COVID-19 Vaccine Platforms:
Strengths &Opportunities

Dr. Manish Gautam
Serum Institute of India Pvt Ltd
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Disclaimer

- *I work for Serum Institute of India Pvt Ltd.*
- *Views and opinions provided in this presentation is of author and not of the employer.*

Objectives

- Introduction to COVID-19
- Overview of vaccine platforms: Development of vaccines for SARS-CoV-2
- Variants of SARS-CoV-2
- India’s response to COVID-19

Introduction to COVID-19

- December, 2019, a new disease of unknown aetiology appeared in Wuhan, China
- Quickly identified as a novel beta coronavirus, and related to severe acute respiratory syndrome (SARS)- a zoonotic disease.
- Global spread and declared a Public Health Emergency by WHO on 30 January 2020
- Jan 2020- Whole genome “2019-nCoV”
- 7 Jan 2021:

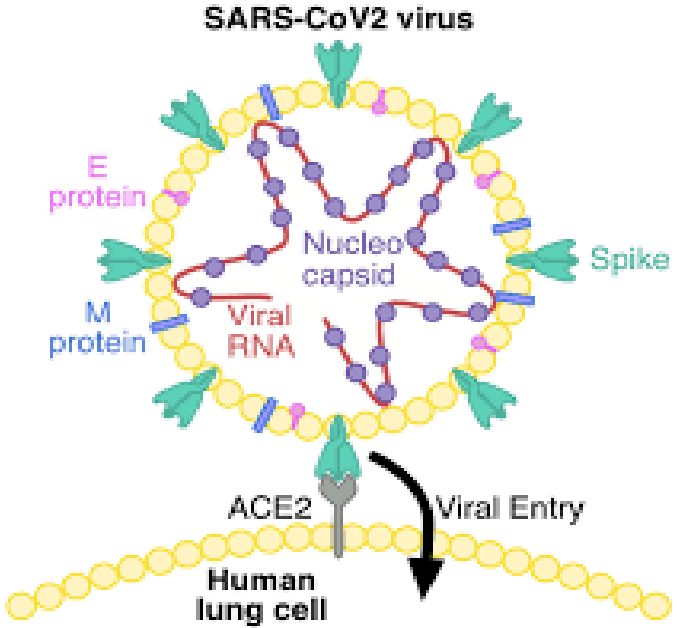
Cases	Recovered	Deaths
89M	49.5M	1.92M

SARS-CoV, SARS-CoV-2 and MERS-CoV

	SARS-CoV	SARS-CoV-2	MERS-CoV
Year of 1st reported case	2002	2019	2012
Country/ region of first reported case	China	China	Middle east
Natural reservoirs	Chinese horseshoe bats	Unclear (possibly bats)	Camels (possibly bats)
Intermediate host	Civet cats	Debatable(possibly pangolins)	Dromedary camels
Primary mode of transmission	Droplet, aerosol, contact	Droplet, aerosol, contact	Droplet, aerosol, contact
Incubation period	2-7 days	2-14 days	2-14 days
Host receptor	ACE2	ACE2	DPP4
Pandemic or epidemic	Spread over 29 countries	Spread over 218 countries and territories around the world	Spread over 27 countries
Virus type	Betacoronavirus	Betacoronavirus	Betacoronavirus
No of ppl affected	~8,000 infections and 774 deaths	88,455,476 infections and 1,905,173 deaths (increasing)	2,519 infections and 866 deaths
Vaccines available	Few vaccines were developed – Phase1	Few licenced, numerous at clinical and pre-clinical stages	No vaccine or specific treatment available (DNA, Non replicating viral vaccines are at development stage)

Structure of SARS-CoV-2

- Spherical, enveloped, positive stranded RNA virus with diameter of ~125 nm
- Spike club-shaped projections emerging from the surface of the virions is characteristic feature
- Primary structural proteins are the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (all coded inside the viral genome's 3′end)
- Spike protein- Immunodominant protein
- Spike protein- Target for neutralization antibodies



Pathogenesis of SARS-CoV-2

- SARS-CoV primarily infects the epithelial cells within the lung
- Not only the lungs; SARS-CoV-2 also binds and affects

  -Digestive system (via ACE-2 receptor)

  -Urogenital system (via ACE2 receptor in renal-tubular cells, mesenchymal cells)

  -Central nervous system

  -Circulatory system
- The virus can invade macrophages and dendritic cells but and activates pro-inflammatory cytokines that can lead to disease
- The exact mechanism of lung damage and the cause of serious illness remains undetermined in humans
- The S proteins (S1, S2, RBD) and the SARS-CoV-2 N protein during infection are the two most immunogenic and predominantly expressed proteins

Pandemics and vaccine development

- Scientific community and the vaccine industry have responded urgently to pandemics of H1N1 influenza, Ebola, Zika, and now SARS-CoV-2.
- H1N1 influenza vaccine was developed relatively rapidly, largely because influenza-vaccine technology was well developed.
- Key regulators had previously decided that vaccines made using egg- and cell-based platforms could be licensed under the rules used for a strain change.
- SARS and Zika epidemics ended before vaccine development was complete, leaving manufacturers with financial losses and setting back other vaccine-development programs.

World Health Organization

R&DBlueprint
Powering research to prevent epidemics

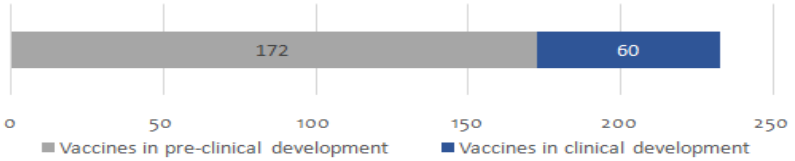
COVID-19 - Landscape of novel coronavirus candidate vaccine development worldwide

29 December 2020

Summary Information on Vaccine Products in Clinical Development

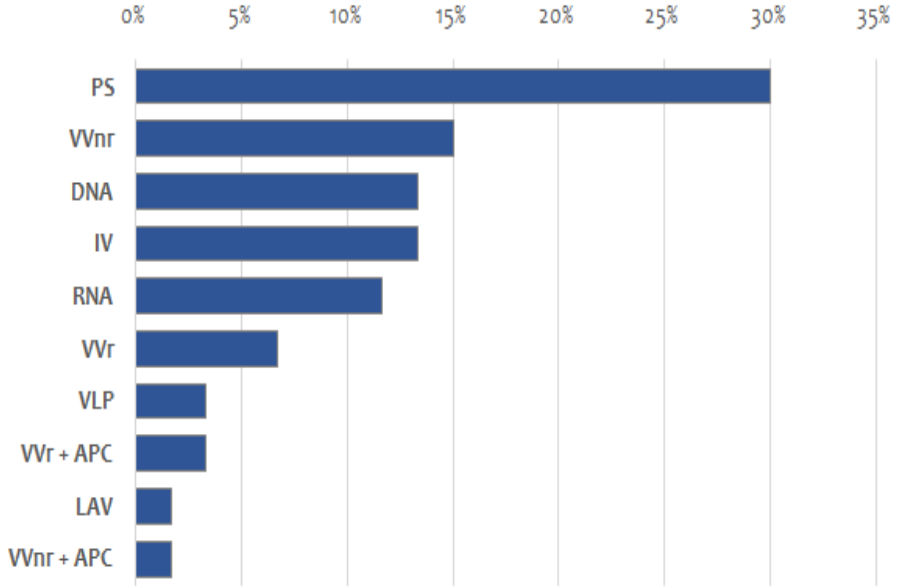
1. - Number of vaccines in clinical development: 60

