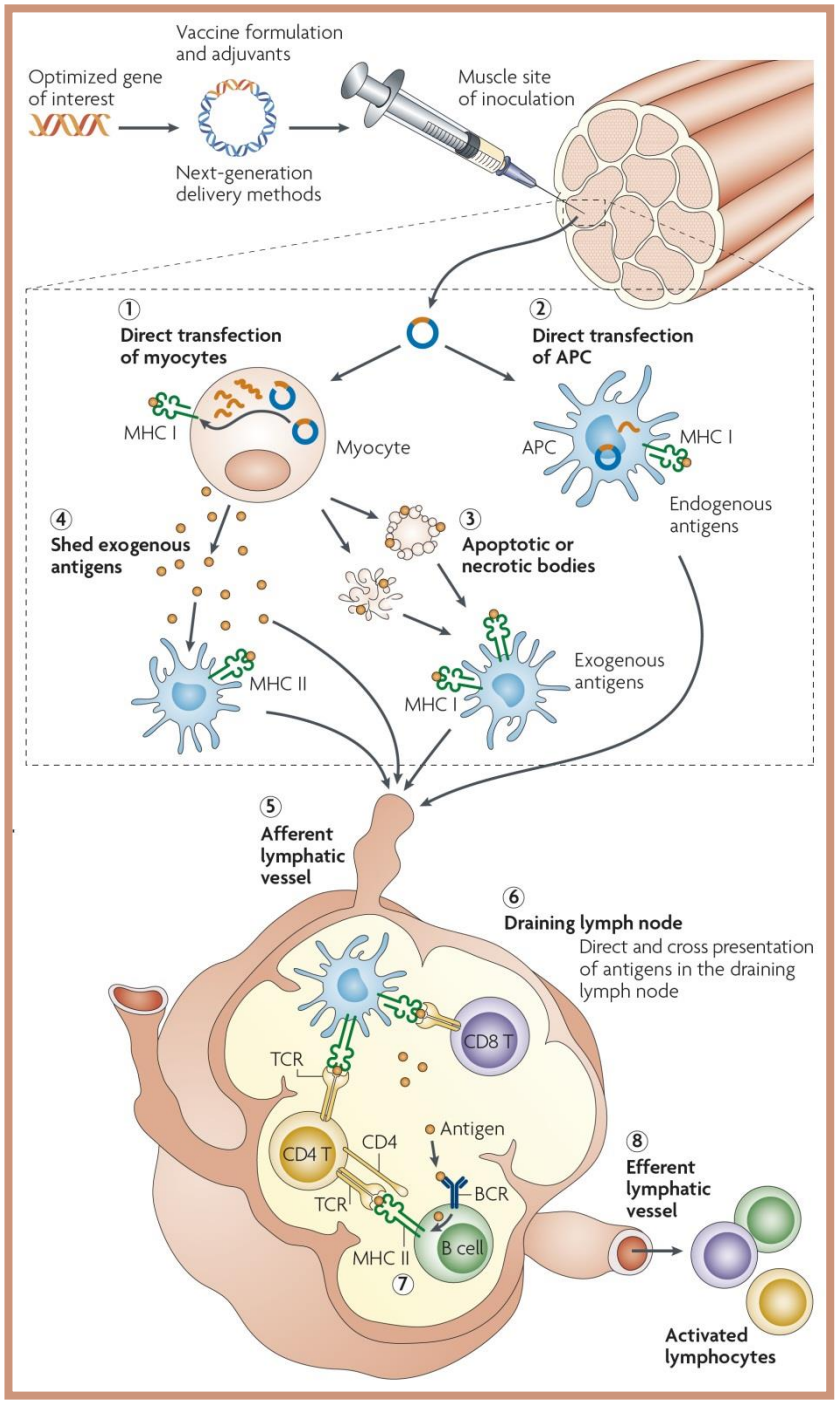
Maheshi N Ramasamy*, Angela M Minassian*, Katie J Ewer*, Amy L Flaxman*, Pedro M Folegatti*, Daniel R Owens*, Merryn Voysey*, Parvinder K Aley, Brian Angus, Gavin Babbage, Sandra Belij-Rammerstorfer, Lisa Berry, Sagida Bibi, Mustapha Bittaye, Katrina Cathie, Harry Chappell, Sue Charlton, Paola Cicconi, Elizabeth A Clutterbuck, Rachel Colin-Jones, Christina Dold, Katherine R W Emary, Sofiyah Fedosyuk, Michelle Fuskova, Diane Gbesemete, Catherine Green, Bassam Hallis, Mimi M Hou, Daniel Jenkin, Carina C D Joe, Elizabeth J Kelly, Simon Kerridge, Alison M Lawrie, Alice Lelliott, May N Lwin, Rebecca Makinson, Natalie G Marchevsky, Yama Mujadidi, Alasdair P S Munro, Mihaela Pacurar, Emma Plested, Jade Rand, Thomas Rawlinson, Sarah Rhead, Hannah Robinson, Adam J Ritchie, Amy L Ross-Russell, Stephen Saich, Nisha Singh, Catherine C Smith, Matthew D Snape, Rinn Song, Richard Tarrant, Yrene Themistocleous, Kelly M Thomas, Tonya L Villafana, Sarah C Warren, Marion E E Watson, Alexander D Douglas*, Adrian V S Hill*, Teresa Lambe*, Sarah C Gilbert*, Saul N Faust*, Andrew J Pollard*, and the Oxford COVID Vaccine Trial Group

Vantaggio si conserva a 4° C

	Clinical trial:phase III (clinicalTrials.gov)
Study Type :	Interventional (Clinical Trial)
Estimated Enrollment :	40051 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment
Intervention Model Description:	Participants are assigned to one of two or more groups in parallel for the duration of the study.
Masking:	Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor)
Masking Description:	Double Blind: two or more parties are unaware of the intervention assignment.
Primary Purpose:	Treatment
Official Title:	A Phase III Randomized, Double-blind, Placebo-controlled Multicenter Study in Adults to Determine the Safety, Efficacy, and Immunogenicity of AZD1222, a Non-replicating ChAdOx1 Vector Vaccine, for the Prevention of COVID-19
Actual Study Start Date :	August 28, 2020
Estimated Primary Completion Date :	December 22, 2020
Estimated Study Completion Date :	October 25, 2022

Vaccini a DNA

Meccanismo di generazione di linfociti T citotossici, T helper, anticorpi

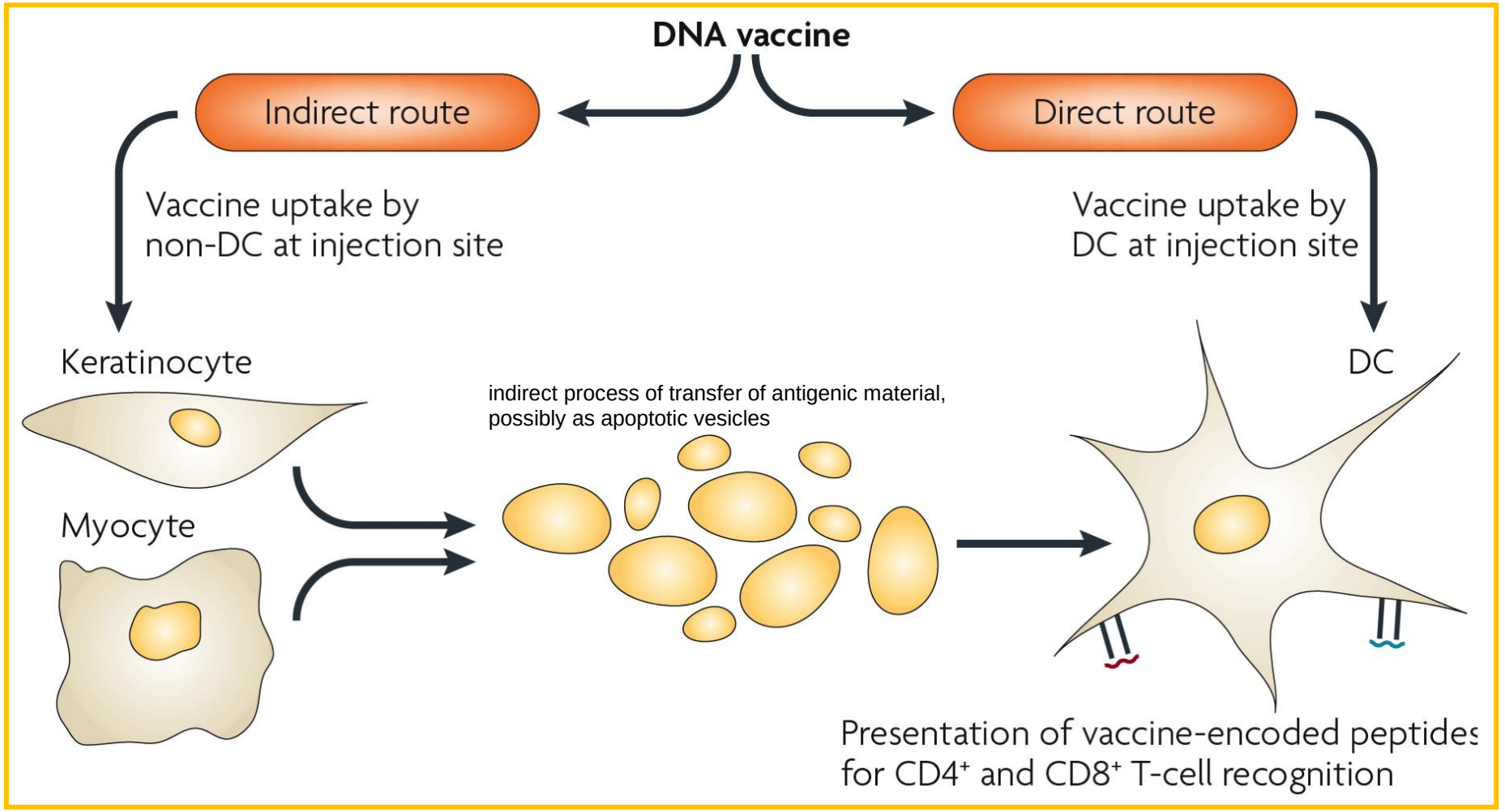


Vantaggi:

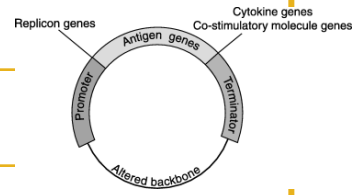
- Più facili da preparare
- Più facili da somministrare
- Più sicuri
- Meno costosi
- Nessun rischio di malattia
- Inducono risposte protettive complete (attivazione di entrambe le branche, B e T, dell'immunità)

Mechanism of antigen presentation for the generation of T lymphocytes following DNA vaccination

injection into skin or muscle

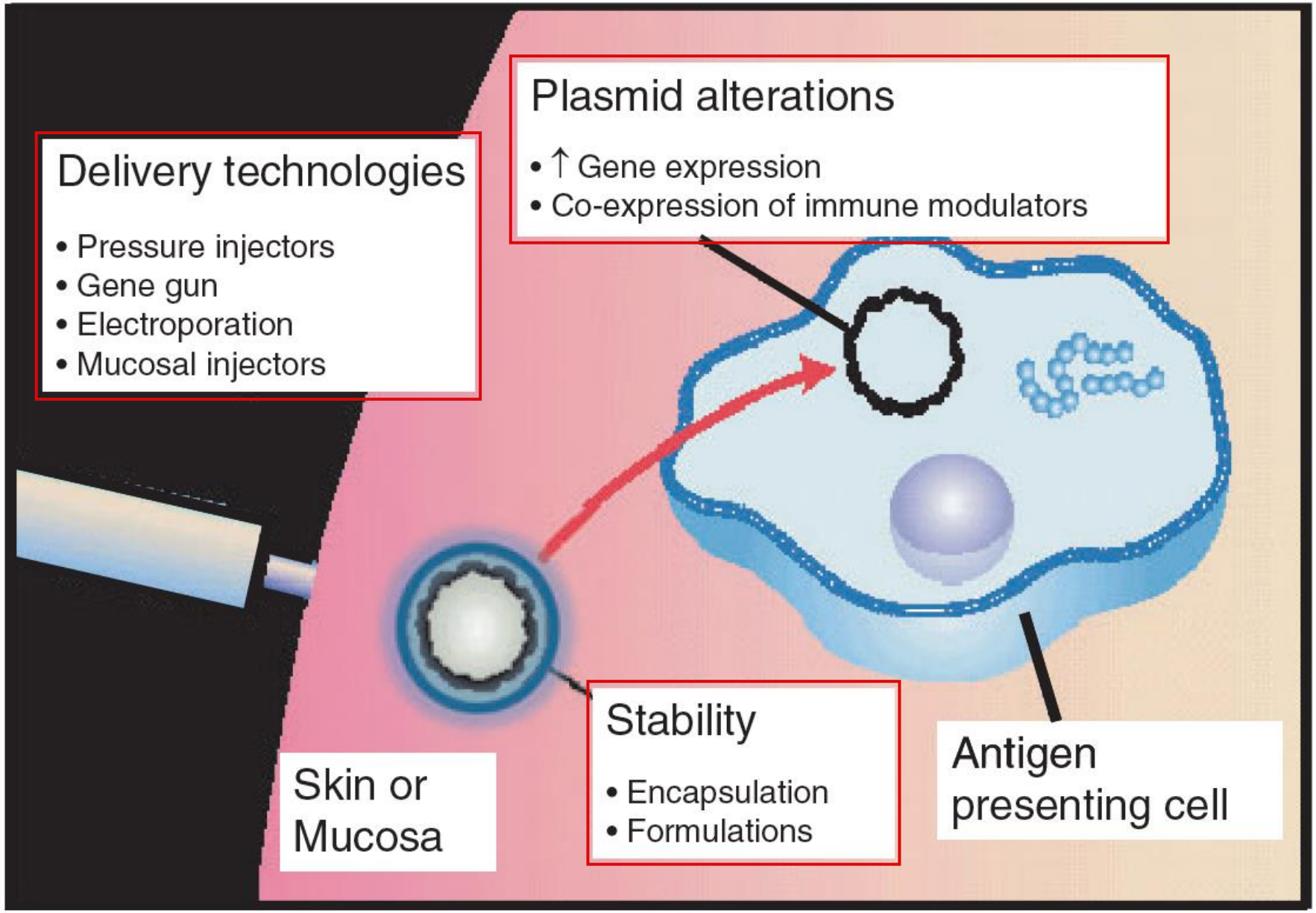


Advantages of DNA vaccines



design	❖ simple engineering & design modification
manufacture	❖ rapid production and formulation; ❖ reproducible, large-scale production and isolation
safety	❖ unable to revert into virulent form (like live vaccine); ❖ does not require toxic treatment (like inactivated vaccine); ❖ no significant adverse events in clinical trials (many thousands vaccinated so far)
stability & mobility	❖ more temperature stable than conventional vaccines; ❖ long shelf-life; ❖ easy storage and transport; ❖ likely not to require a cold chain
immunogenicity	❖ induction of antigen-specific T and B cell responses similar to those elicited by live attenuated vaccines

Approaches to increase the potency of DNA vaccines



“Vaccine design”: considerazioni

Efficacia:

- Anticorpi: attenuato > ucciso > subunità > DNA/RNA

- T citotossici: attenuato > DNA/RNA > ucciso > subunità

Tossicità: attenuato > ucciso > DNA/RNA > subunità
(infiammazione)

Sicurezza: subunità > ucciso > DNA/RNA > attenuato
(biologica)

I diversi tipi di vaccini stimolano in maniera diversa le principali componenti della risposta immunitaria

Table 2 Vaccines that induce CD8 T cells

Type of vaccine	Dose	Need for boost[a]	Need for adjuvants	Risks
Live-attenuated	Low	No	No	Reversion to virulence, disease in immunocompromised
Replication competent live-vectored	Low	No	No	Reversion to virulence, disease in immunocompromised
Replication-defective live-vectored	High	Varies	No	Low
DNA	High	Yes	Helpful	Low
Heterologous prime-boost	High	Yes	Helpful	Varies

Smallpox, yellow fever, oral polio, measles, mumps, rubella, varicella

yellow fever virus vectors with Japanese encephalitis virus, dengue virus, West Nile virus, in human trials

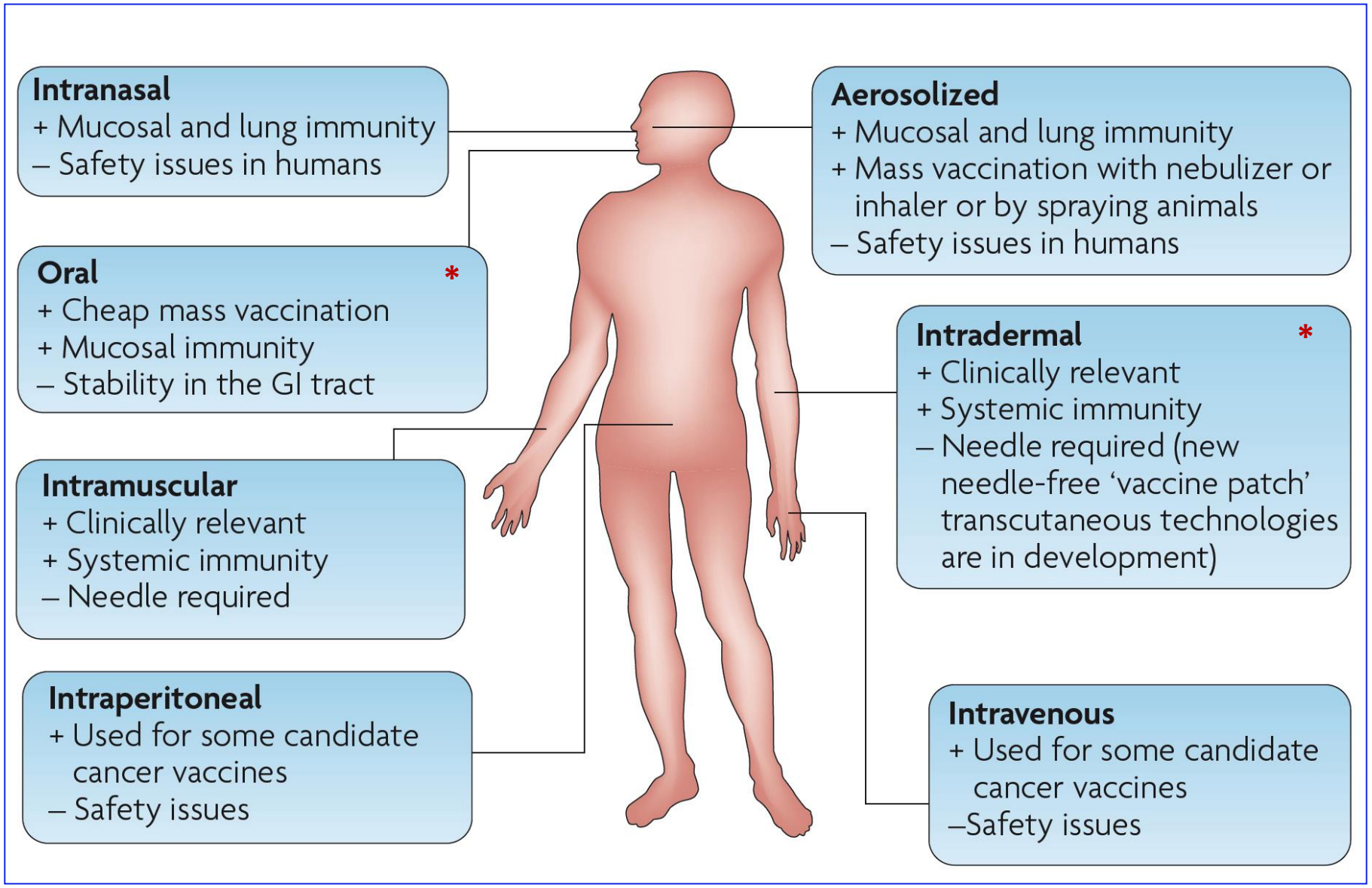
Problems with pre-existing immunity, unless vaccination has stopped or the vector has derived from another species