2. - Number of vaccines in pre-clinical development: 172

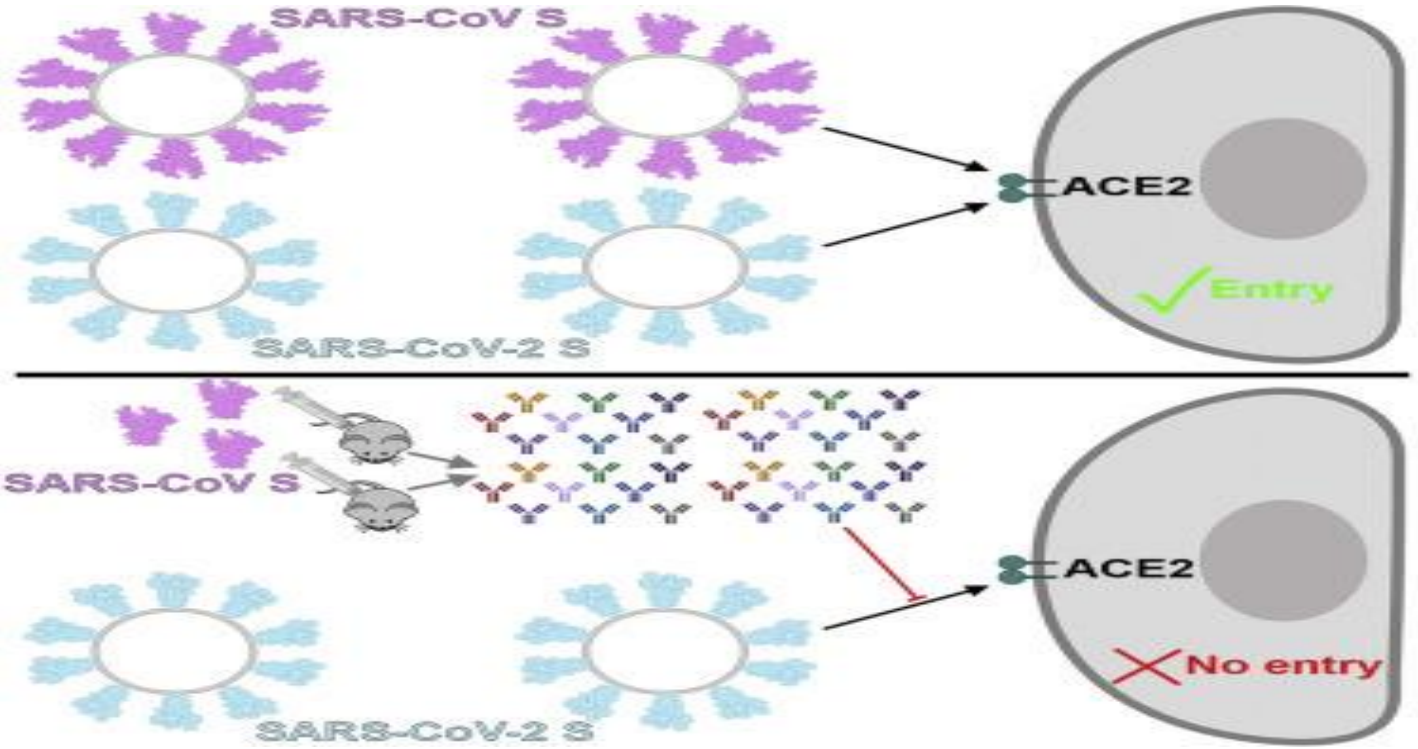


3. - Candidates in clinical phase

Platform		Candidate vaccines (no. and %)	
PS	Protein subunit	18	30%
VVnr	Viral Vector (non-replicating)	9	15%
DNA	DNA	8	13%
IV	Inactivated Virus	8	13%
RNA	RNA	7	12%
VVr	Viral Vector (replicating)	4	7%
VLP	Virus Like Particle	2	3%
VVr + APC	VVr + Antigen Presenting Cell	2	3%
LAV	Live Attenuated Virus	1	2%
VVnr + APC	VVnr + Antigen Presenting Cell	1	2%
		60	