Not robust in humans as in mice, can be used in heterologous prime boost regimens

Immense potential to elicit high titer T and B responses. Need for two different agents in the correct order and interval

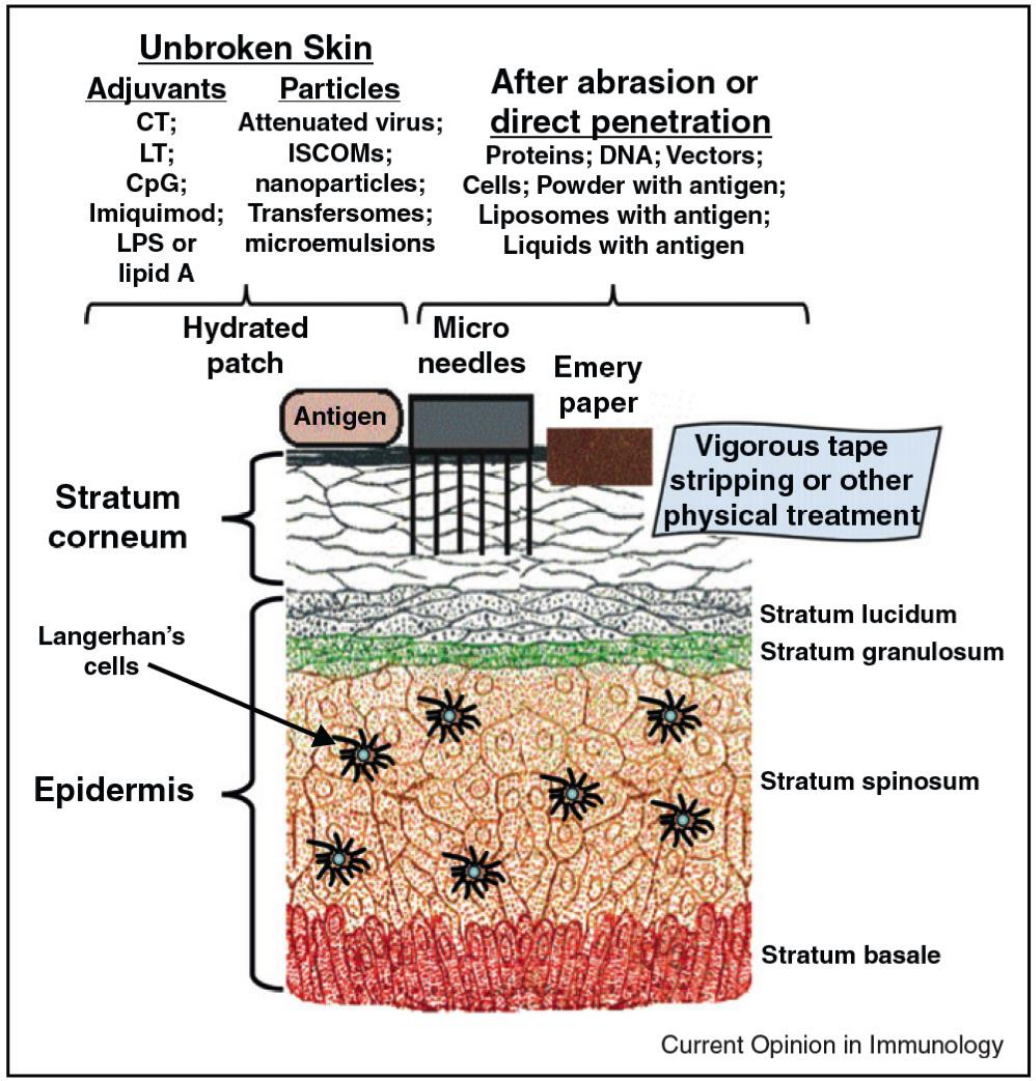
Possible routes of immunization for human vaccines

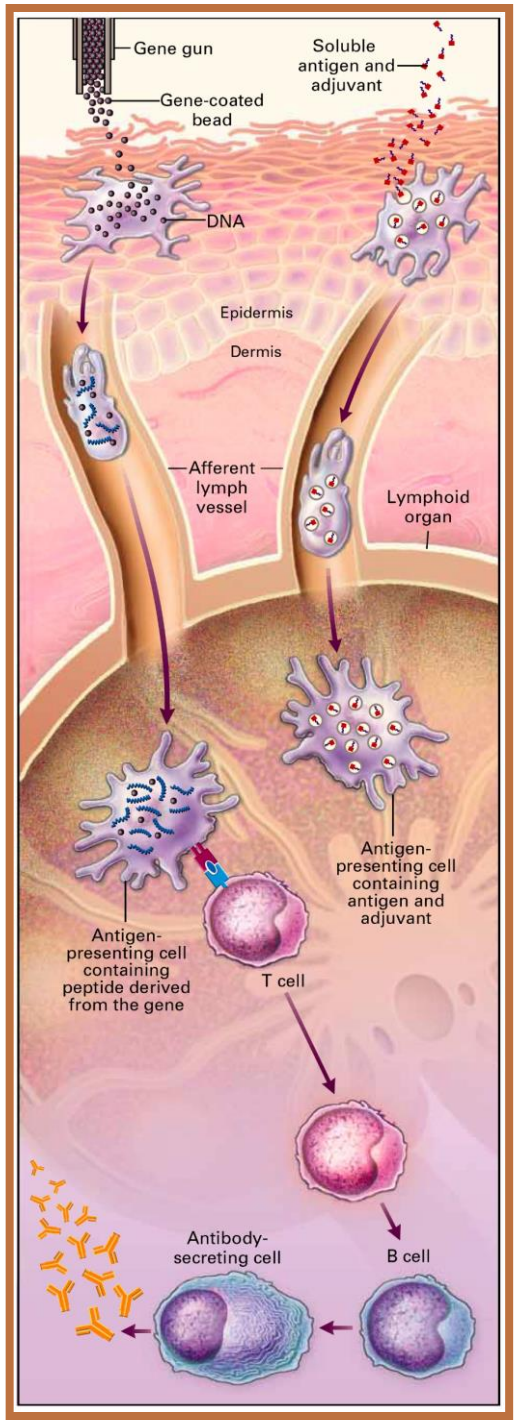
Potential advantages (+) and disadvantages (–)



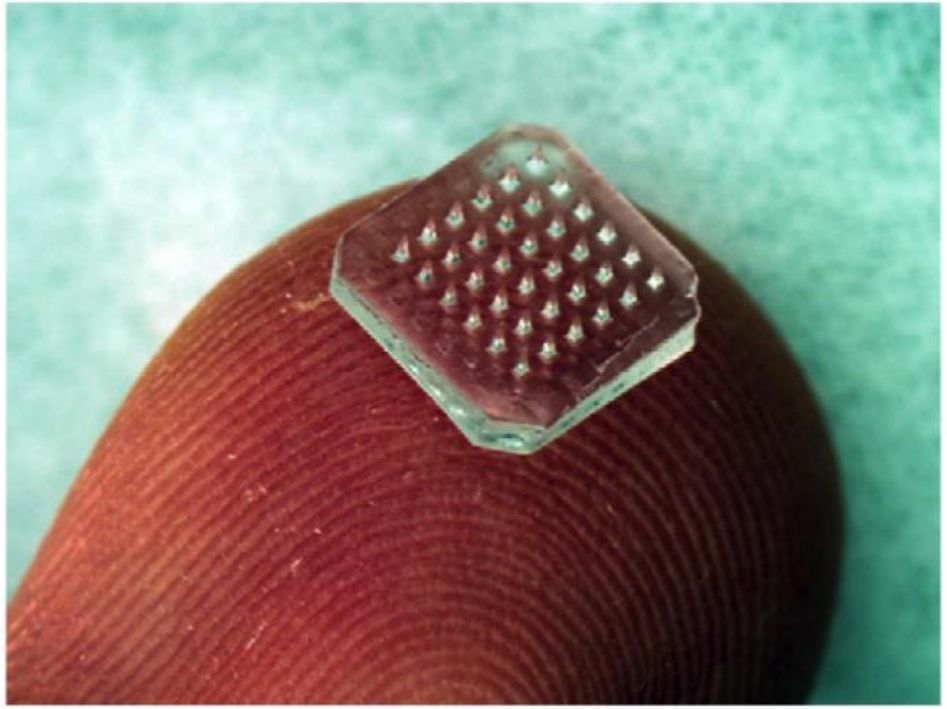
Alternatives to vaccination with a needle

- *"gene gun"* (DNA vaccines)
- transcutaneous immunization

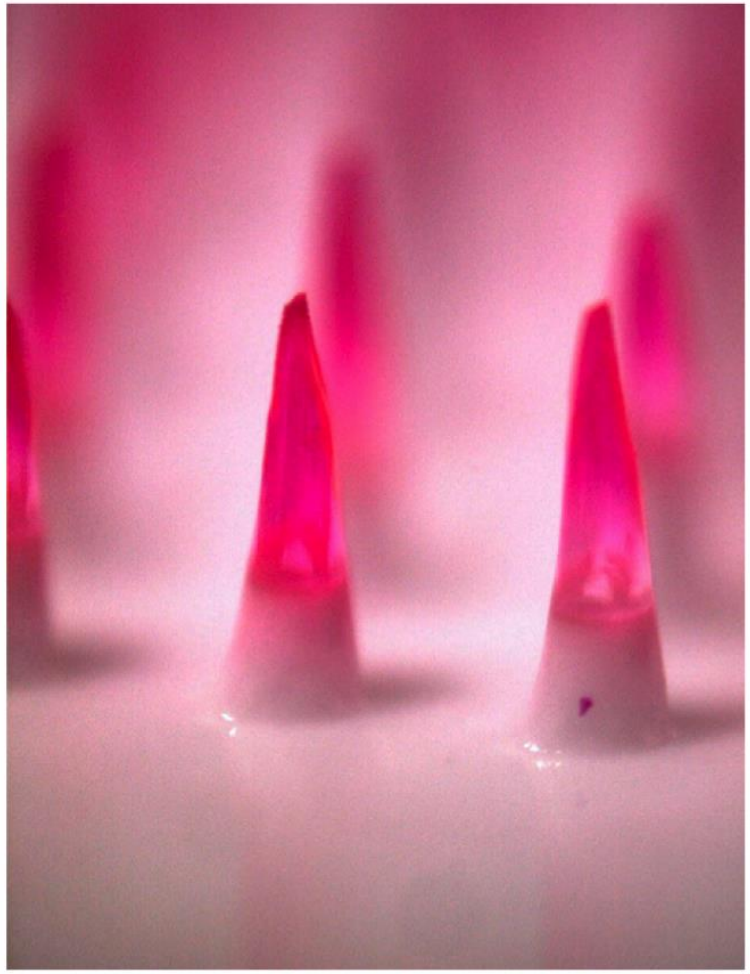




A patch containing 36 dissolving microneedles is shown on a fingertip

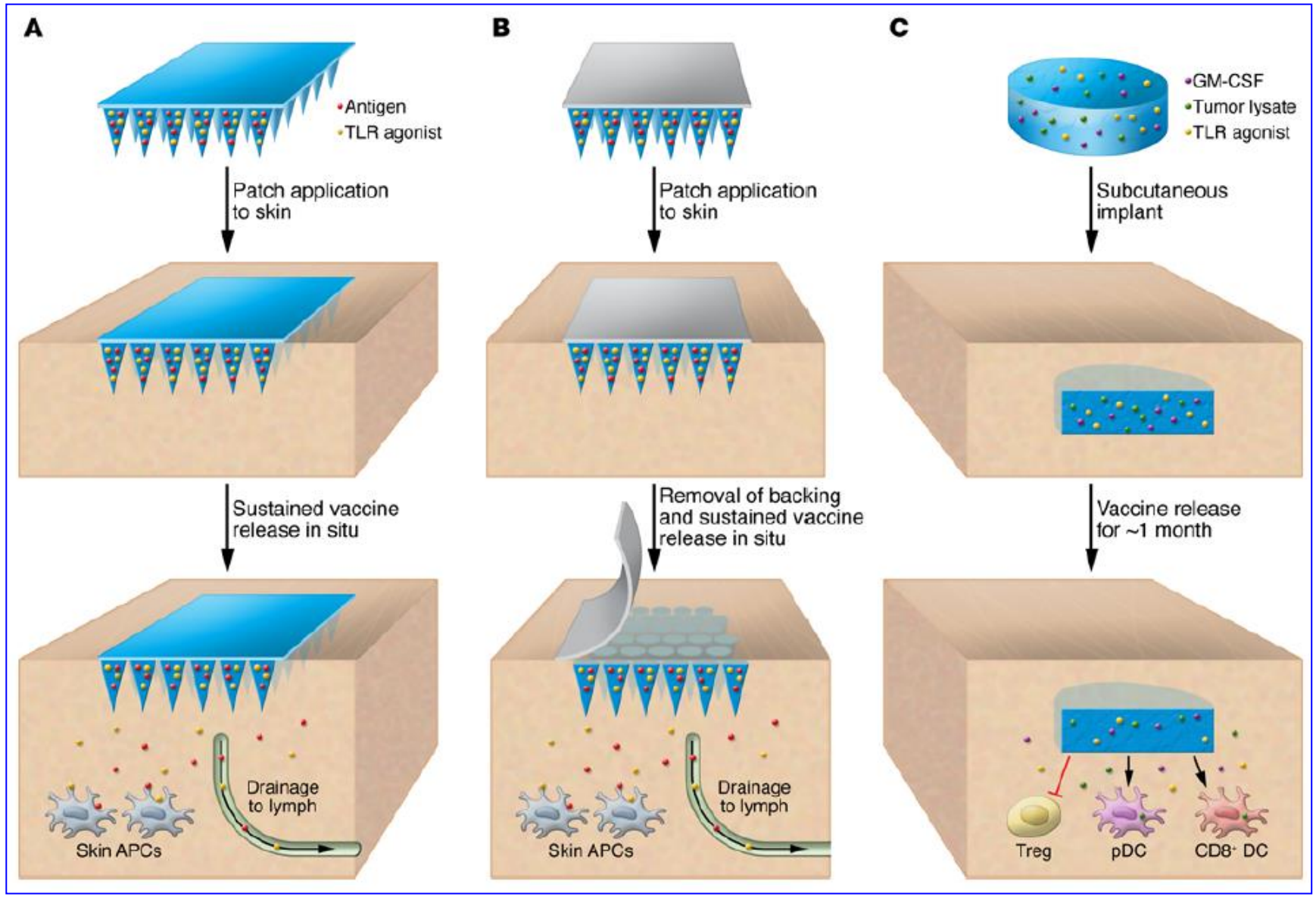


The microneedles dissolve within minutes after insertion into skin to release encapsulated drug or vaccine. Each microneedle is 900-µm tall. (Image courtesy of Jeong-Woo Lee, Laboratory for Drug Delivery, Georgia Institute of Technology.)

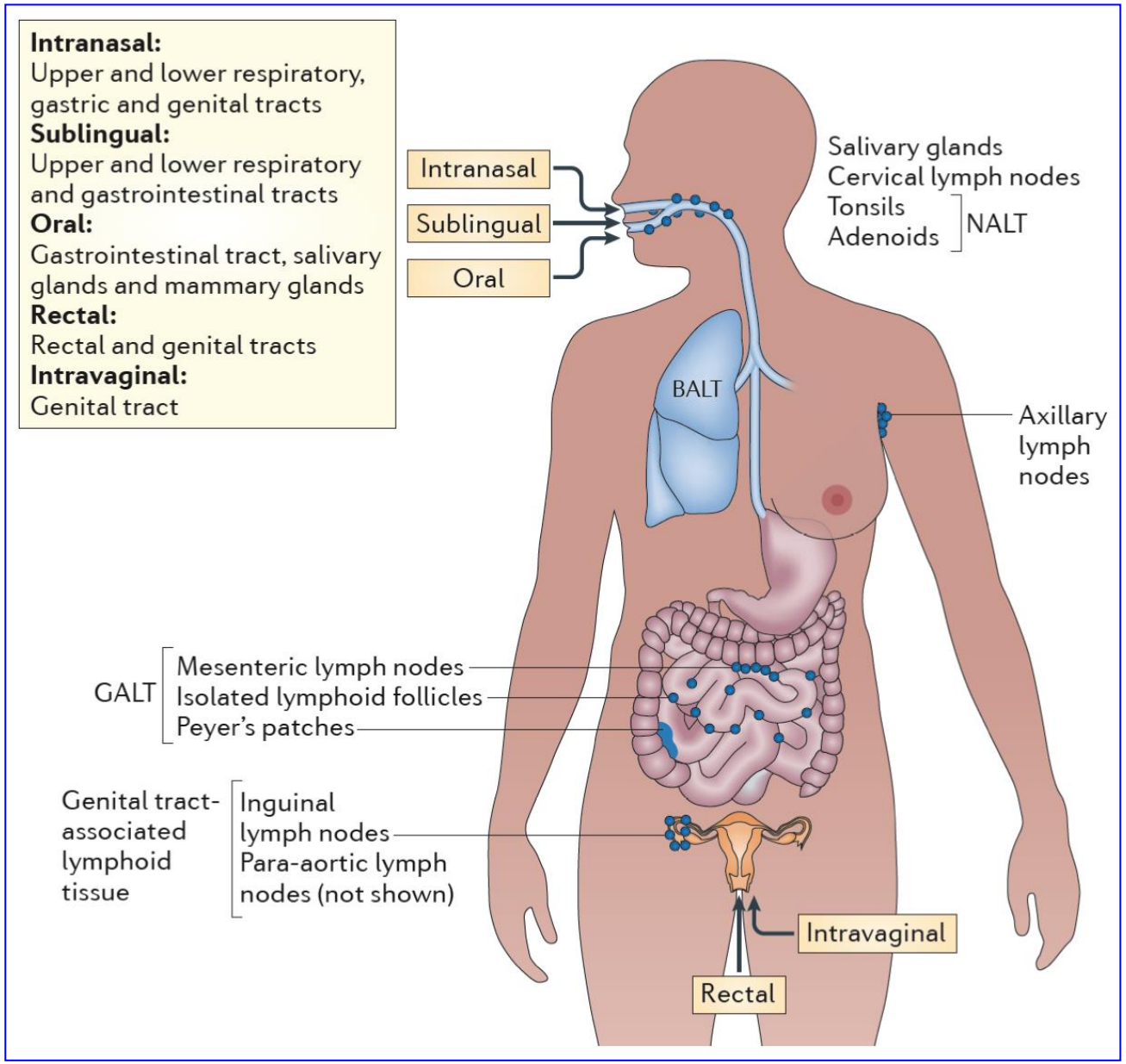


Microscope image shows dissolving microneedles encapsulating a pink dye used to simulate how a drug or vaccine would be incorporated into the needles. Each microneedle is 650-µm tall.
(Image courtesy of Sean Sullivan, Laboratory for Drug Delivery, Georgia Institute of Technology.)

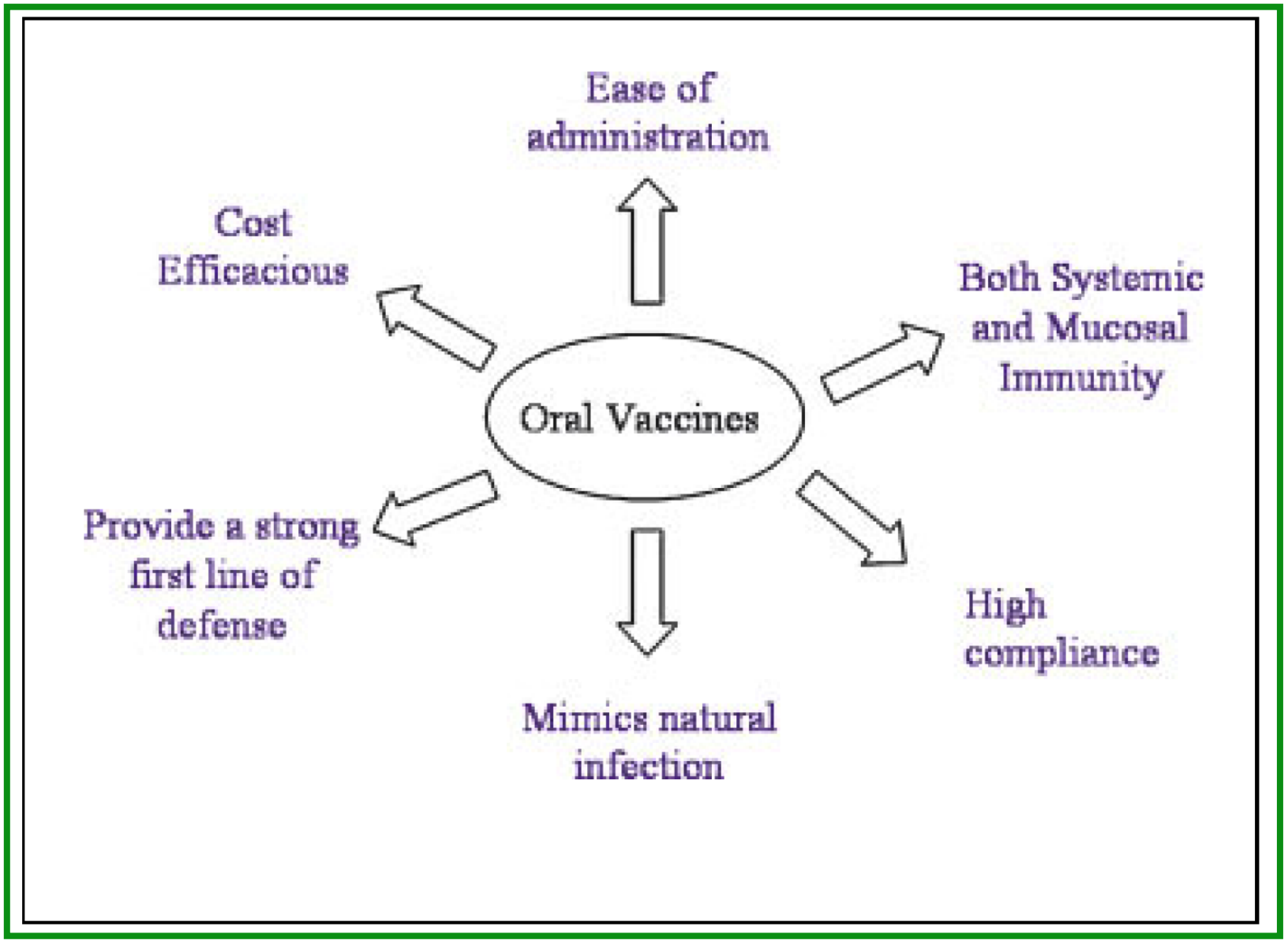
Alternatives to vaccination with a needle



Mucosal immunization routes and compartmentalization of effector functions



Why oral vaccines?



N.B. serum Abs do not efficiently protect against mucosal infections!

Possible alternative (needle-free) routes for vaccination

Table 4 Nonparenteral routes of administration

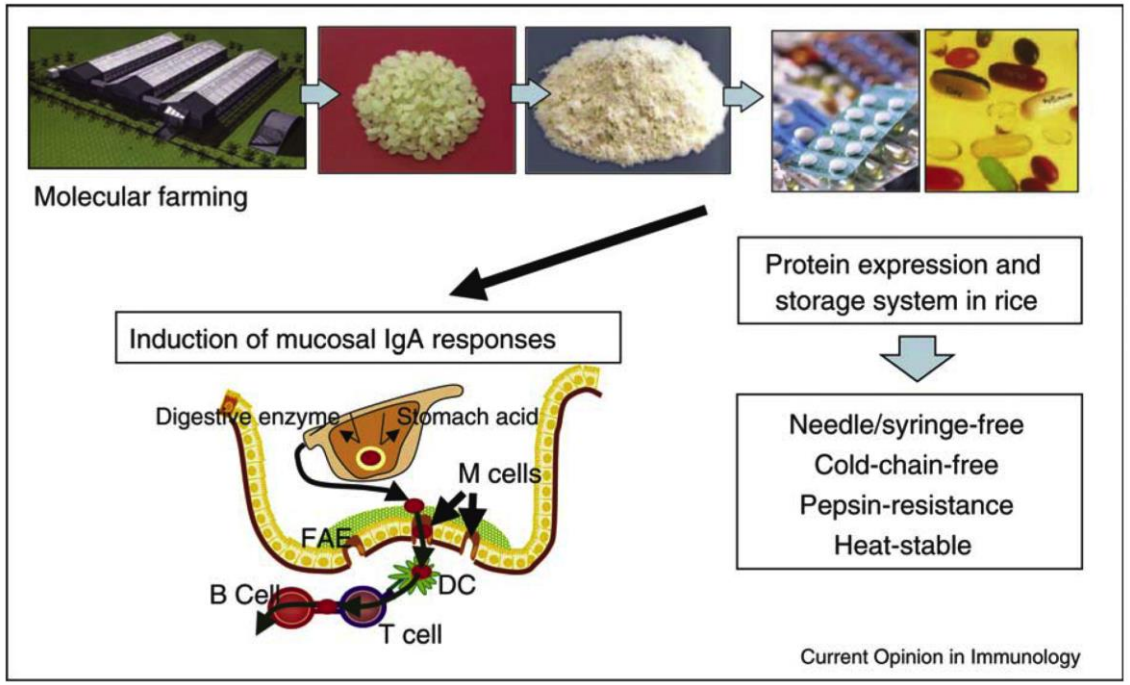
Route	Example of use
Intranasal	Live influenza
Aerosol	Measles
	Rubella
Oral	Plants transgenic for Hepatitis BsAg
Transcutaneous (patches, microneedles, powder)	Hepatitis B, anthrax

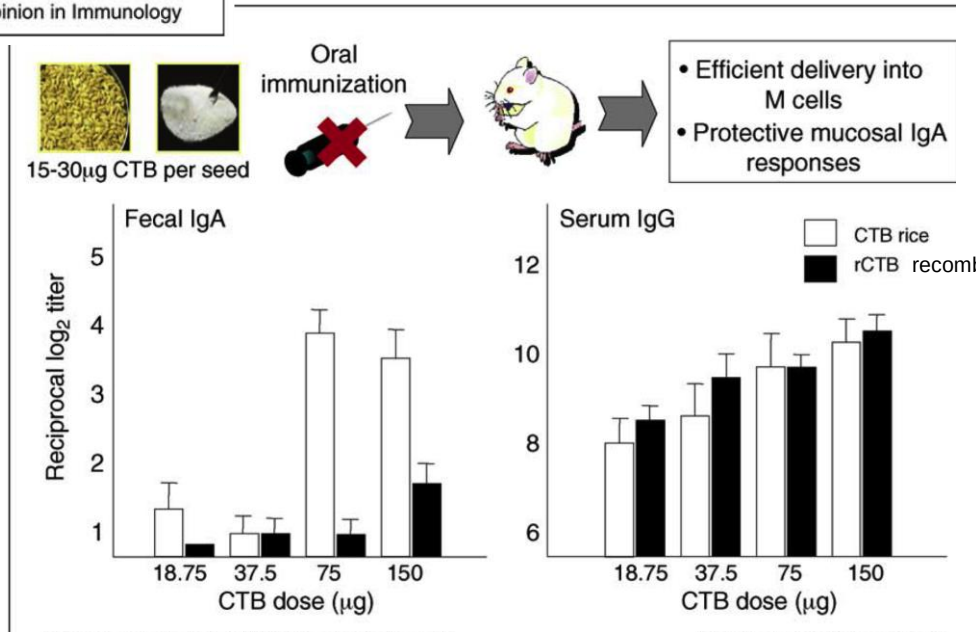
Table 1. Edible transgenic plant vaccines

Vaccine	Edible plant
Norwalk virus particle	Potato Tomato
Heat-labile enterotoxin B subunit	Potato Maize Soybean
Cholera toxin B subunit	Rice Potato
Enterotoxigenic *Escherichia coli* fimbrial subunit	Soybean
Japanese cedar pollen peptide	Rice

- **NO requirement of professional skill and syringe/needle for administration**
- **NO requirement for cold-chain (or refrigeration storage)**
- **HIGHER physico-chemical stability**

Overview of rice-based mucosal vaccine development





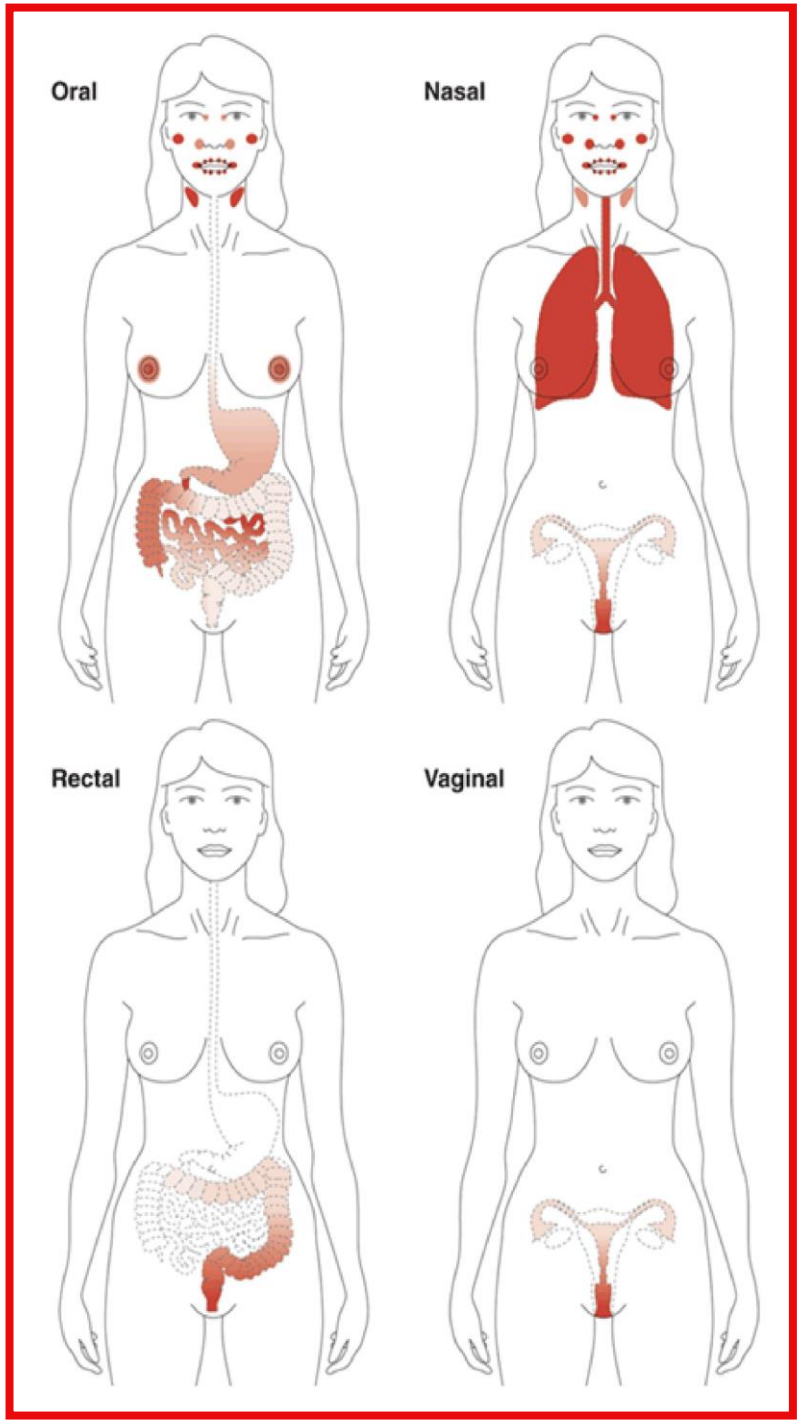
Orally administered rice-based Cholera Toxin B subunit (CTB) (or MucoRice-CTB)

After 20 years, golden rice nears approval

Bangladesh may become the first country to adopt transgenic rice enriched in vitamin A

A serving of golden rice contains half the beta-carotene children need daily.

- Golden rice, a genetically modified (GM) crop that could help prevent childhood blindness and deaths in the developing world.
- Golden rice was developed in the late 1990s to combat vitamin A deficiency, the leading cause of childhood blindness, by equipping the plant with beta-carotene genes from maize
- Low levels of vitamin A also contribute to deaths from infectious diseases such as measles.
- Spinach, sweet potato, and other vegetables supply ample amounts of the vitamin, but in some countries, particularly those where rice is a major part of the diet, vitamin A deficiency is still widespread.
- in Bangladesh it affects about 21% of children

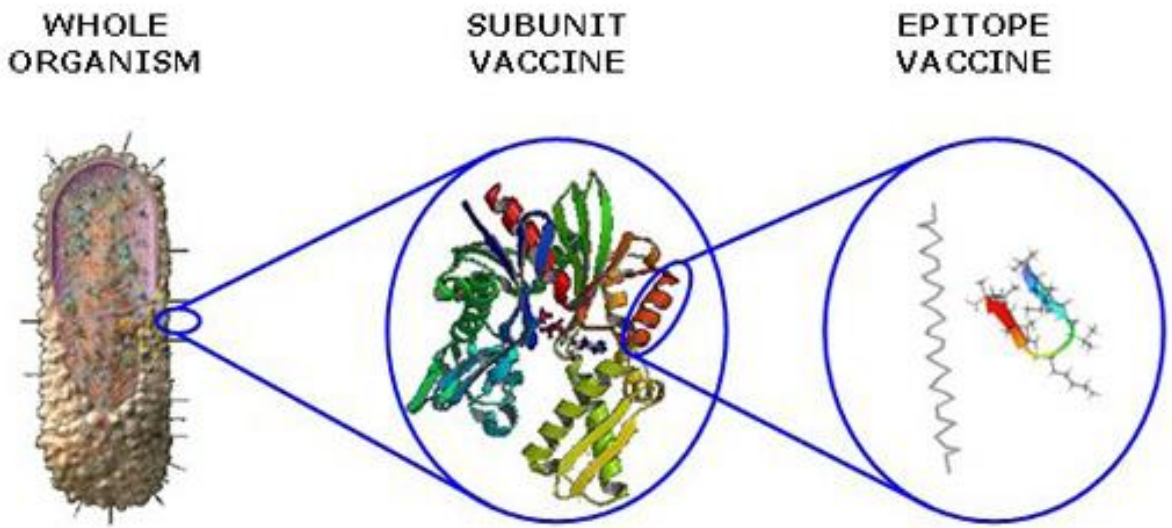


Expression of mucosal IgA immune responses after different routes of vaccination

Table 2. Currently approved oral/nasal vaccine against mucosal infectious diseases

	Administration route	
Pathogen	**Oral**	**Nasal**
Polio virus	Live attenuated polio virus (strain: Sabin)	–
Vibrio cholera	Heat or formalin inactivated *V. cholerae* O1 (strain: Inaba and Ogawa) and cholera toxin B subunit (CT-B) (Dukoral®)	–
Rotavirus	Live attenuated rotavirus Strain: human rotavirus 89–12 (RotaRix®) Bovine rotavirus WC3 (RotaTeq®)	–
Influenza virus	–	Live attenuated influenza virus (strain: Fashion species) (FluMist®)

Gli adiuvanti



+ ***Delivery Mechanism***:
recombinant protein,
viral or bacterial vector,
naked DNA,
loaded onto APCs or liposome,
etc.