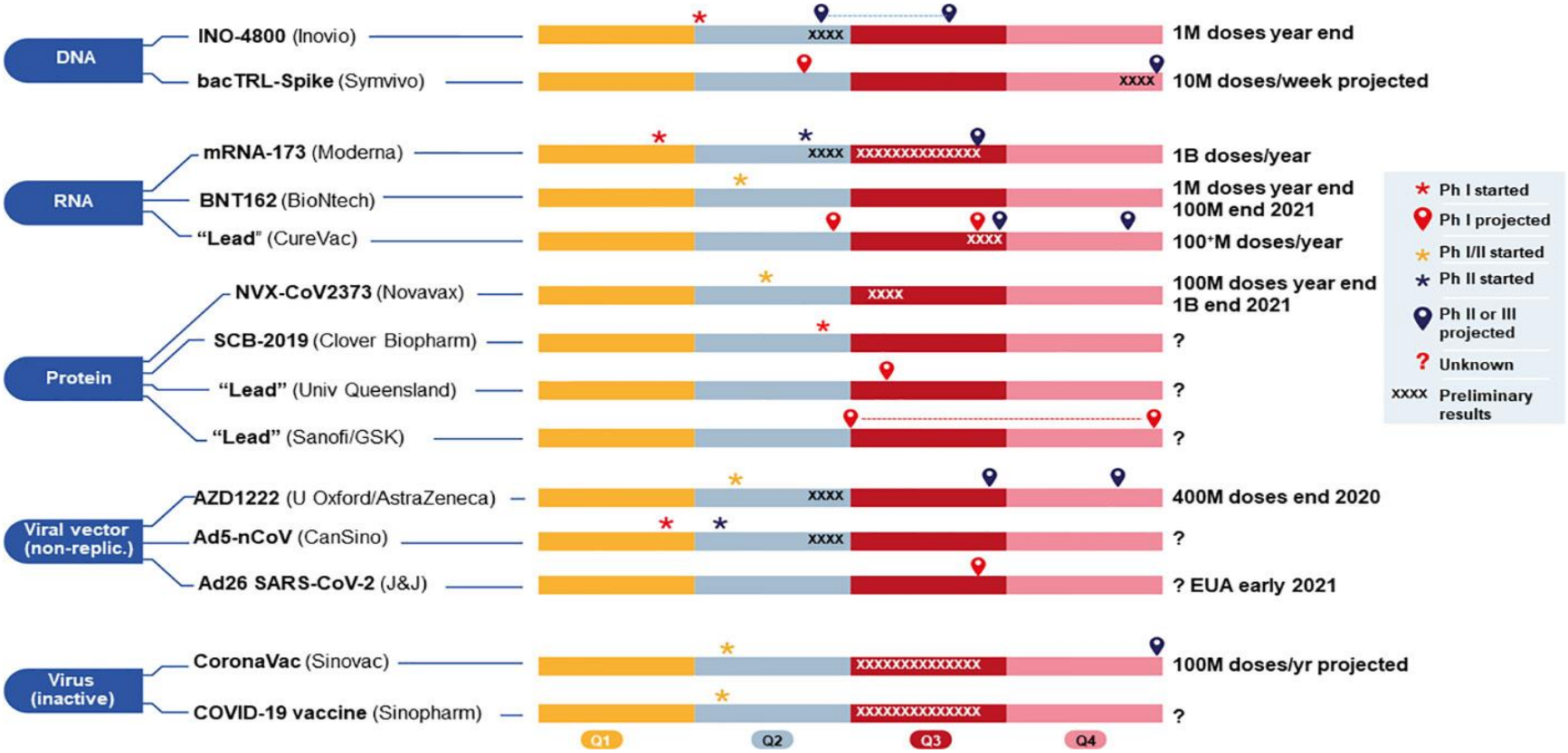
Spike protein: Key target



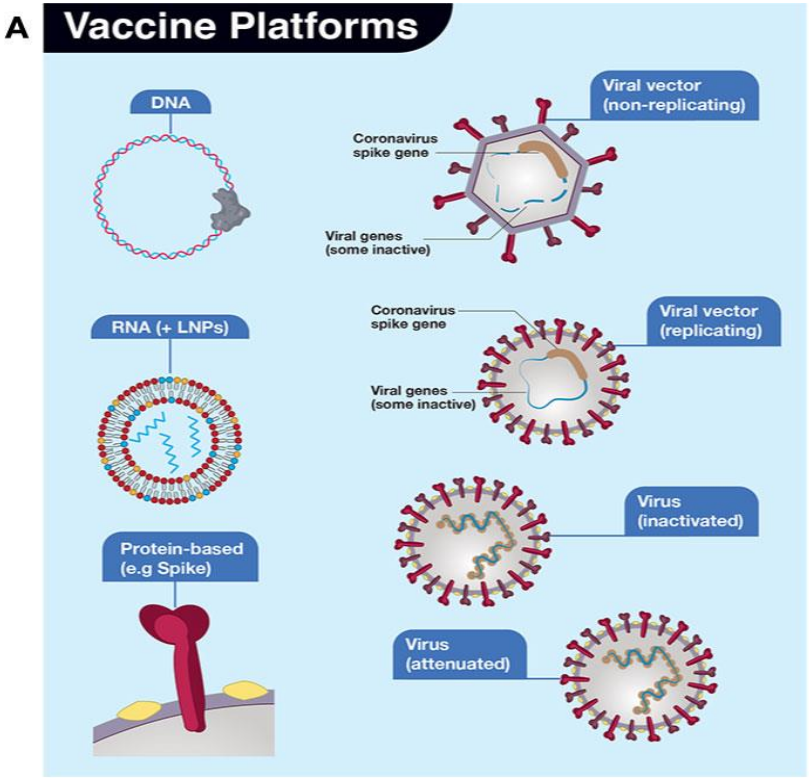
SARS-CoV-2 Vaccine Development

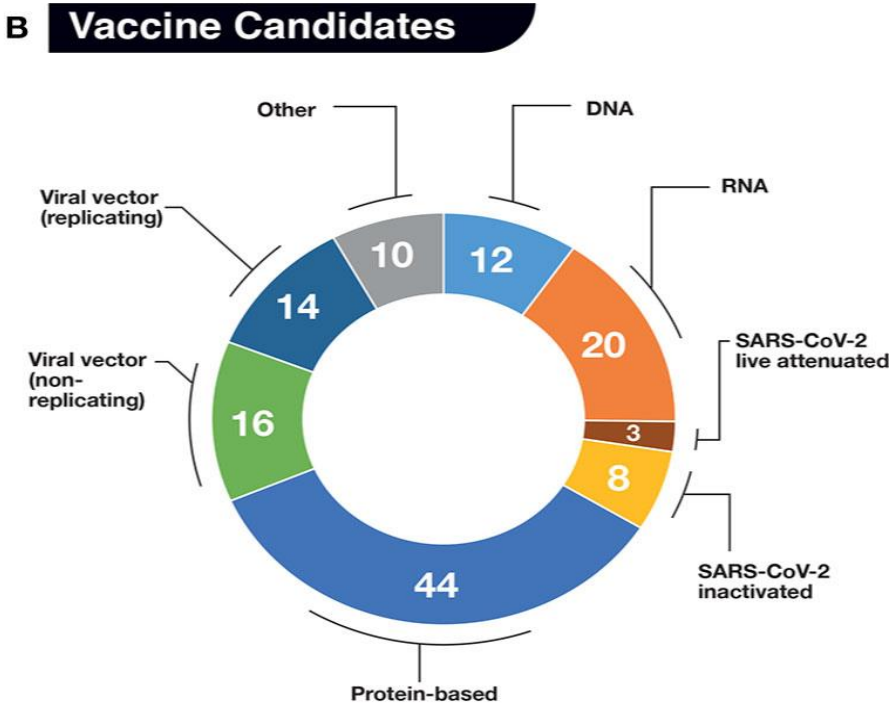
- Multiple New platforms are under development.
- Few platforms- were under studies during MERS/SARS pandemics
- RNA and DNA platforms- lot of interest-potential of scale up- -require no culture or fermentation, use synthetic processes.
- Some experience in developed world with RNA vaccines coming from few vaccine candidates- rabies, HIV, Zika and oncology vaccines targeting leukaemia, prostate cancer, etc.
- Developing word- experience with RNA vaccine platforms is limited.

Vaccine candidates



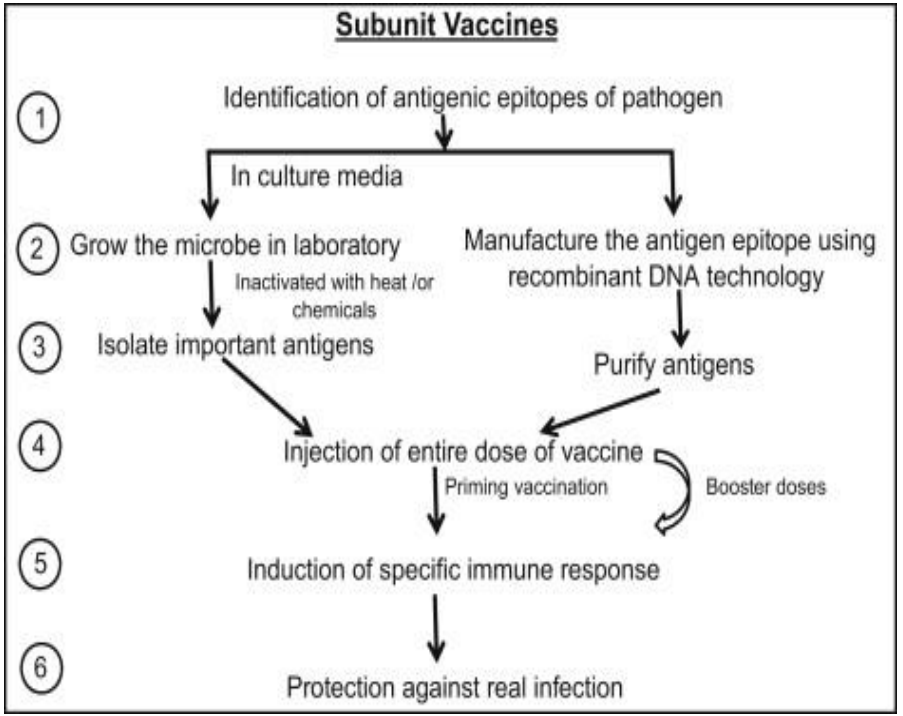
Vaccine Platforms





Protein Subunit vaccines

- Contain an antigenic portion of the pathogen meant to induce a protective immune response
- they include only the parts of the virus or bacteria necessary to elicit a protective immune response
- Side effects are less common because subunit vaccines contain only the antigen and no other pathogen molecules
- Application of recombinant proteins is common now a days
- Often require adjuvants to enhance their immune response
- Leading eg.: Novavax, SIIPL, Sanofi Pasteur, GSK etc.
- Examples: Hepatitis B, Polysaccharide conjugate based vaccines, etc.
- Subunit vaccine based on spike protein, RBD and trimeric spike protein are under advanced development.