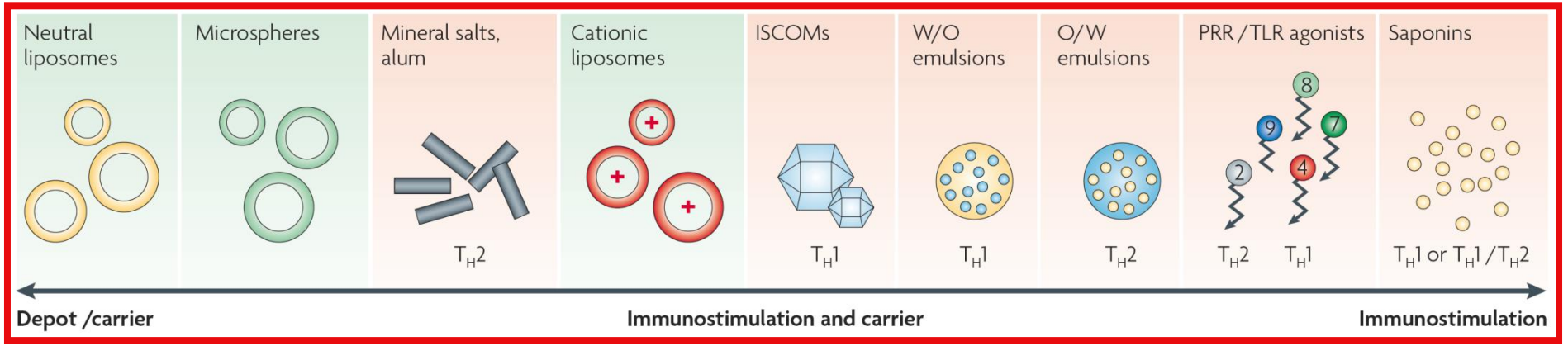
+ ***Adjuvant***

Gli adiuvanti

- Adiuvante di Freund
- Idrossido di Alluminio

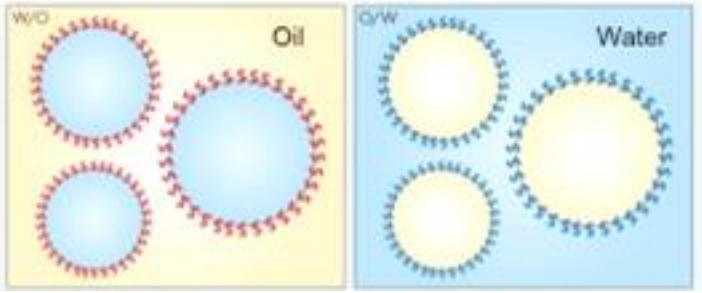
- Citochine/chemochine
- Prodotti batterici
- CpG
- Liposomi

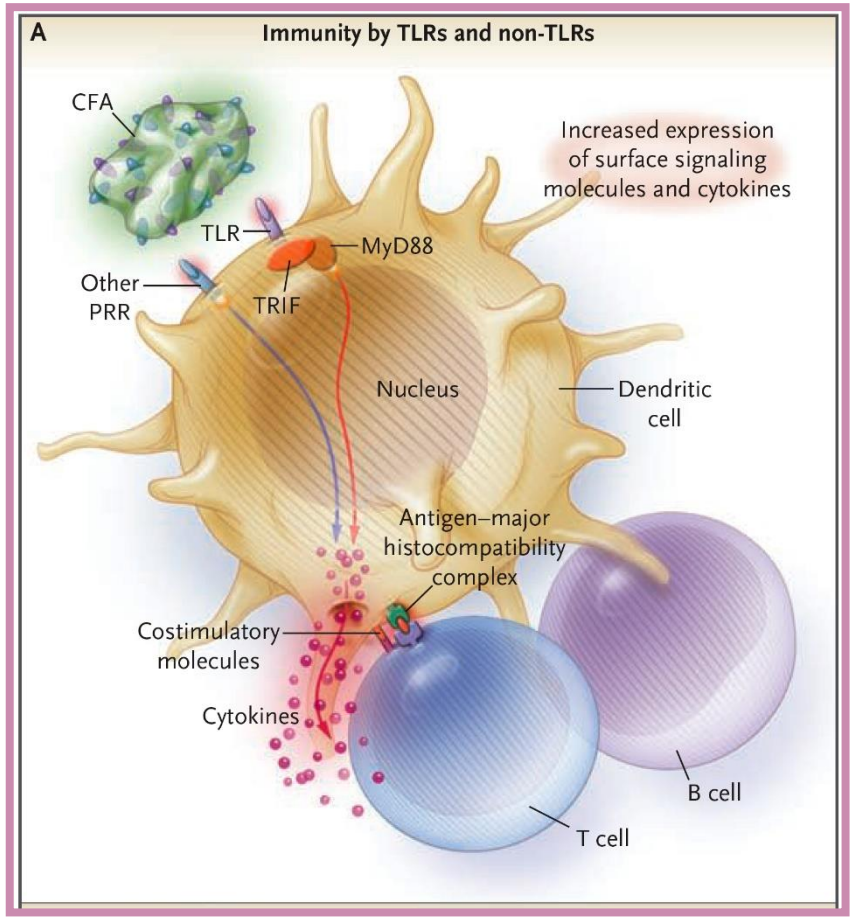
Gli adiuvanti sono sostanze che, quando somminstrate con l'antigene, ne aumentano l'immunogenicità



Come funzionano gli adiuvanti?

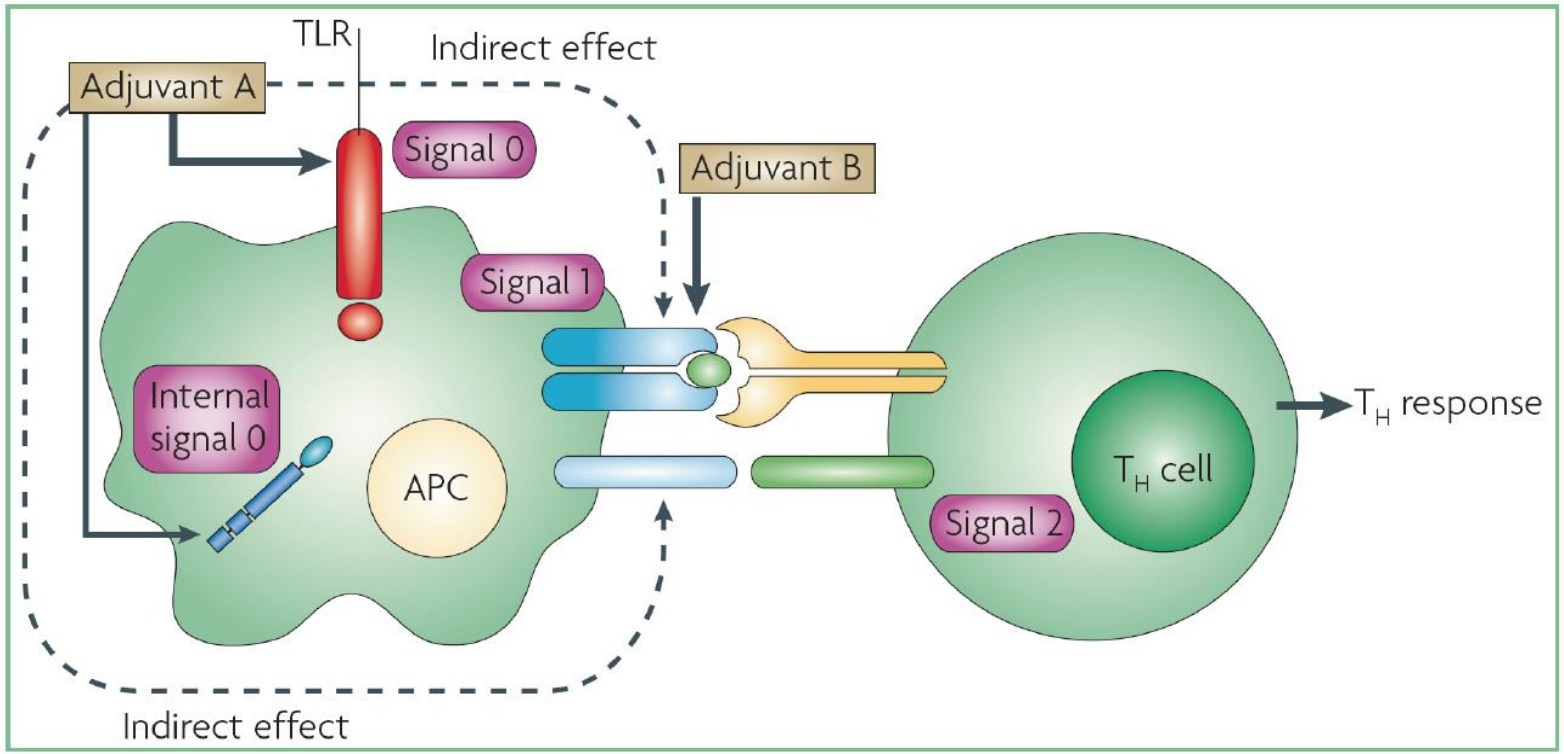
Adjuvant name	Composition	Mechanism of action
Incomplete Freund's adjuvant	Water-in-oil emulsion	Delayed release of antigen; enhanced uptake by macrophages
Complete Freunds adjuvant	Water-in-oil emulsion with dead mycobacteria	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages





1. Determinano un lento rilascio dell'antigene.
2. Stimolano l'immunità innata (attivazione/maturazione delle APC).
3. Aumentano la cattura dell'antigene (liposomi, microsfere).
4. Forniscono segnali co-stimolatori o citochine.

Dove e come agiscono gli adiuvanti?



***A*: attiva le APC**
B: favorisce la presentazione dell'antigene (liposomi, ISCOM)

Alcuni esempi

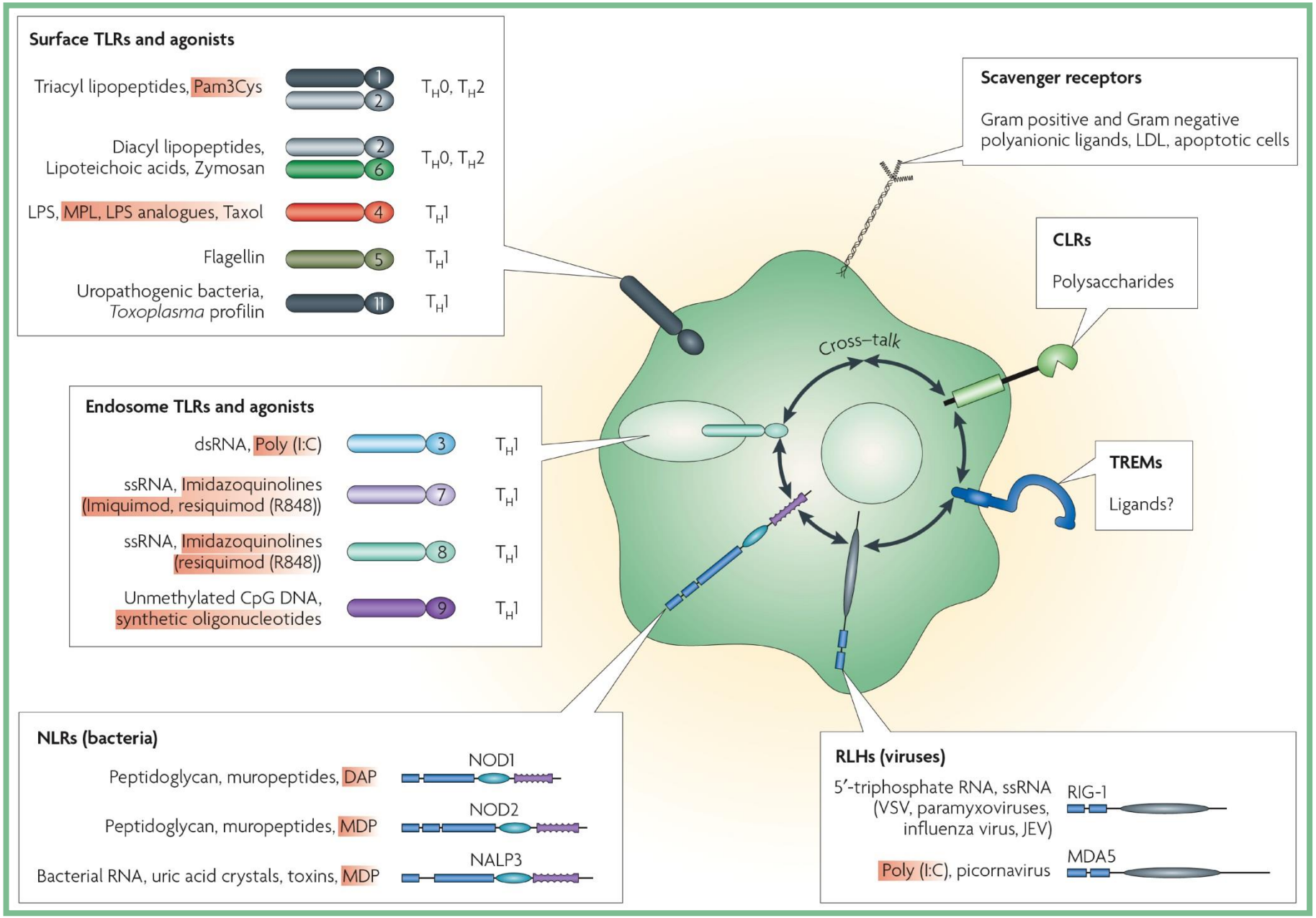
Il mix perfetto: i progressi nella ricerca sugli adiuvanti

Adjuvants				
Year licensed	**Name**	**Class**	**Contents**	**In vaccines against**
1924	Alum	Mineral salt	Aluminum phosphate or hydroxide	Many infectious diseases
1997	MF59	Oil-in-water emulsion	Squalene, polysorbate 80, sorbitan trioleate	Influenza
2000	Virosomes	Liposomes	Lipids, hemagglutinin	Influenza, hepatitis A
2005	AS04	Alum-absorbed TLR4 agonist	Aluminum hydroxide, monophosphoryl lipid A	Hepatitis B, human papilloma
2009	AS03	Oil-in-water emulsion	Squalene, polysorbate 80, α-tocopherol	Influenza
In development	CpG 7909	TLR9 agonist	CpG nucleotides	
	Imidazoquinolines	TLR7 and TLR8 agonist	Small molecules	
	PolyIC	TLR3 agonist	Double-stranded RNA analogs	
	Pam3Cys	TLR2 agonist	Lipopeptide	
	Flagellin	TLR5 agonist	Bacterial protein linked to antigen	

(LPS modificato)

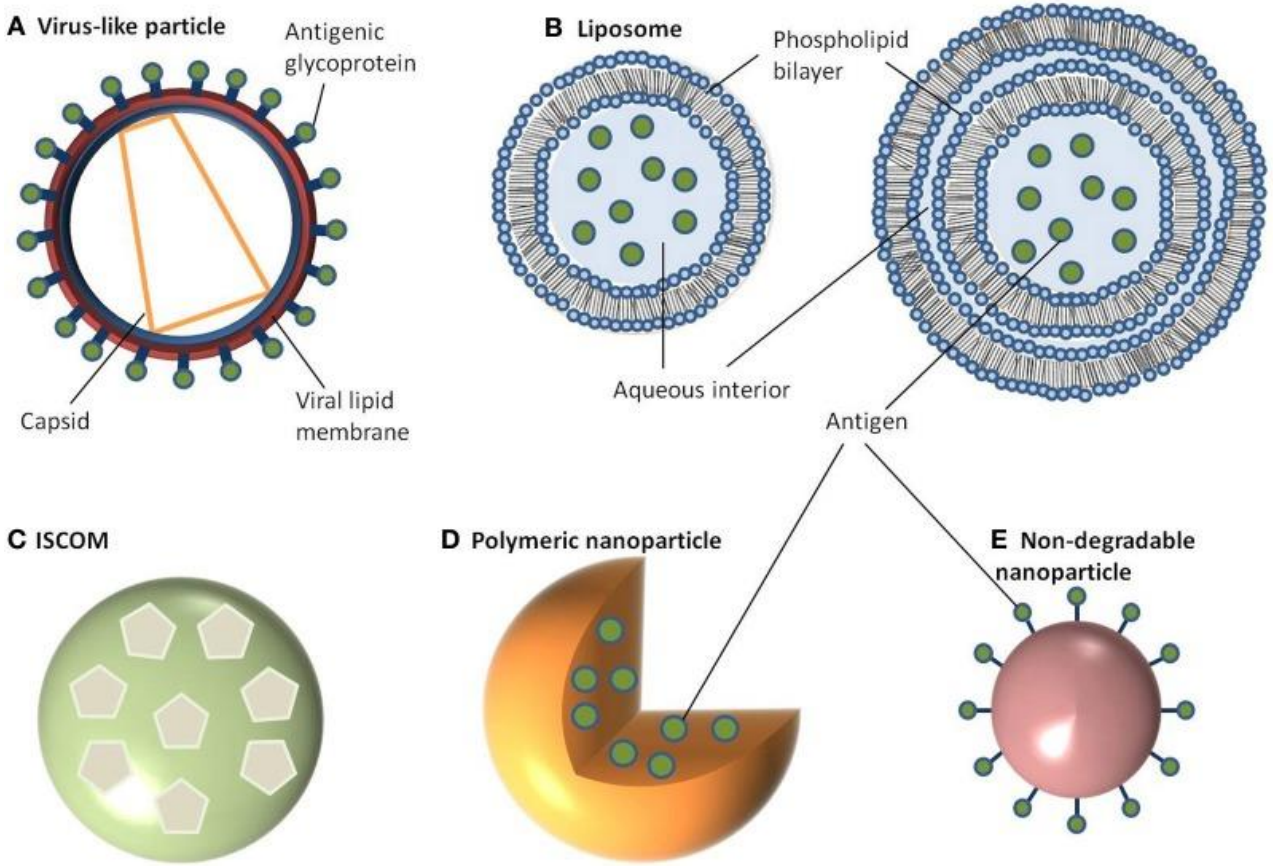
Alcuni esempi

Agonisti dei PRR come adiuvanti



Alcuni esempi

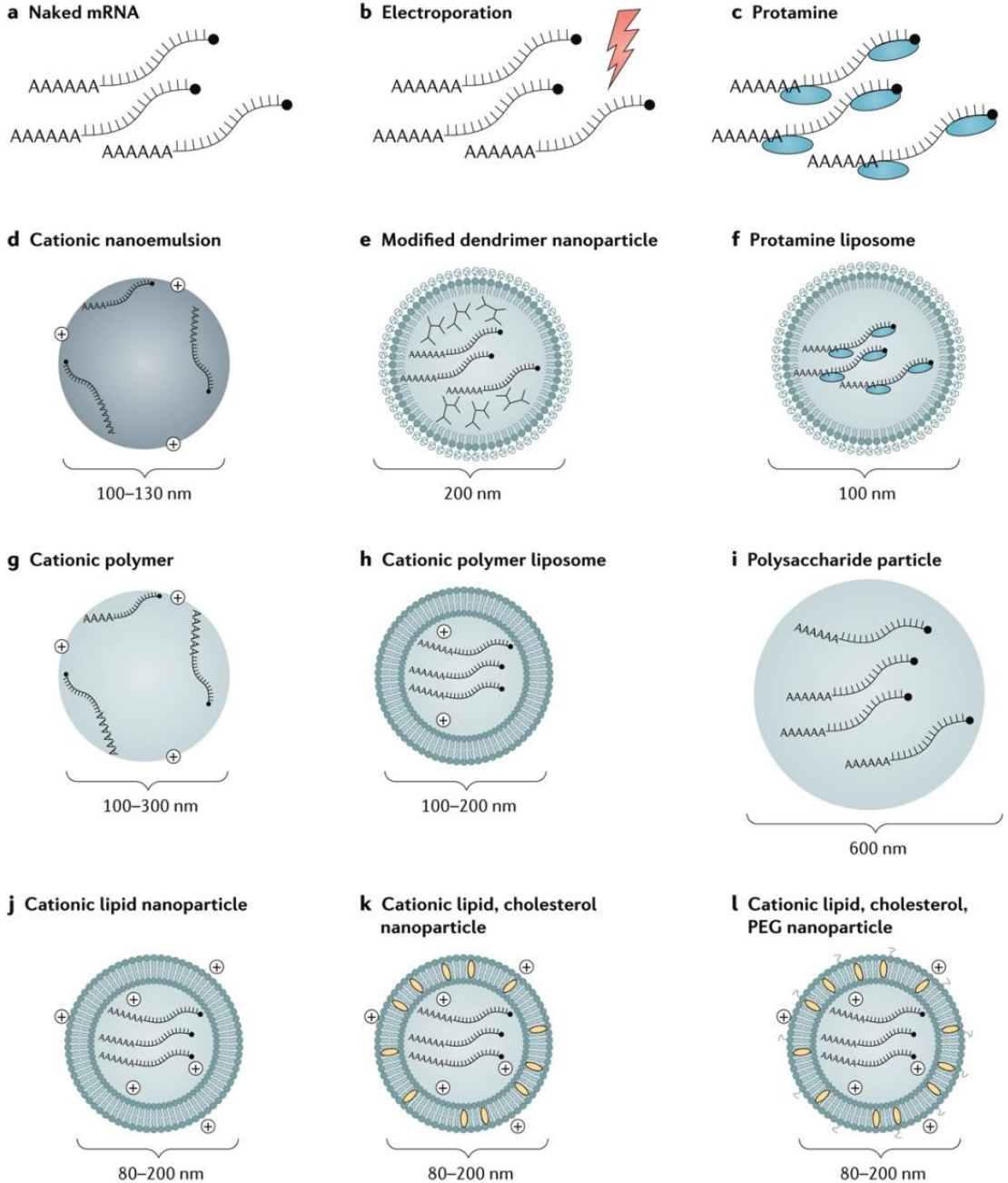
Rappresentazione schematica dei diversi sistemi di rilascio basati su nanoparticelle



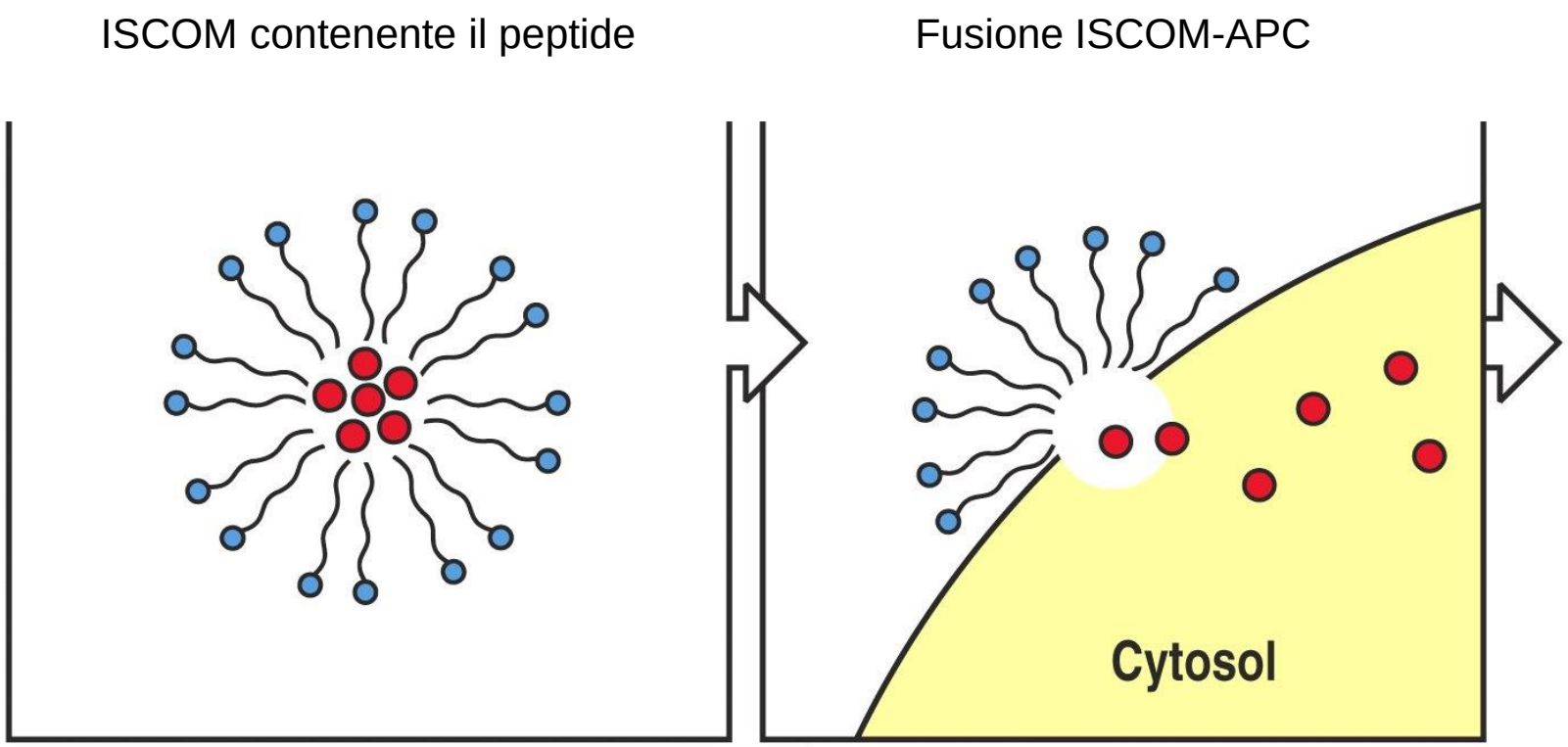
Data la scarsa immunogenicità dei vaccini a peptidi e la scarsa efficacia dei vaccini a sub-unità di indurre una risposta cellulo-mediata, sono state sviluppate nuove tecnologie, come **complessi antigene/anticorpo su matrice solida** che espongono epitopi sia per linfociti T che per linfociti B, o l'impiego di vescicole lipidiche **(liposomi**) o immunostimolanti (*immunostimulating complex*-i**scom**).

mRNA vaccines — a new era in vaccinology

Norbert Pardi[1], Michael J. Hogan[1], Frederick W. Porter[2] and Drew Weissman[1]



Un diverso approccio per ottenere peptidi antigenici presentati attraverso la via dell'MHC di classe I

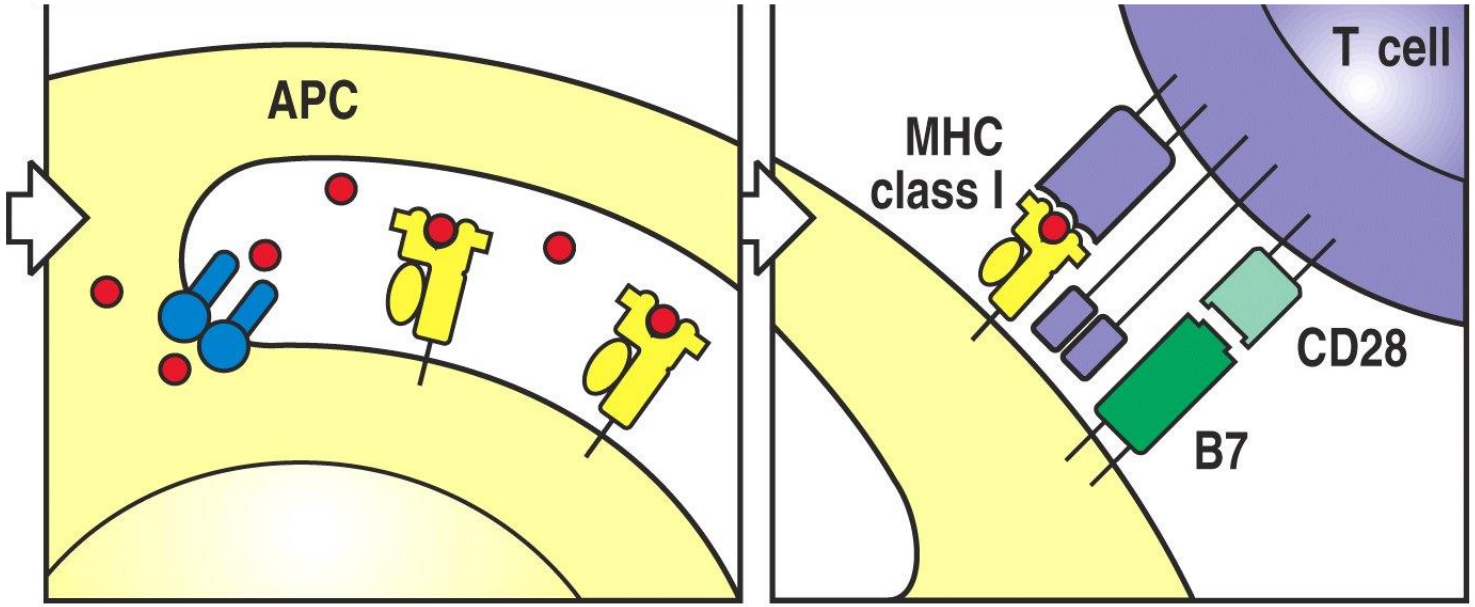


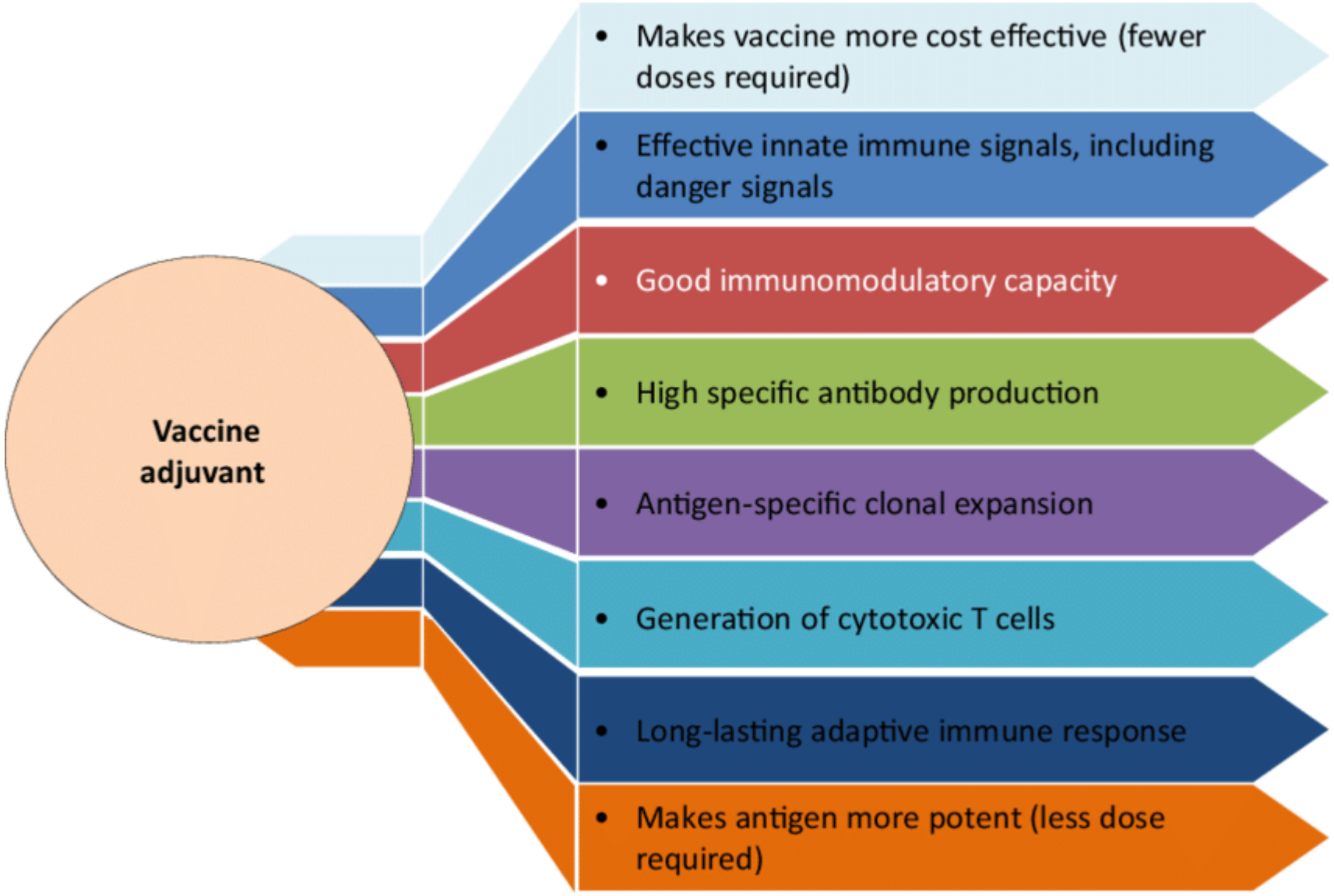
ISCOM, un complesso immunostimolatorio

ISCOM: Immunostimulating Complex Adjuvant (complesso di sostanze immunostimolanti adiuvante)

Il peptide è trasportato nel reticolo endoplasmatico

Il peptide presentato in MHC di classe I sulla superficie cellulare è riconosciuto dal linfocita T CD8+

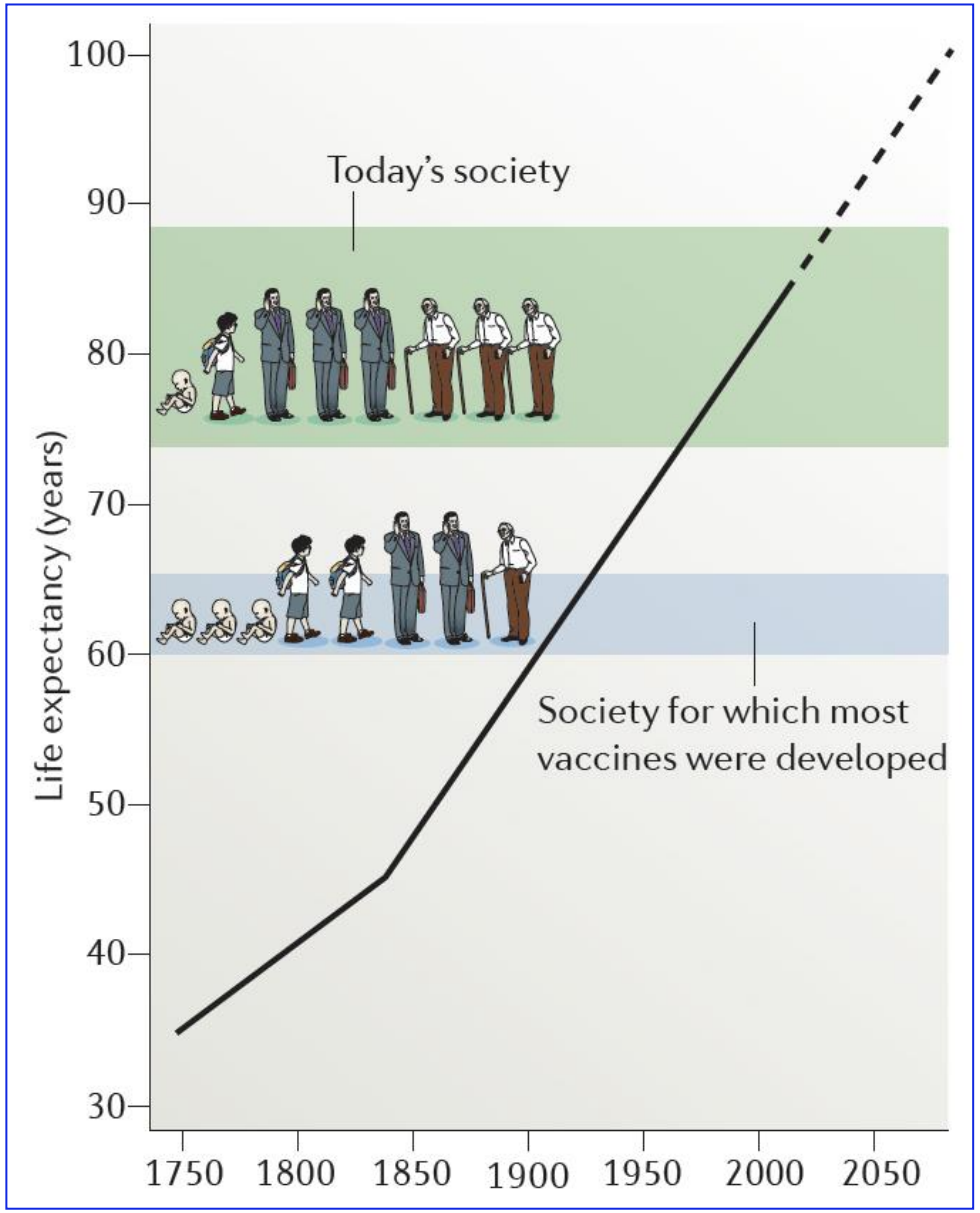




Trends in Pharmacological Sciences

Vaccines have been developed for children

With an aging society, we need a new model for health care



The average life expectancy for individuals in the society for which most vaccines were developed was 60–65 years.

This society was characterized by a high proportion of children and young people, and is quite different from today's society, which is characterized by a high proportion of elderly people and a life expectancy of more than 80 years.