Viral -vector vaccine-platforms

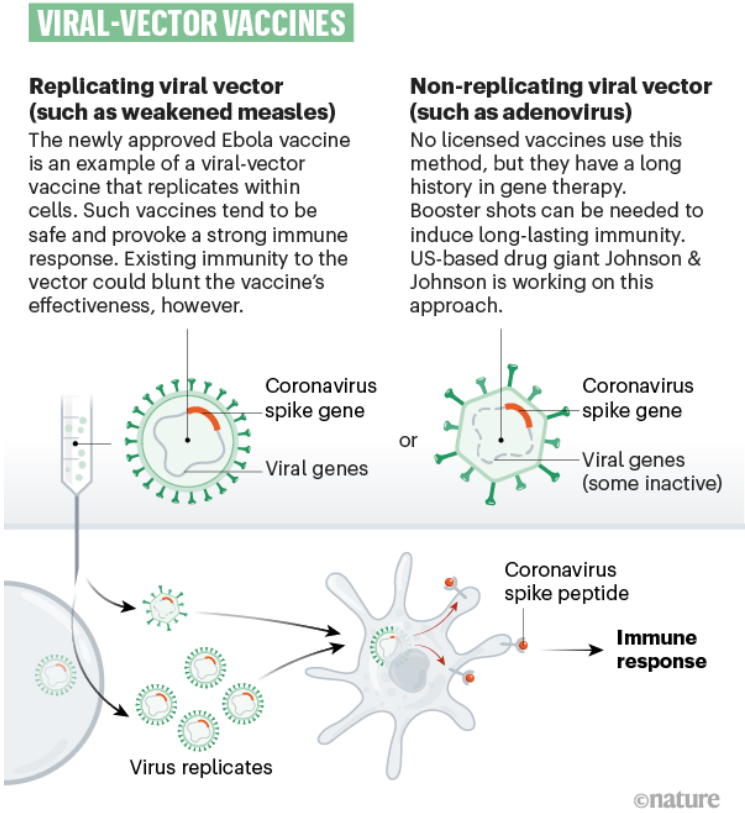
- Adenoviruses: non-enveloped, double-stranded DNA viruses (genome size: 34-43 kb; virion size: 70-90 nm), first discovered in the human adenoid tissue in 1953 by Rowe and his colleagues.
- Relatively large-sized and well-characterized genome, adenoviruses are easy to manipulate genetically.
- In humans, adenoviruses generally cause mild respiratory and gastrointestinal tract infections. Also present in other mammalian species, ranging from simians, chimpanzees.

Adeno-vector vaccines

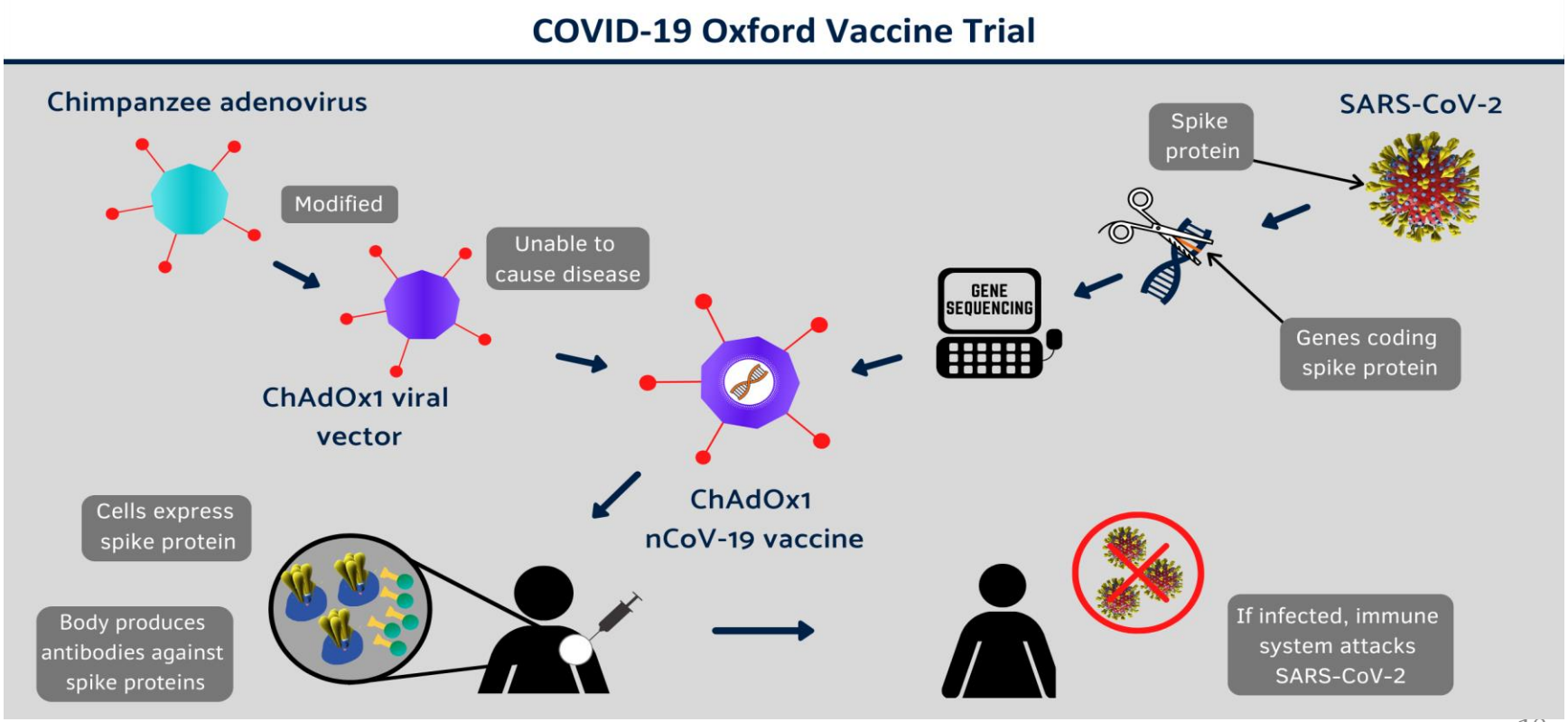
- Adenoviruses express two types of genes: early genes and late genes. Early genes (E1A, E1B, E2, E3, and E4) are necessary for supporting viral replication inside host cells; whereas, late genes are required for host cell lysis, viral assembly, and virion release.
- Recombinant adenoviruses that are generated in the laboratory as vectors can be either replication-deficient or replication-competent.
- Higher thermostability, ability to grow to high titers, and easy application through systemic or respiratory mucosal routes.
- Induce both CD4+ T cell- and CD8+ T cell-mediated immune responses.