Vaccini per ogni età…

a Age groups

Pre-birth
- Cytomegalovirus
- Group B streptococcus
- Hepatitis B virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pertussis
- Respiratory syncytial virus
- Tetanus

Infants and children
- Diphtheria
- Group A streptococcus
- *H. influenzae* type b
- *Helicobacter pylori*
- Hepatitis A virus
- Hepatitis B virus
- Inactivated poliovirus vaccine
- Influenza virus
- Measles
- Meningococcus serogroups A, B, C, Y and W135
- Mumps
- Pertussis
- Pneumococcus
- Respiratory syncytial virus
- Rotavirus
- Rubella
- Tetanus
- Varicella zoster virus

Adolescents
- Cytomegalovirus
- Diphtheria, tetanus acellular pertussis
- Epstein–Barr virus
- Herpes simplex virus
- Human papilloma virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Parvovirus B19

Adults
- Diphtheria
- Hepatitis B virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pertussis
- Respiratory syncytial virus
- Tetanus

Elderly

Recurrent infections:
- Group B streptococcus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pneumococcus
- Respiratory syncytial virus
- Varicella zoster virus

Antibiotic resistance:
- *Acinetobacter baumannii*
- *C. difficile*
- *Candida* spp.
- Enterotoxigenic *E. coli*
- *Klebsiella pneumoniae*
- *P. aeruginosa*
- *S. aureus*

Cancer:
- Breast cancer
- Colorectal cancer
- Prostate cancer

… e vaccini per ogni gruppo

b Special target groups

Travellers	Patients with chronic diseases	Patients with HIV	Emerging infections	Poverty
• Cholera	• Cytomegalovirus	• Influenza virus	• AIDS	• Cholera
• Dengue	• Fungal infections	• Pneumococcus	• Anthrax	• Dengue
• Enterotoxigenic *E. coli*	• Influenza virus	• Pneumocystosis	• Avian influenza	• Enterotoxigenic *E. coli*
• Hepatitis A virus	• *P. aeruginosa*	• Tuberculosis	• Cholera	• Hepatitis A virus
• Hepatitis B virus	• Parainfluenza		• Dengue	• Hepatitis B virus
• Influenza virus	• Parvovirus B19		• Diphtheria	• Hepatitis E virus
• Japanese encephalitis virus	• Respiratory syncytial virus		• Ebola virus disease	• Influenza virus
• Malaria	• *S. aureus*		• EV71	• Japanese encephalitis virus
• Meningococcus serogroups A, B, C, Y, W135 and X	• Tuberculosis		• Malaria	• Malaria
• Paratyphoid fever			• Meningococcus serogroup X	• Meningococcus serogroups A, B, C, Y, W135 and X
• Rabies			• Plague	• Parasitic infections
• *Shigella* spp.			• SARS	• Paratyphoid fever
• Tick-borne encephalitis virus			• Smallpox	• Rabies
• Tuberculosis			• Swine influenza	• Rotavirus
• Typhoid fever			• Tuberculosis	• *Salmonella* spp.
• Yellow fever			• West Nile	• *Shigella* spp.
				• Tuberculosis
				• Typhoid fever
				• Yellow fever

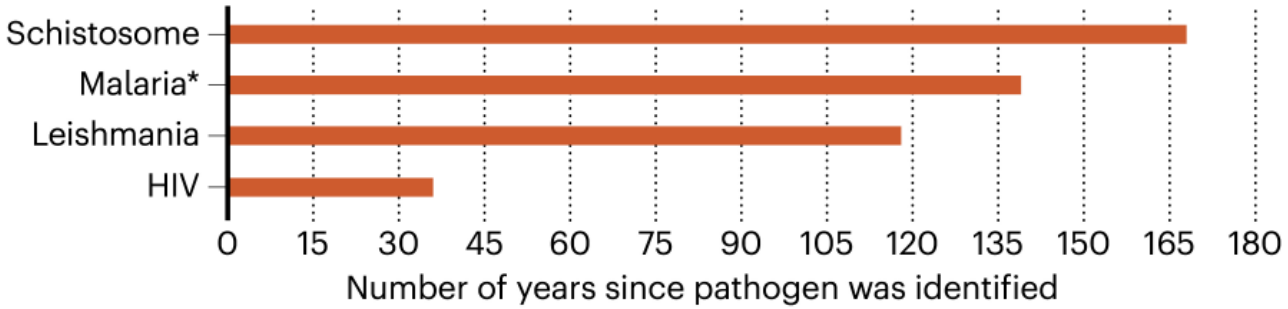
Nature Reviews | Immunology

… e non ancora disponibili!

VIRAL	*BACTERIAL*	*PARASITIC*
HIV	*Leprosis*	*Ascaris*
Cytomegalovirus	*Gonorrhea*	*Malaria*
Herpes simplex	*Urinary infections*	
		Schistosomes
Genital herpes	*Enterotoxigenic E. Coli*	*Hookworm*
HCV	*Streptococcus pyogenes*	*Trichuris*
Parainfluenza	*Streptococcus mutans*	*Filarias*
Respiratory syncytial virus	*Streptococcus agalactiae*	*Giardia*
Rotavirus	*Pseudomonas*	*Leishmania*
Ebola virus		
Zika virus	*Shigella*	
	Campylobacter	
Coronavirus (SARS)	*Lyme disease*	
	H. pylori	
	S. aureus	
	Chlamydia	
	MenB	
	Klebsiella	

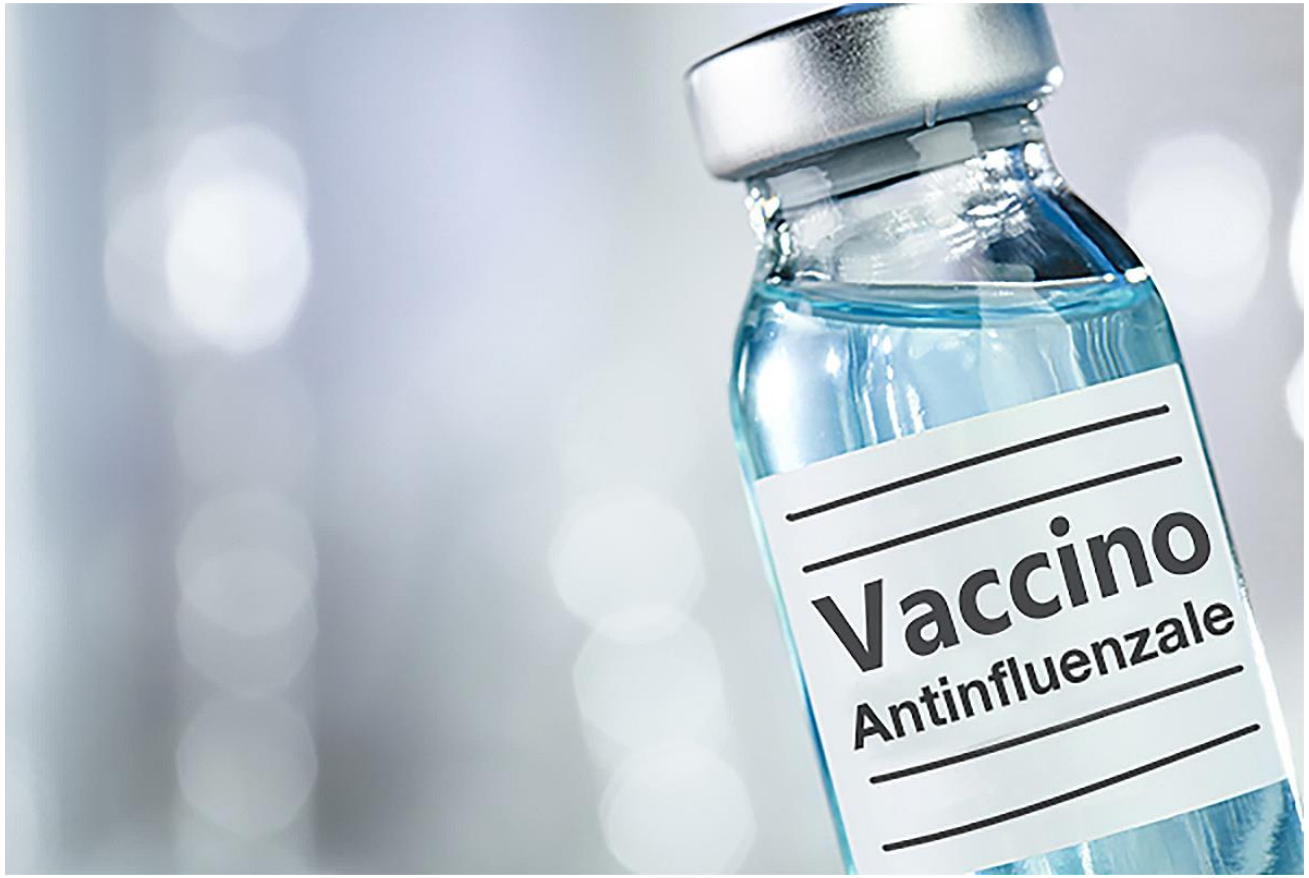
Some diseases for which effective vaccines are not yet available		
Disease	**Estimated annual mortality**	**Estimated annual incidence**
Malaria*	1,124,000	300–500 million
Schistosomiasis	15,000	no numbers available
Worm infestation	12,000	no numbers available
Tuberculosis	1,644,000	~8 million
Diarrheal disease	2,001,000	~4,100 million
Respiratory disease	3,947,000	~362 million
HIV/AIDS	2,866,000	~2 million
Measles†	745,000	~44 million

Figure 14-22 Immunobiology, 6/e. (© Garland Science 2005)



~1.5 MILLION
deaths from these four diseases in 2016.

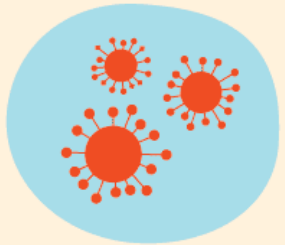
*A pilot study of a malaria vaccine began in three countries in 2019.



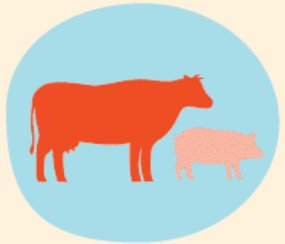
I vaccini anti-influenzali

Different Types of Flu

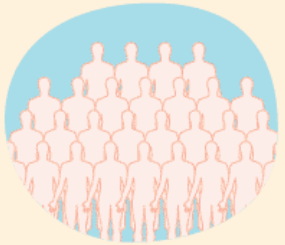
Seasonal Flu



Influenza C



Influenza D



Flu Pandemic

Influenza A

H1N1 Swine Flu

Influenza B

H5N1 Bird Flu

I virus influenzali

- Esistono quattro tipi di virus influenzali: **A, B, C and D.**

- I virus dell'influenza umana **A e B** causano epidemie stagionali di malattia quasi ogni inverno negli Stati Uniti (e in Europa).

- I **virus dell'influenza A** sono gli unici virus influenzali noti per causare pandemie influenzali (epidemie globali di malattia influenzale). Una pandemia può verificarsi quando emerge un virus dell'influenza A nuovo e molto diverso, in grado di infettare e diffondersi in modo efficiente tra le persone.

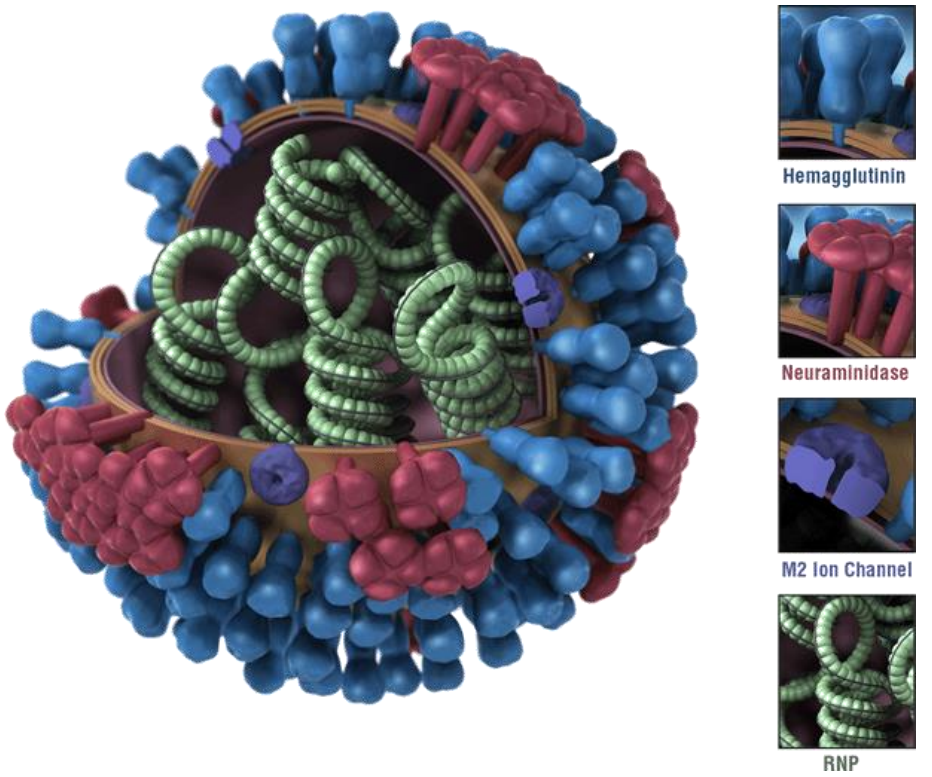
- **I virus dell'influenza di tipo C** generalmente causano malattie lievi e non si ritiene che causino epidemie di influenza umana.

- I **virus dell'influenza di tipo D** colpiscono principalmente i bovini e non sono noti per infettare o causare malattie nell'Uomo.

Fonte: CDC, Atlanta, USA

I virus influenzali

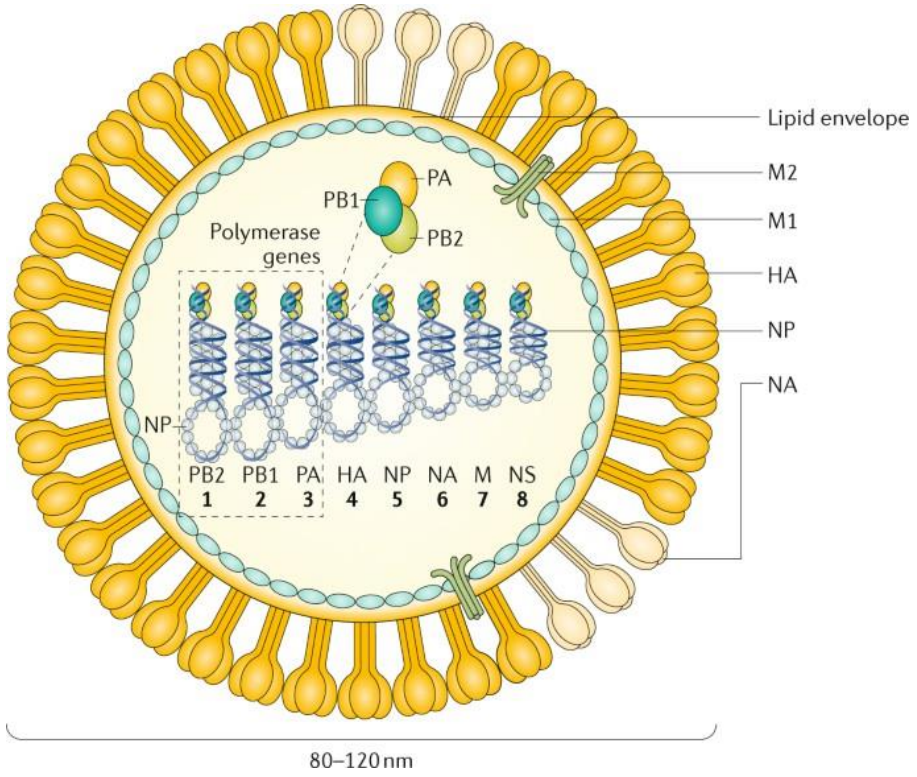
- I virus dell'influenza A sono suddivisi in sottotipi, in base a due proteine della superficie del virus: emagglutinina (H) e neuraminidasi (N).
- Esistono 18 sottotipi diversi di emagglutinina e 11 di neuraminidasi (H1-18 e N1-11).
- Sebbene esistano potenzialmente 198 diverse combinazioni di sottotipi di influenza A (18x11), in natura ne sono stati rilevati solo 131.
- Attualmente, i sottotipi di virus dell'influenza A che circolano abitualmente tra gli individui includono: A (H1N1) e A (H3N2).
- I sottotipi di influenza A possono essere ulteriormente suddivisi in diversi *clade* e *subclade* genetici (o «gruppi» e «sottogruppi»).



HA: glicoproteina espressa sulla superficie del virus. Responsabile del legame all'acido sialico (o acido neuraminico) della cellula (e quindi dell'ingresso del virus). Media anche la fusione in endosomi-lisosomi e il rilascio del genoma del virus all'interno della cellula.

NA: enzima che taglia i gruppi di acido sialico dalle glicoproteine a cui si è attaccata HA. Media l'ingresso del virus nella cellula.

Influenza virus & its antigenic variation



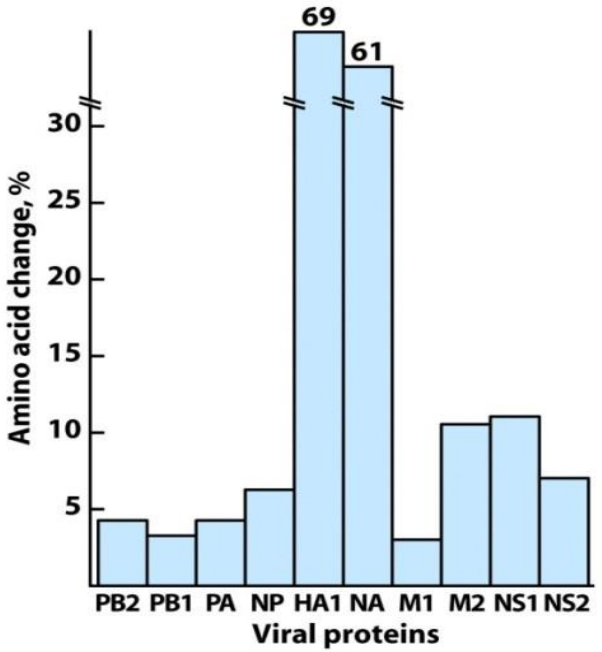
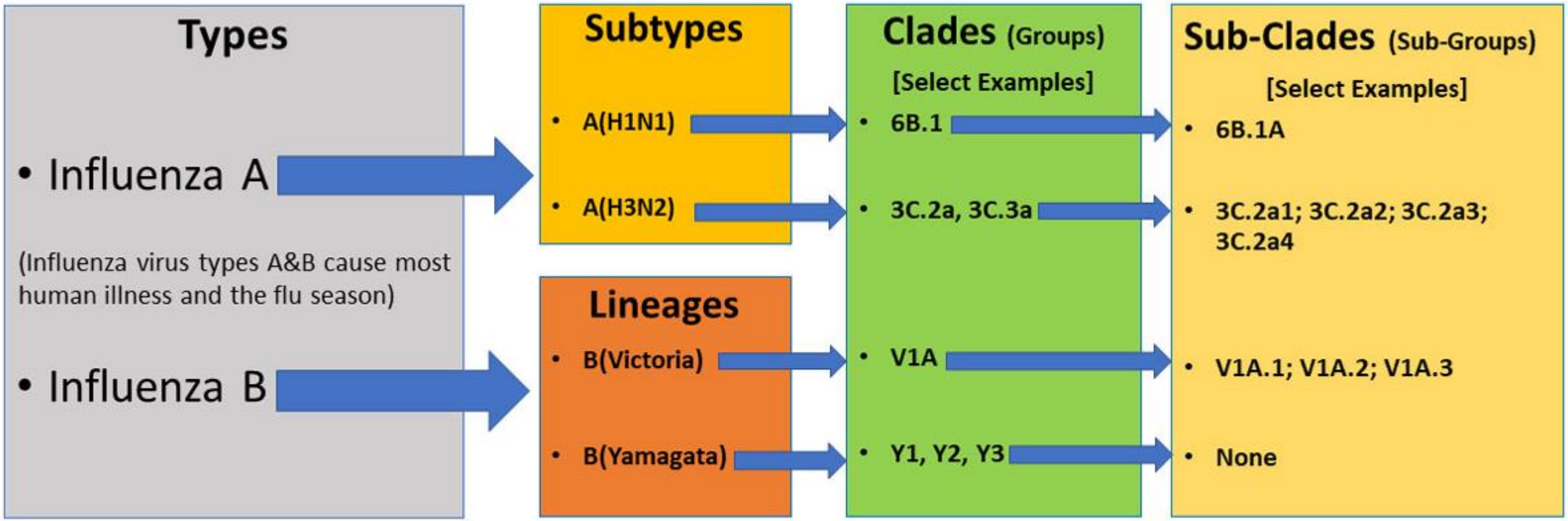


TABLE 18-2 Some influenza A strains and their hemagglutinin (H) and neuraminidase (N) subtype

Species	Virus strain designation	Antigenic subtype
Human	A/Puerto Rico/8/34	H0N1
	A/Fort Monmouth/1/47	H1N1
	A/Singapore/1/57	H2N2
	A/Hong Kong/1/68	H3N2
	A/USSR/80/77	H1N1
	A/Brazil/11/78	H1N1
	A/Bangkok/1/79	H3N2
	A/Taiwan/1/86	H1N1
	A/Shanghai/16/89	H3N2
	A/Johannesburg/33/95	H3N2
	A/Wuhan/359/95	H3N2
	A/Texas/36/95	H1N1
	A/Hong Kong/156/97	H5N1
Swine	A/Sw/Iowa/15/30	H1N1
	A/Sw/Taiwan/70	H3N2
Horse (equine)	A/Eq/Prague/1/56	H7N7
	A/Eq/Miami/1/63	H3N8*
Bird	A/Fowl/Dutch/27	H7N7
	A/Tern/South America/61	H5N3
	A/Turkey/Ontario/68	H8N4
	A/Chicken/Hong Kong/258/97	H5N1†

*H3N8 has recently been shown to cause flu-like illness in dogs; the species shift occurred with no reassortment of genes.
†As of 2006, a dangerous new H5N1 avian strain has infected approximately 175 humans with 50% mortality.