Viral Vector Vaccines

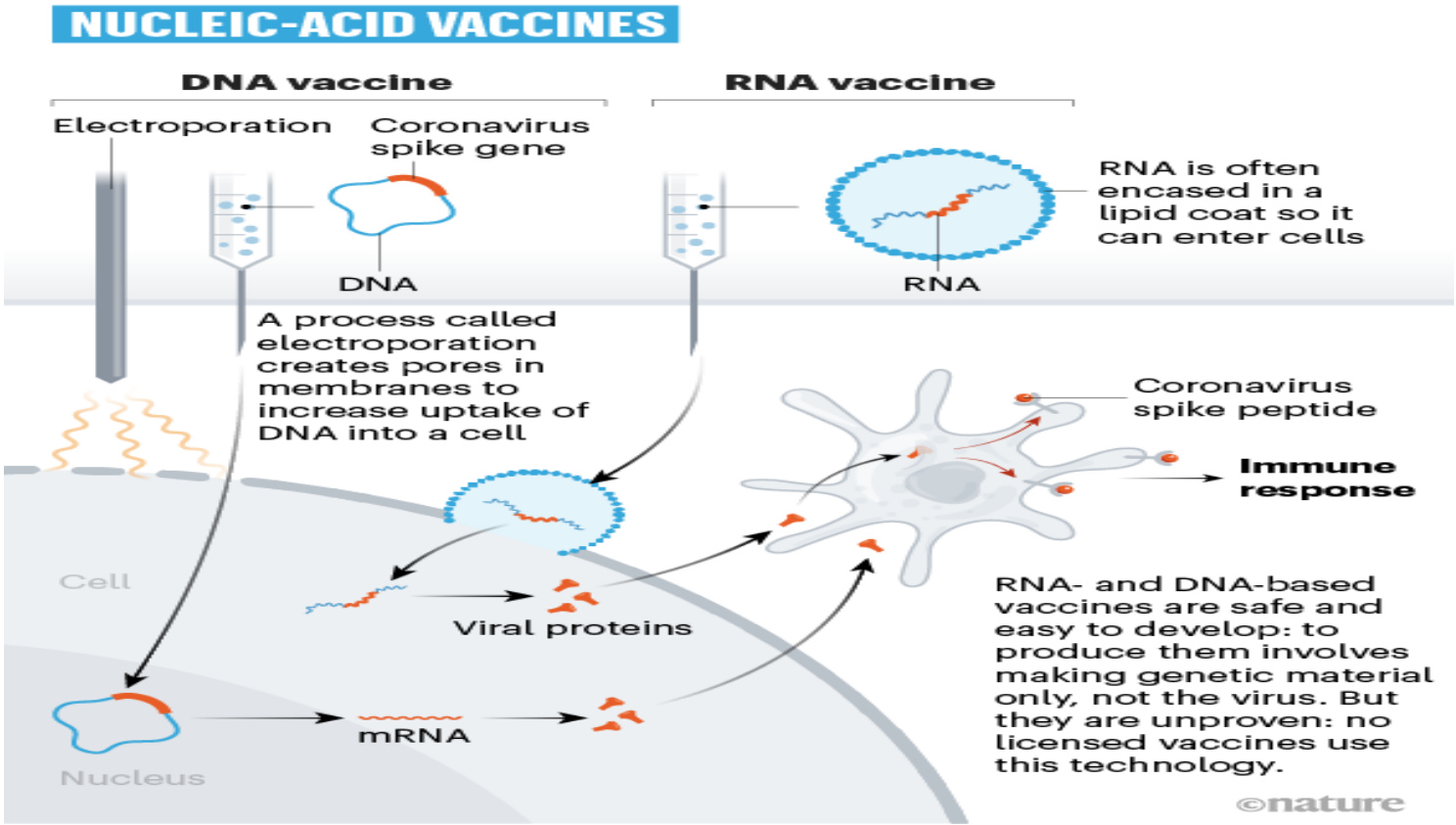
- Adenovirus-based vaccines are prepared by inserting a transgene cassette into the adenoviral backbone through direct cloning or homologous recombination.
- Commonly carried out in mammalian cells
- Use of microcarrier bioreactor systems and suspension cell culture bioreactor systems for large scale cultivation of cell lines that support the growth of viral vectors
- Two-step process for removal of process-related impurities
- Leading eg.: University of Oxford-Astrazeneca, CanSino Biologics, Gamaleya Research Institute



Adenovector- vaccine

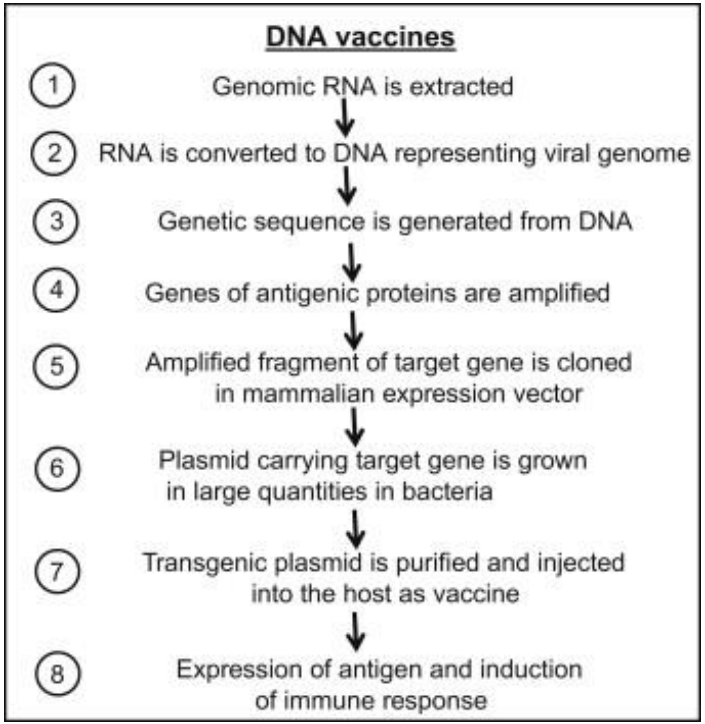


Nucleic acid Vaccines

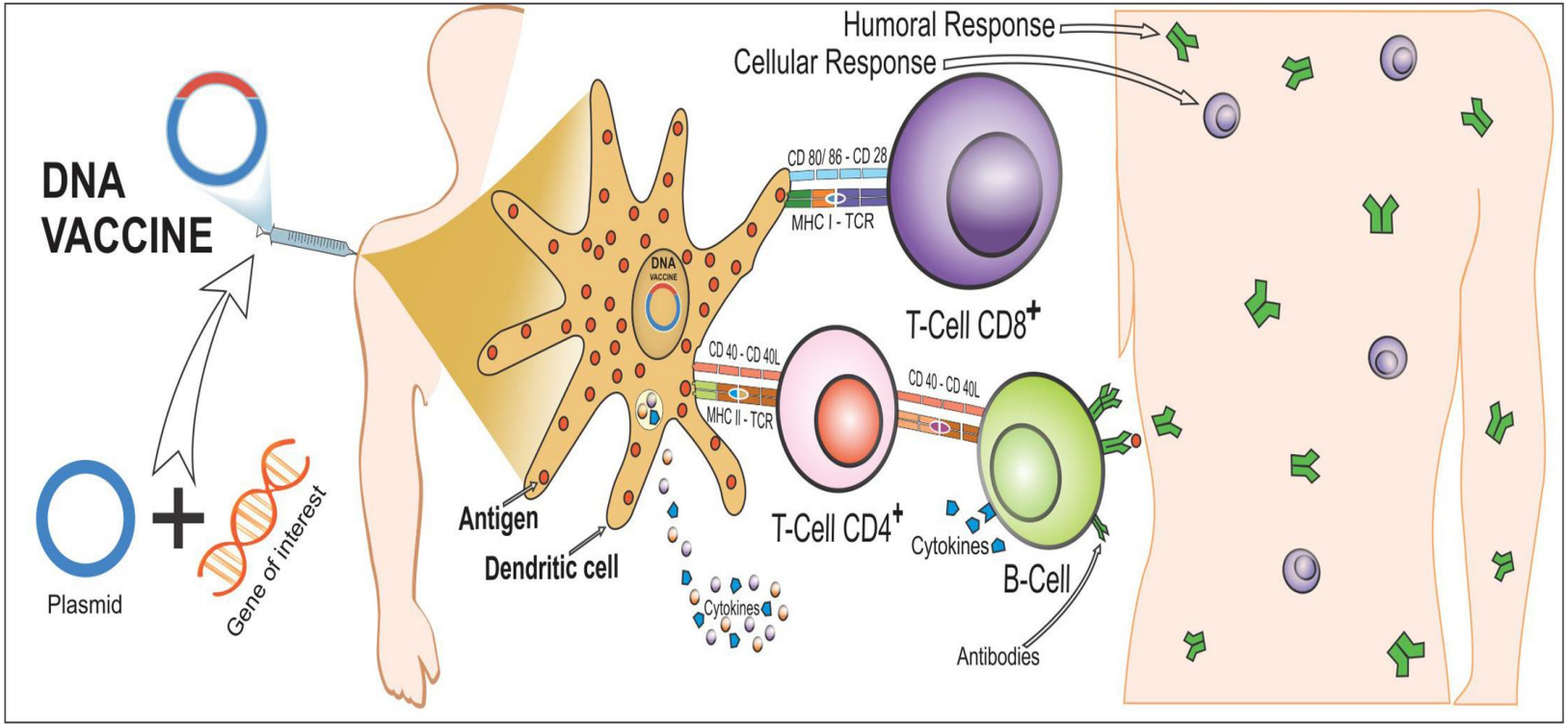


DNA Vaccines

- Bacterial plasmids that encode a vaccine antigen driven by a viral-derived RNA polymerase II promoter
- When administered, plasmid DNA is taken up by a limited number of cells at the injection site
- Injected DNA is transcribed into mRNA and translated into the antigen of interest intracellularly
- The synthesized protein is secreted out of the cell and immune response is triggered by DCs
- Leading eg.: Zydus Candila Healthcare Ltd., Invio Pharmaceuticals, etc.

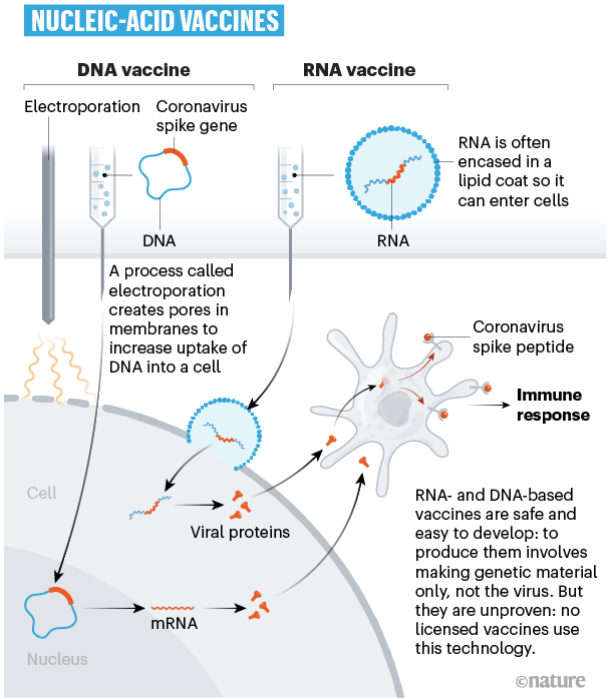


DNA Vaccine

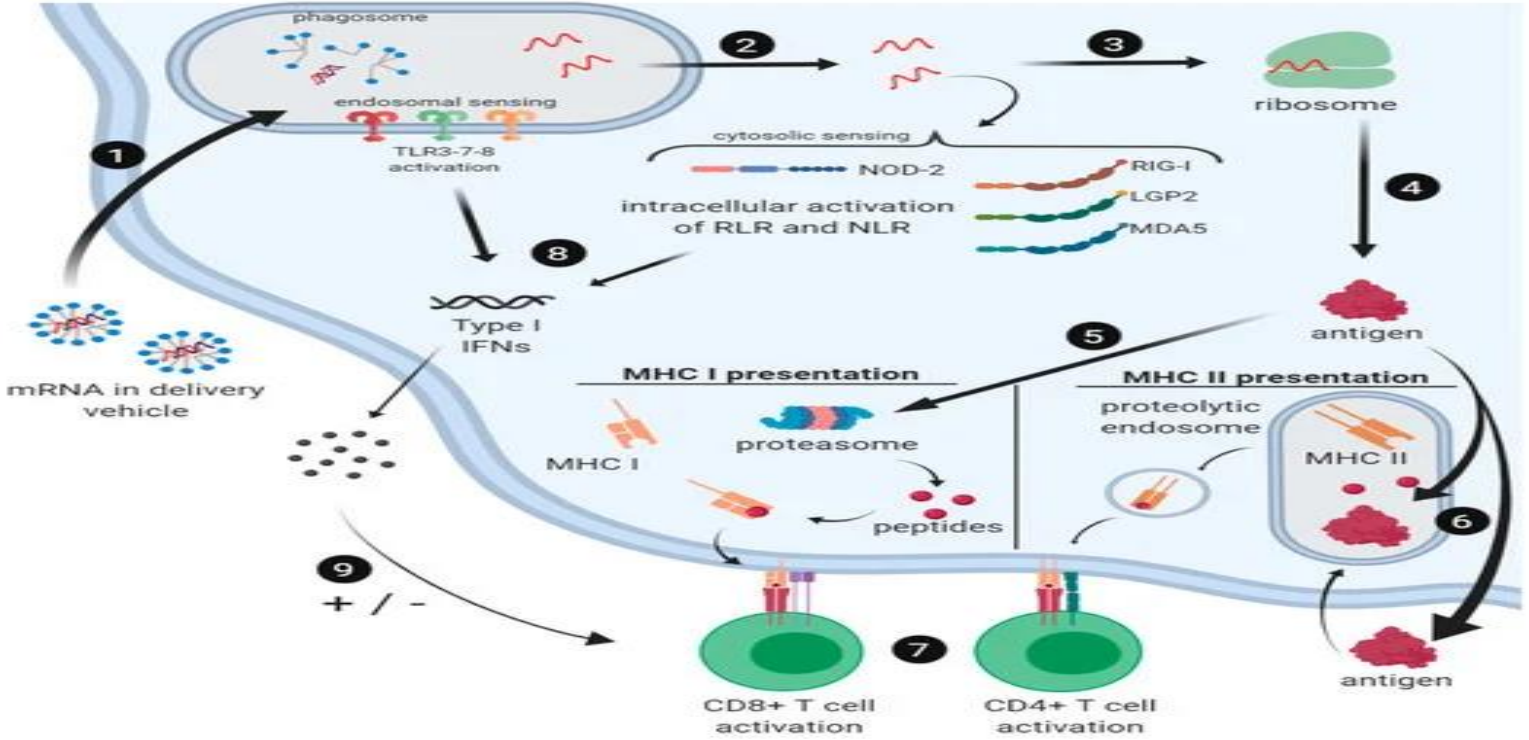

Humoral Response
Cellular Response
DNA VACCINE
Plasmid
Gene of interest
DNA VACCINE
CD 80/ 86 - CD 28
MHC I - TCR
T-Cell CD8+
CD 40 - CD 40L
MHC II - TCR
CD 40 - CD 40L
Antigen
Dendritic cell
T-Cell CD4+
Cytokines
B-Cell
Cytokines
Antibodies