I virus influenzali stagionali umani



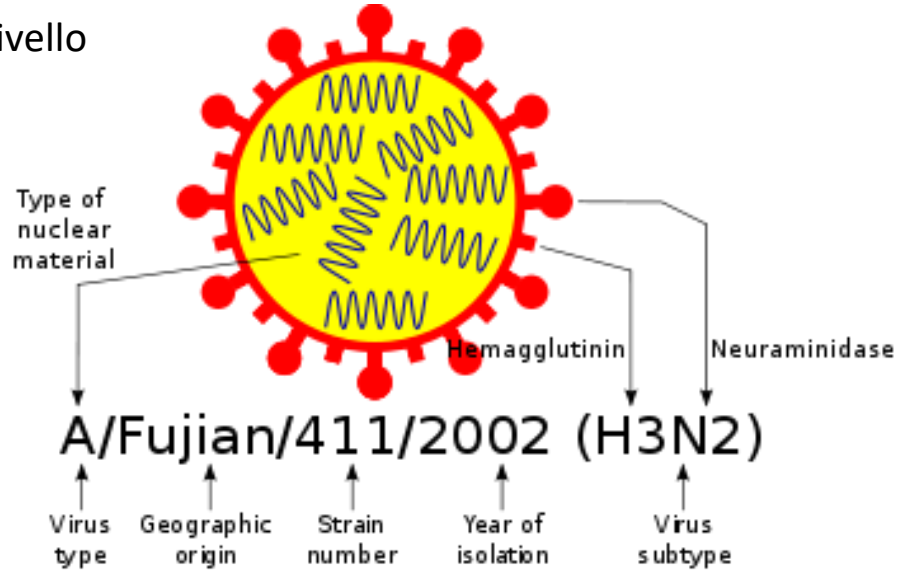
Esistono due tipi di virus influenzali (A, B) che nell'Uomo causano la maggior parte delle malattie e che sono responsabili della stagione influenzale ogni anno:

- I virus dell'influenza A, ulteriormente classificati in sottotipi
- I virus dell'influenza B, ulteriormente classificati in due «linee»: B/Yamagata e B/Victoria.
- Entrambi i virus dell'influenza A e B possono essere ulteriormente classificati in gruppi e sottogruppi.

Denominare i virus influenzali

- Il *Center for Disease Control* (CDC, Atlanta, USA) segue una convenzione riconosciuta a livello internazionale per la denominazione dei virus influenzali. Questa convenzione è stata accettata dall'OMS nel 1979.

- L'approccio utilizza i seguenti aspetti:



- Il **tipo antigenico** (es., A, B, C, D)
- L'**ospite di origine** (es., suini, equini, polli, ecc.). Per i virus di origine umana, non viene fornita alcuna denominazione di origine. Per esempio:

(*Esempio anatra): influenza aviaria A (H1N1), A / duck / Alberta / 35/76*
(Esempio umano): influenza stagionale A (H3N2), A / Perth / 16/2019

- **Origine geografica** (es. Denver, Taiwan, Sidney, ecc.)
- **Numero di ceppo** (es. 7, 15, ecc.)
- **Anno di raccolta** (es., 57, 2009, ecc.)
- Per i **virus dell'influenza A**, la descrizione degli antigeni H ed N è fornita tra parentesi (es.: virus dell'influenza A(H1N1) o virus dell'influenza A(H5N1))
- Al **virus pandemico del 2009** è stato assegnato un nome distinto: **A(H1N1)pdm09** per distinguerlo dai virus dell'influenza stagionale A(H1N1) che circolavano prima della pandemia.
- Quando gli esseri umani sono infettati da virus influenzali che normalmente circolano nei suini (suini), questi virus sono chiamati **virus varianti** e sono designati con una lettera **"v"** (ad esempio: un virus A (H3N2)v).

Circulating Influenza Viruses

Influenza A

- **I virus dell'influenza A (H1N1) attualmente in circolazione sono correlati al virus pandemico H1N1 del 2009** che è emerso nella primavera del 2009 e ha causato una pandemia influenzale. Questo virus, scientificamente chiamato **"virus A (H1N1) pdm09"** o più generalmente **"2009 H1N1",** da allora ha continuato a circolare stagionalmente. Questi virus H1N1 hanno subito nel tempo cambiamenti genetici e antigenici relativamente piccoli.

- Di tutti i virus influenzali che circolano abitualmente e causano malattie nell'Uomo, i **virus dell'influenza A (H3N2)** tendono a cambiare più rapidamente, sia geneticamente che antigenicamente. Negli ultimi anni questi virus hanno formato molti gruppi separati e geneticamente differenti, che continuano a «co-circolare».

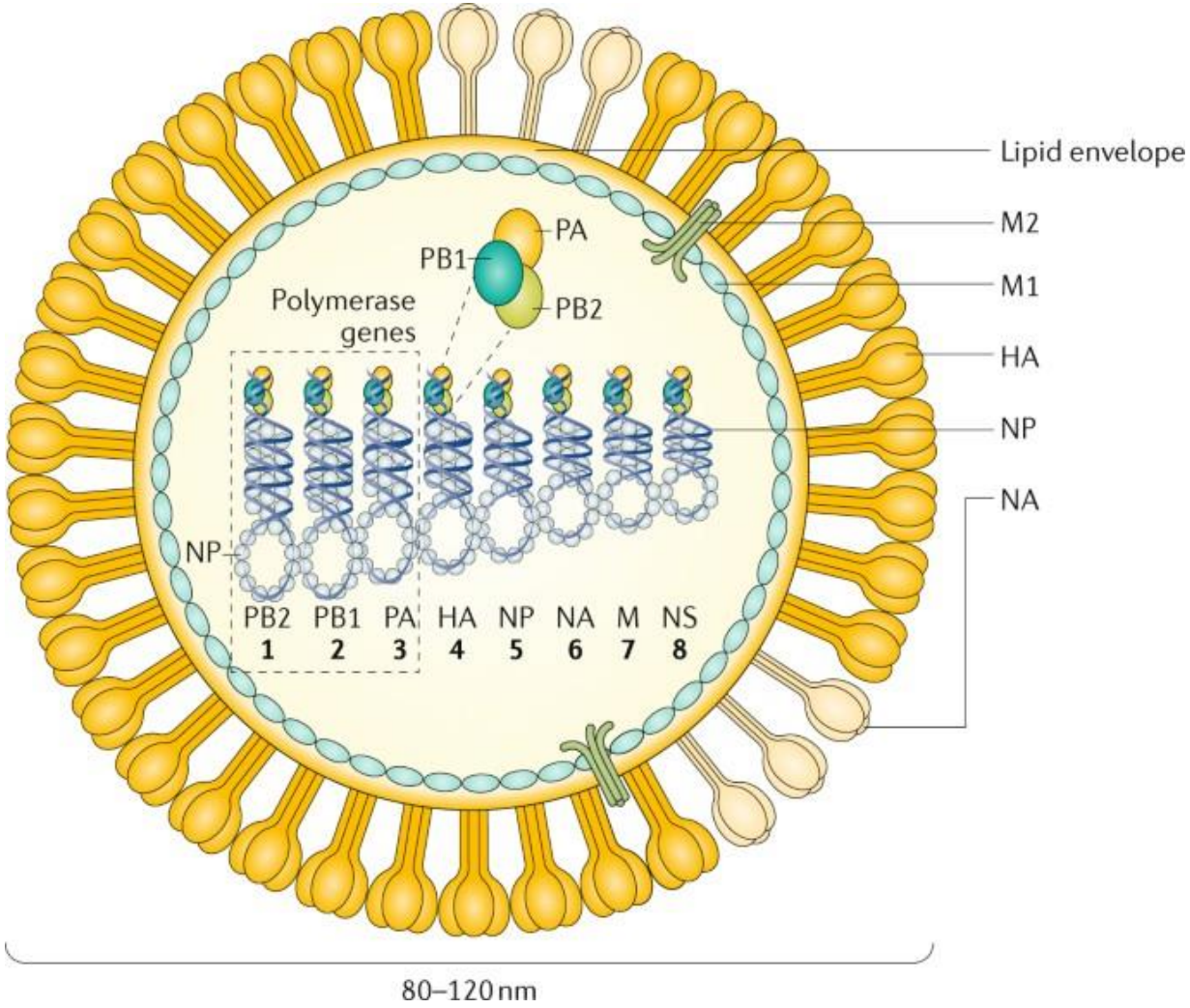
Influenza B

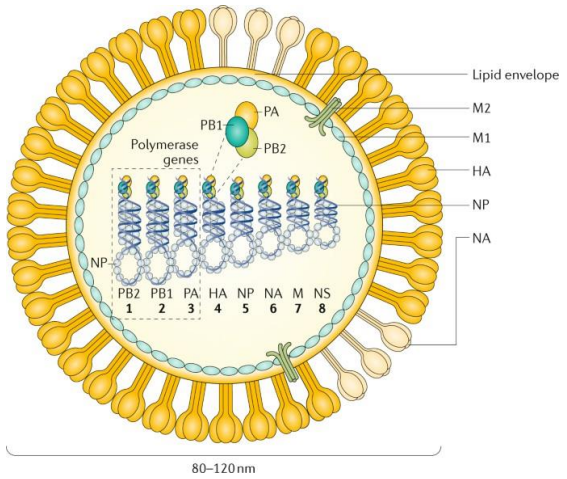
- I **virus dell'influenza B** non sono suddivisi in sottotipi, ma sono classificati in due linee: **B/Yamagata e B/Victoria**. Analogamente ai virus dell'influenza A, i virus dell'influenza B possono essere ulteriormente classificati in gruppi e sottogruppi.

- I virus dell'influenza B cambiano più lentamente in termini di proprietà genetiche e antigeniche rispetto ai virus dell'influenza A, ed in particolare ai virus dell'influenza A (H3N2).

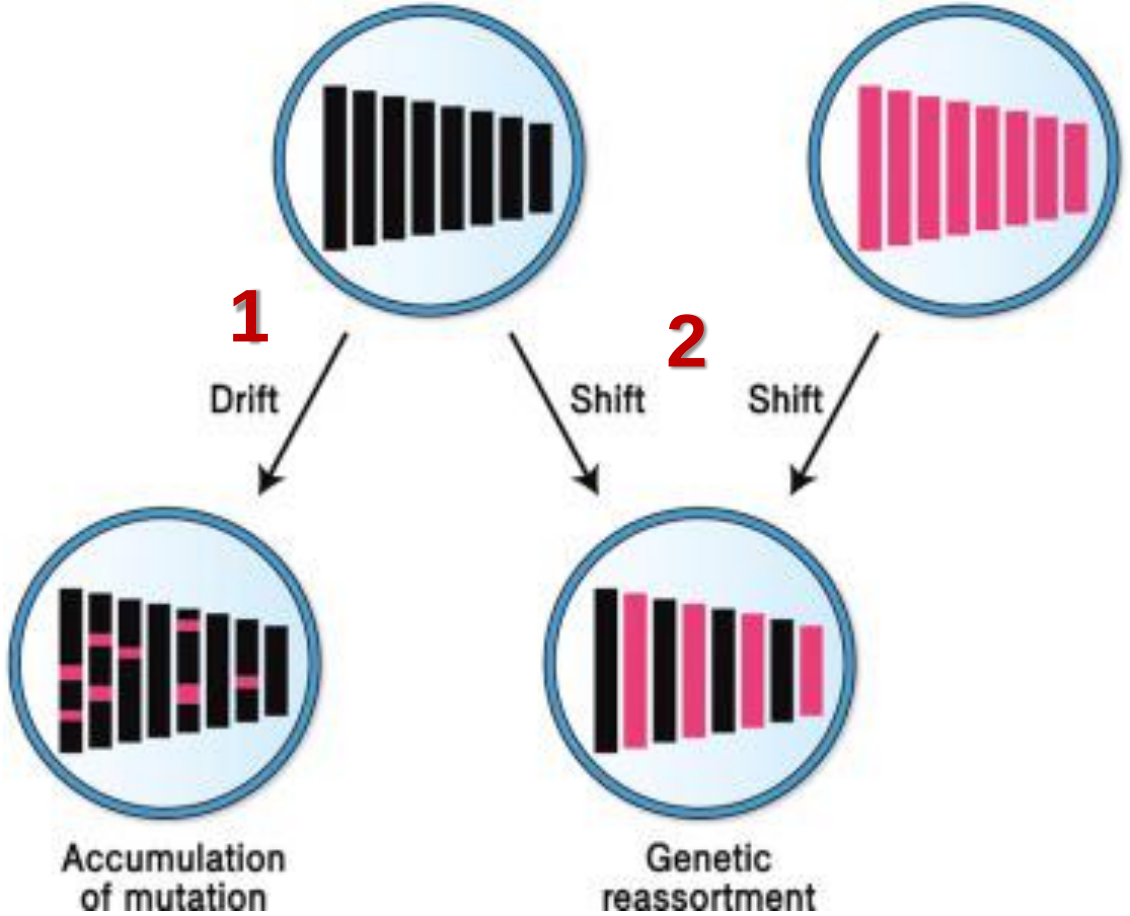
- I dati degli ultimi anni sulla sorveglianza dell'influenza mostrano la co-circolazione dei virus dell'influenza B di entrambe le linee negli Stati Uniti e in tutto il mondo. Tuttavia, la percentuale di virus dell'influenza B di ciascuna linea in circolazione può variare in base alla posizione geografica.

Le mutazioni nei virus dell'influenza





Le mutazioni nei virus dell'influenza



MUTAZIONI

1: la deriva antigenica

(antigenic drift)

Il virus dell'influenza infetta rapidamente molte persone.

Poiché l'immunità protegge dalla re-infezione, il virus esaurirà rapidamente gli ospiti da infettare.

"La soluzione è mutare in modo tale che ogni anno vengano presentati nuovi antigeni".

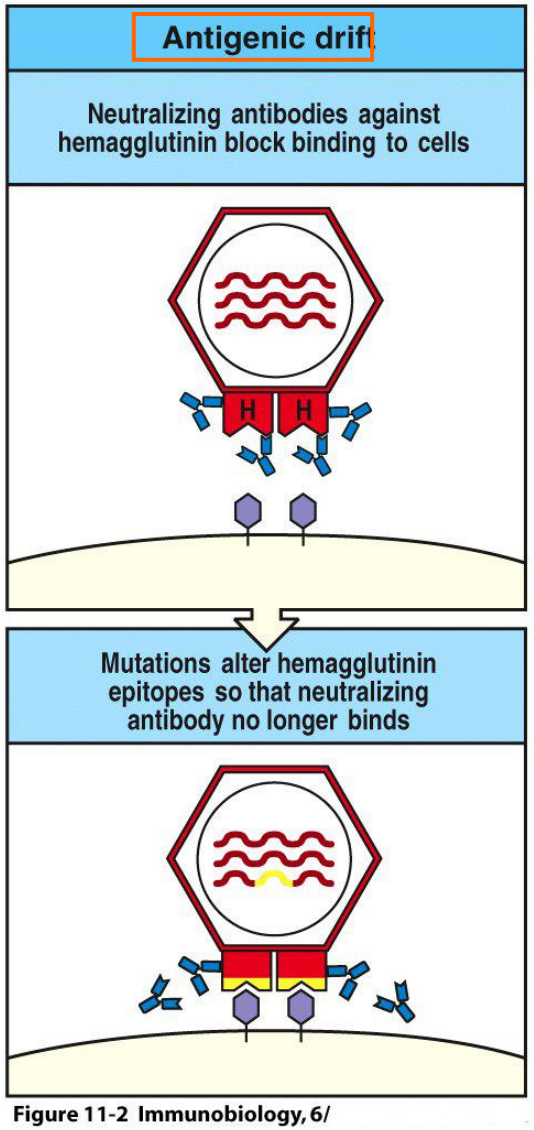


Figure 11-2 Immunobiology, 6/

La **deriva antigenica** lascia alcuni epitopi del ceppo precedente. Pertanto, una precedente esposizione (immunità) a ceppi correlati può mitigare la gravità della malattia e dei sintomi ...

..ma consente comunque al virus di replicarsi e diffondersi.