RNA Vaccines

- Messenger RNA (mRNA) vaccines are a newer technology engineered to mimic the nucleic acid transcripts of eukaryotic cells
- Resulting vaccine contains the mRNA for the gene of interest and factors required for elements for post-translational modifications (a 5' cap, and the poly(A) tail)
- After post-translational modification, protein can act in the cell, or can be secreted to act via autocrine, paracrine, or endocrine mechanisms
- mRNA vaccine can be made self-amplifying by inserting viral replication machinery into the DNA template
- self-replication permits lower quantities of a vaccine to be administered per dose
- Leading eg.: Pfizer, Moderna, CureVac, etc.



RNA Vaccine

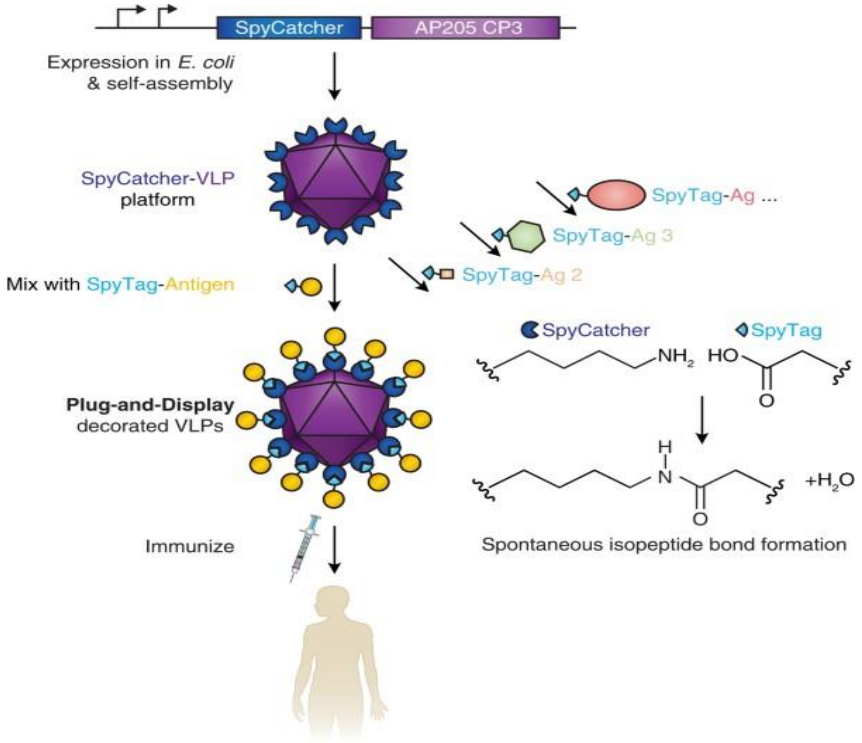

phagosome
endosomal sensing
TLR3-7-8 activation
cytosolic sensing
NOD-2
RIG-I
LGP2
MDA5
intracellular activation of RLR and NLR
ribosome
mRNA in delivery vehicle
Type I IFNs
antigen
MHC I presentation
MHC II presentation
proteolytic endosome
MHC I
proteasome
MHC II
peptides
+ / -
CD8+ T cell activation
CD4+ T cell activation
antigen
1
2
3
4
5
6
7
8
9

Virus Like Particle (VLP) Vaccines

- Virus-like particles (VLPs) are nanoparticles composed of multiple copies of recombinant viral structural proteins with diameters of 25–100 nm.
- Their immunogenicity stems from multiple factors, including their particulate structure, repetitive surface units that stimulate B cells.
- The efficacy of the antibody response generated by VLP vaccines is evident in the success of Gardasil and Cervarix in preventing human papilloma virus infection (Bessa et al., 2008).
- In addition to inducing immunity to the structural proteins from which they were derived, VLPs can be designed with encapsulated or surface-conjugated antigens, thereby inducing responses to these additional antigens.

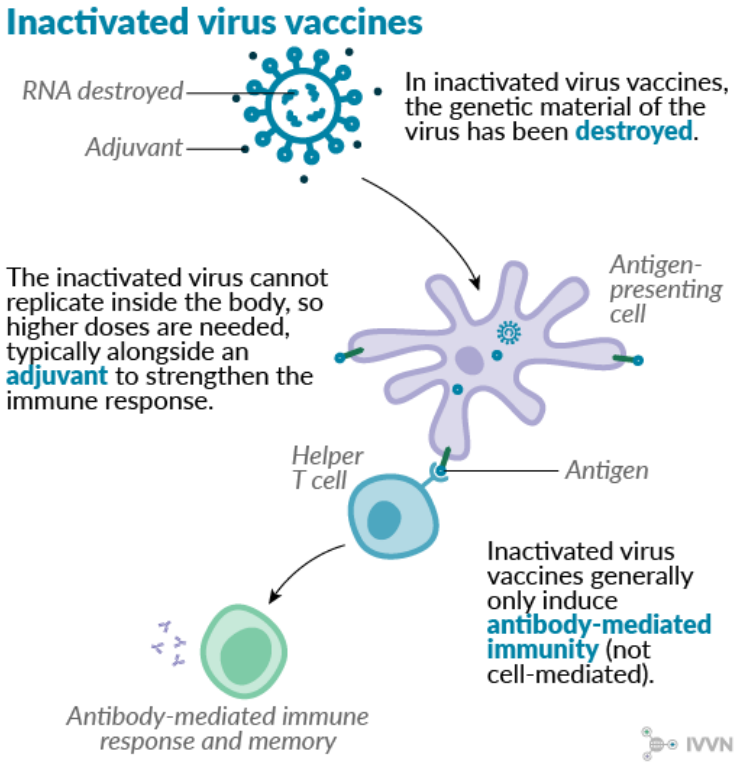
VLP Based Vaccines

- Similar to viral vector systems and present the protective antigen in the context of an intact viral particle
- No replication adds an extra level of safety but requires higher doses of vaccine
- The assembled VLPs have a similar morphology to natural viruses
- VLPs can mimic the presentation of antigenic epitopes of the virus and achieve tissue-specific targeting and effective cell penetration
- Induction of a healthy cellular and humoral immune response
- Leading eg.: SII RBD spytag vaccine