SCAMBIO DI SEGMENTI GENICI

2: lo shift antigenico

(antigenic shift)

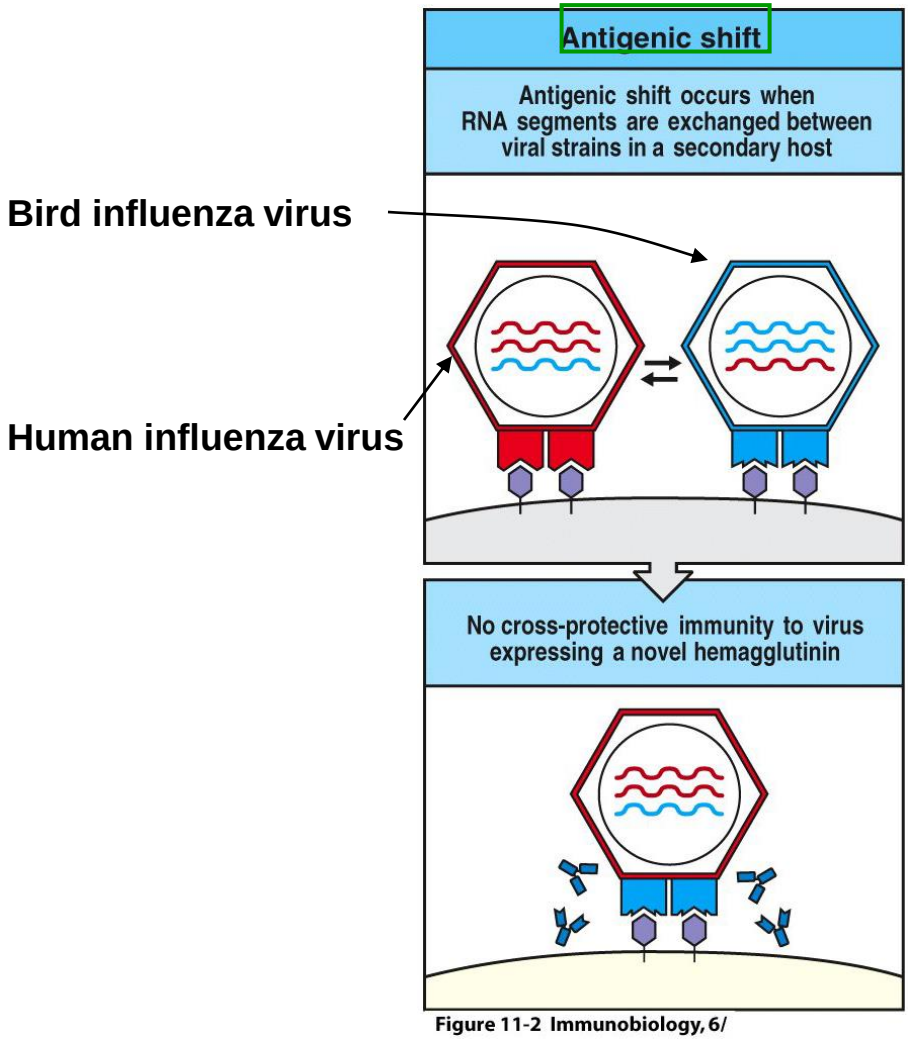


Figure 11-2 Immunobiology, 6/

Lo **shift antigenico** è facilitato dalla divisione del genoma in 8 segmenti. E può avvenire durante una co-infezione con due ceppi diversi (ad es. nei suini e nell'Uomo).

Può creare virus che non condividono epitopi con ceppi precedenti (nessuna reazione crociata).

Pertanto, non esiste una protezione parziale derivante dalle infezioni precedenti, e la malattia è molto più grave rispetto agli anni in cui c'è solo deriva antigenica.

La pandemia influenzale del **1918-19** ha ucciso > 40.000.000 ... la peggiore pandemia del secolo scorso.

Cambiamenti antigenici importanti anche **nel 1957 e nel 1968.**

1. DERIVA ANTIGENICA

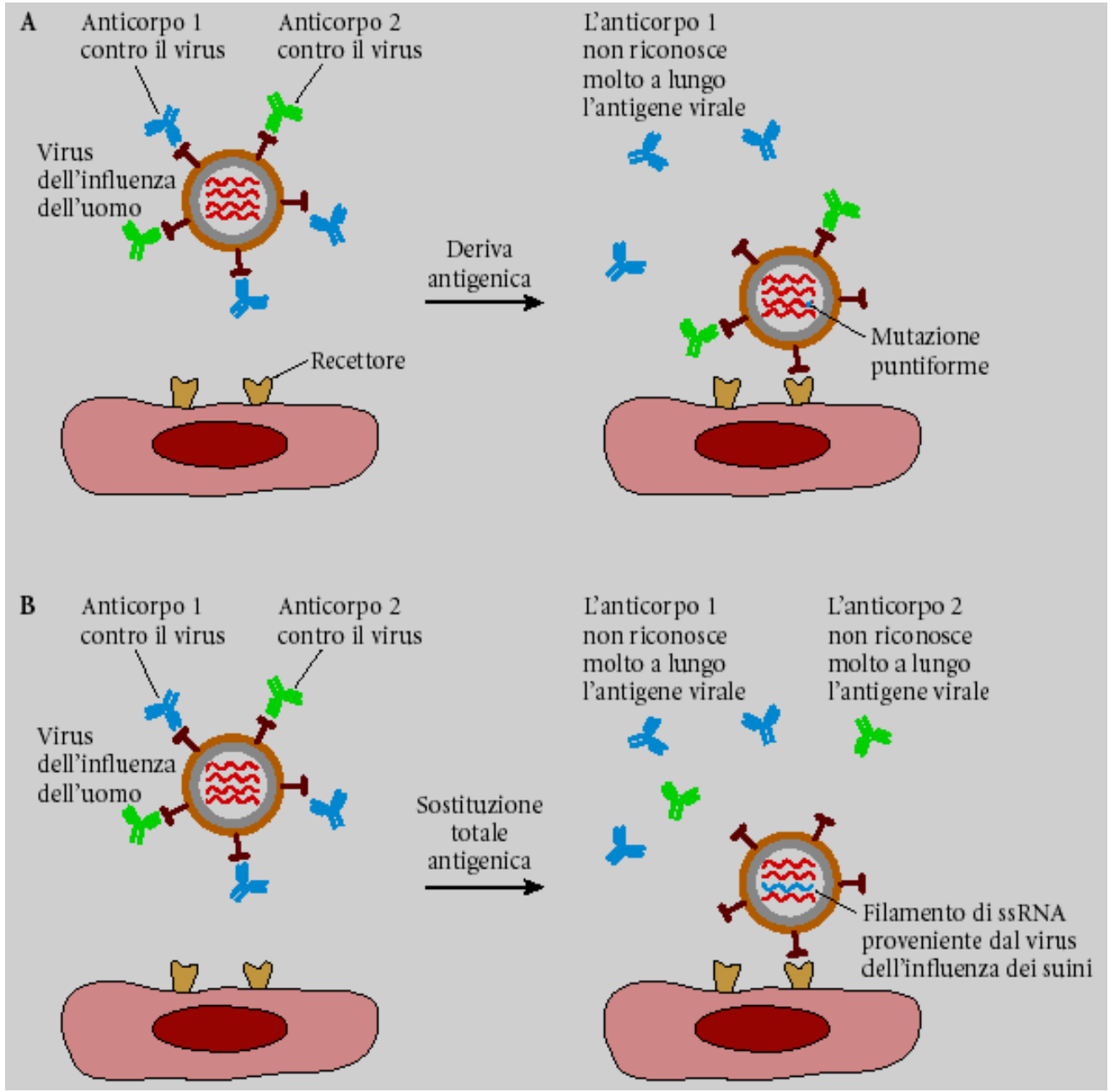
(ANTIGENIC DRIFT)

(mutazioni di epitopi)

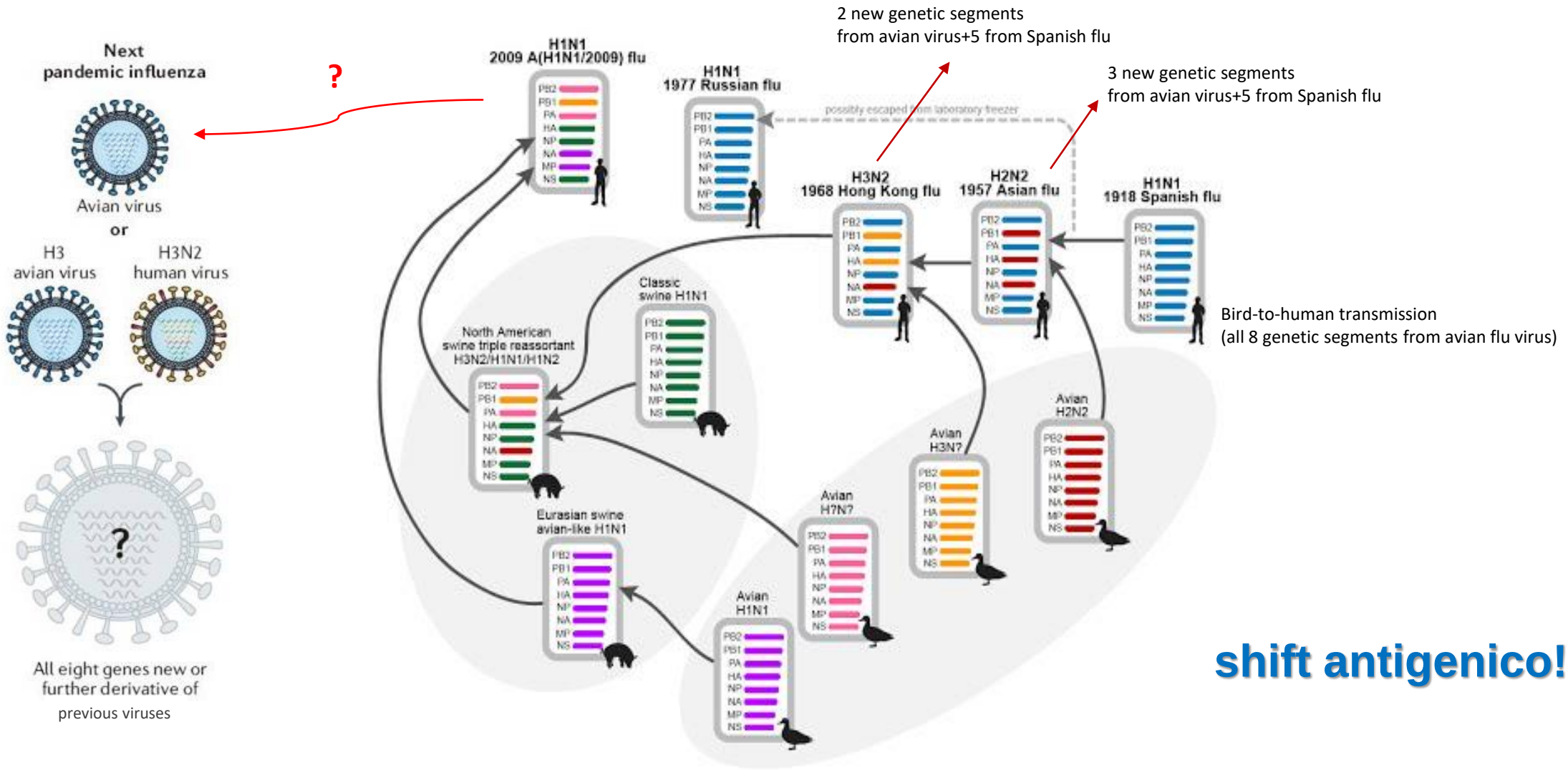
2. SHIFT ANTIGENICO

(ANTIGENIC SHIFT)

(mutazioni radicali per scambio di segmenti genici)

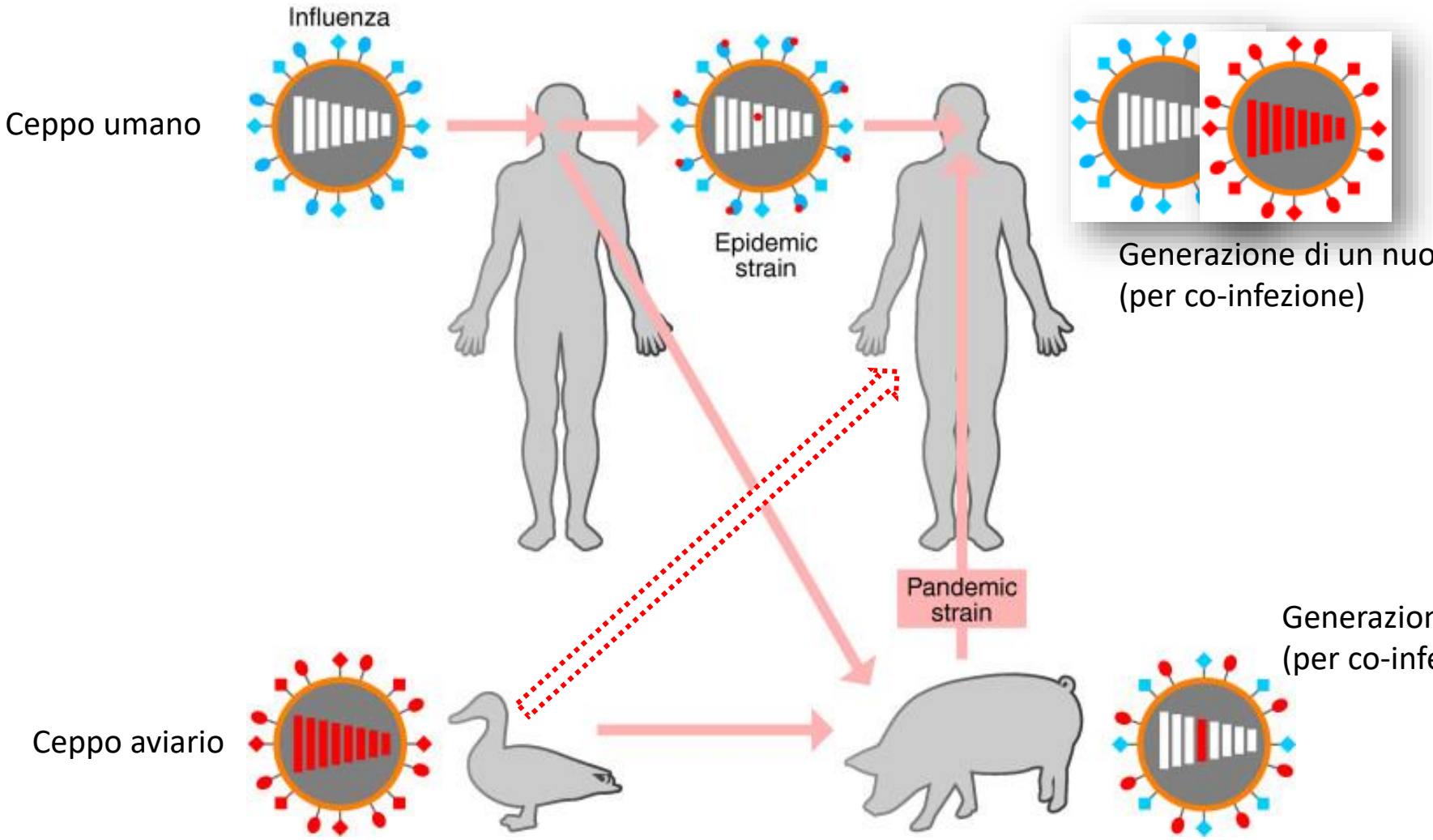


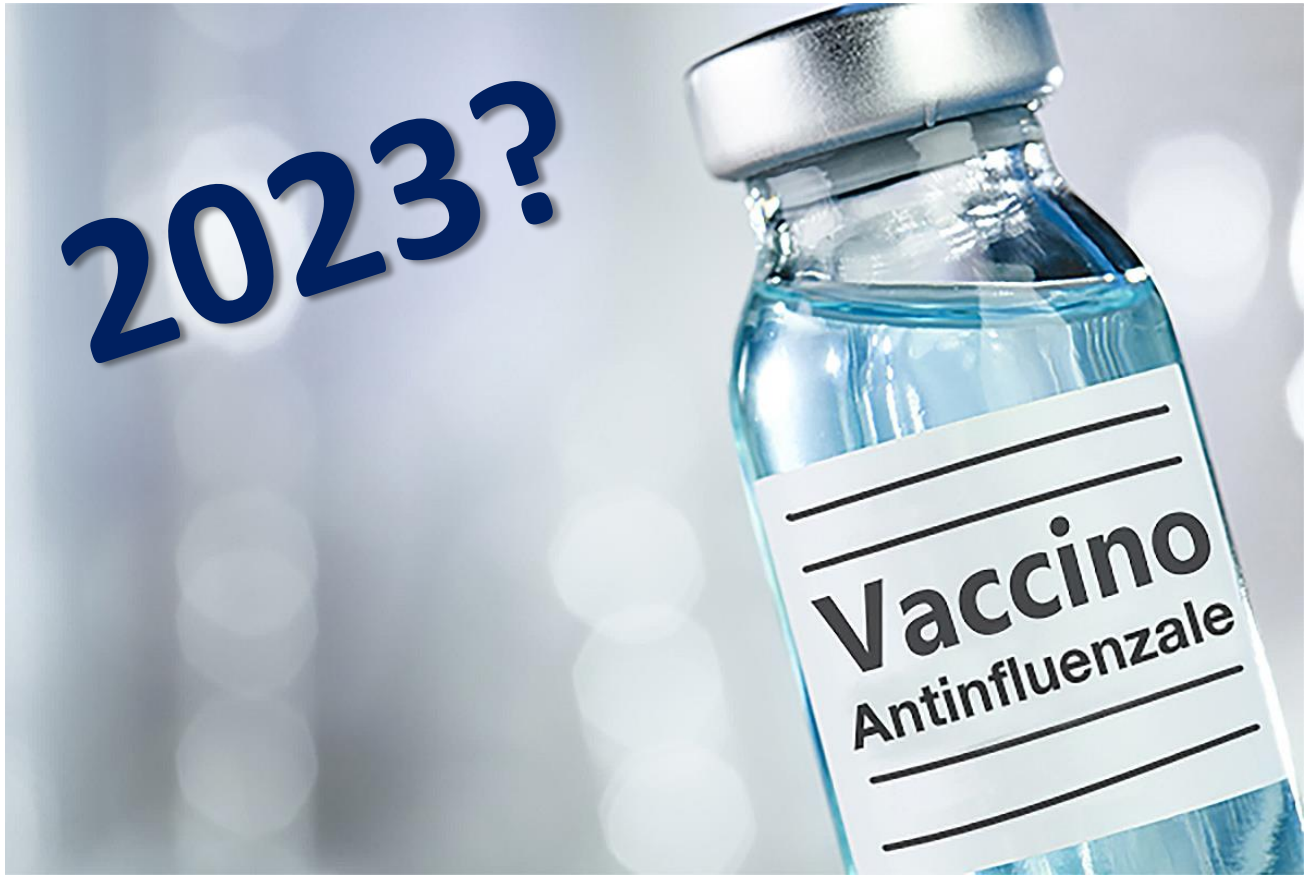
Storia del riassortimento genetico dei ceppi di virus influenzali pandemici



shift antigenico!

Generazione di un ceppo influenzale potenzialmente pandemico attraverso il riassortimento dei geni tra ceppi aviari e umani (shift antigenico)





I vaccini anti-influenzali