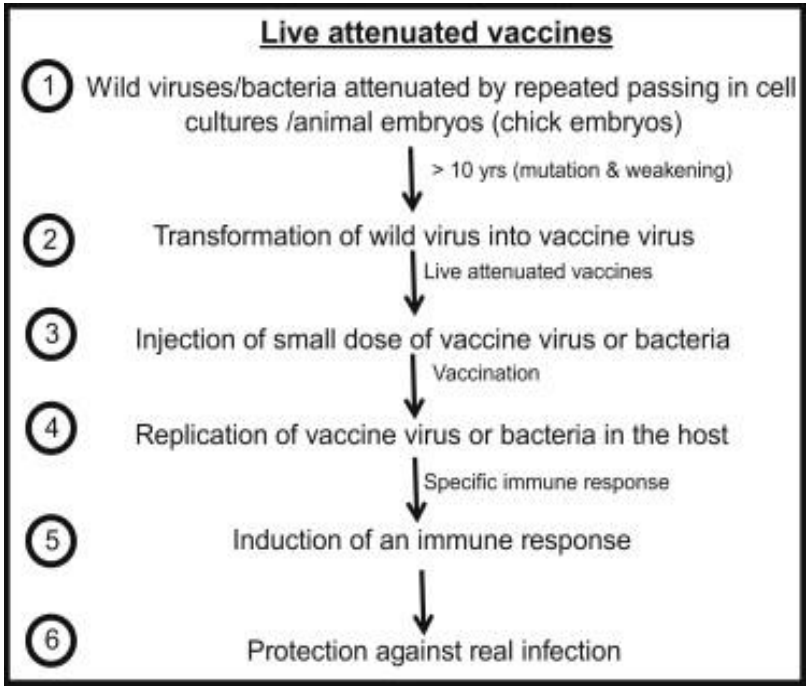
Inactivated Pathogen Vaccines

- Isolation, sequencing, plaque purification, and passaging of individual viral strain in a mammalian cell line to create a viral stock
- Monitoring the genetic stability of the virus through several more passages
- Inactivation of virus using a wide range of reagents or methods (eg. scorbic acid, hydrogen peroxide, gamma irradiation, UV treatment, heat, formaldehyde, and β-Propiolactone etc.)
- Purification of inactivated virus from cell culture using filtration and chromatography
- Treatment with adjuvant if required
- Leading eg.: Sinovac Biotech Ltd., Bharat Biotech-ICMR and many more



Live Attenuated Vaccines

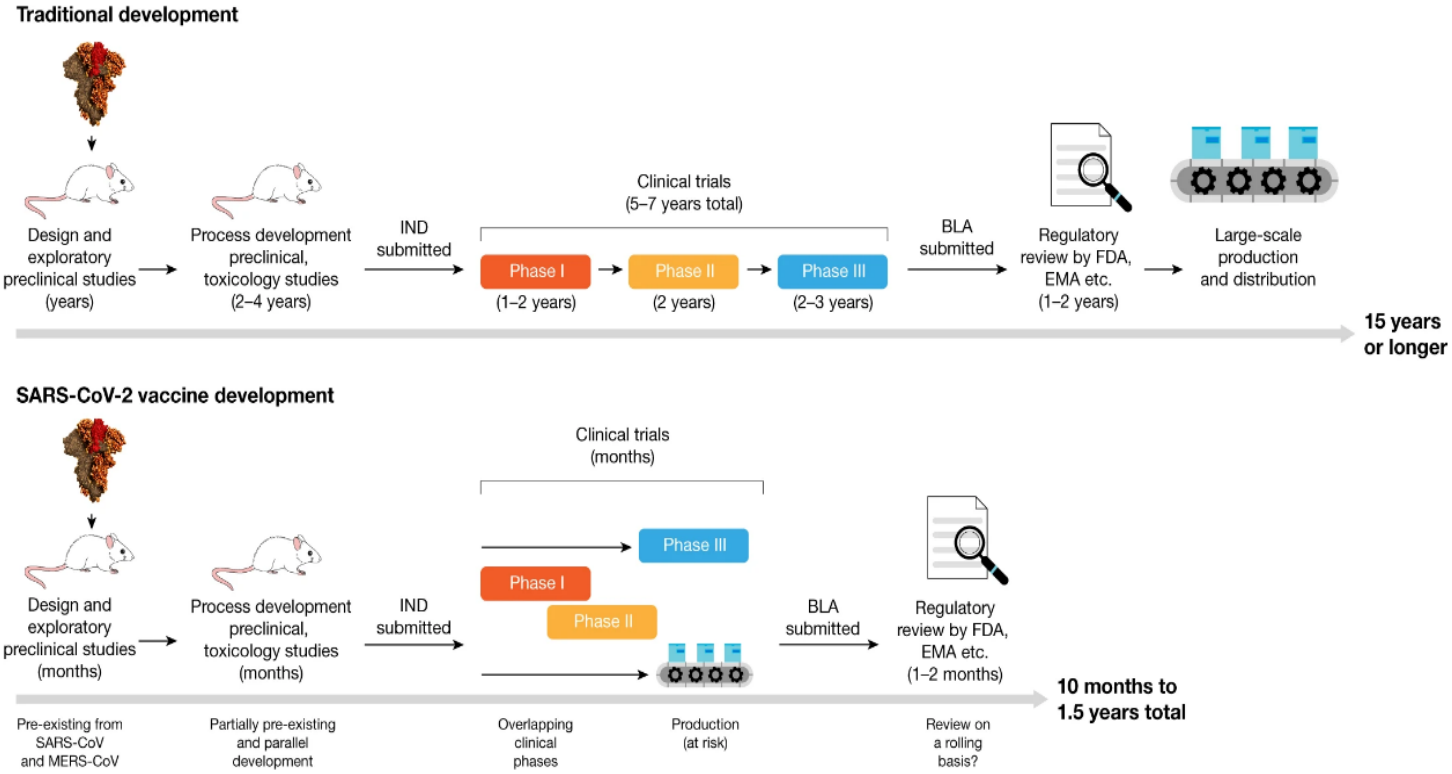
- Live viruses are weakened to reduce virulence, selected by growth in heterologous species and/or in tissue culture cells
- Contain a weakened version of the live virus that does not cause severe disease in people with healthy immune systems
- Attenuated vaccines is often created by passaging the wild-type virus in a novel cell line or adapting the virus to non-natural environmental conditions such as low temperatures
- Closest to a natural infection
- SII- Codigenix vaccine



Benefits and challenges with vaccine platforms

Vaccine Platform	Benefits	Challenges
Protein Subunit Vaccine	• Safe vaccine • Well characterized vaccine •Regulators- like such vaccines •Long shelf life •Storage: refrigerated conditions	•Booster shots may be necessary thus increasing the cost of vaccination Response- primarily humoral.
DNA Vaccine	• Induction of B and T cell response • Long shelf life • Heat stable than RNA vaccines • Inexpensive	• Weaker induction of immunity • Risk for integration into recipient's chromosomal DNA resulting insertional mutagenesis • Require specific delivery devices
Inactivated Viral Vaccine	• Reduced risk of infectivity without loosing antigenicity • Stable and safe vaccine	• Booster(s) may be required thus increasing cost • Use of adjuvants may cause unwanted inflammatory response •Inactivation kinetics •Needs specialized production facilities
RNA Vaccine	• Induction of B and T cell response • Enhanced antigen expression • Does not interact with the genome	• Lack of interaction with endosomal RNA receptors may weaken immunostimulation • Necessity to keep at cooler temperatures
Viral Vector vaccine	• Long term gene expression •Induction of B and T cell response	• Large-scale manufacturing of viral vectors may be expensive • Recombinant viruses may cause disease in immunocompromised hosts
Virus Like Particle (VLP) Vaccine	• Present antigens in a dense, repetitive manner, enabling the cross linking of BCRs • Stimulate protecting neutralizing antibodies • Safe with self-adjuvanting properties	• Risk of presence of host cell-derived particles • Challenges to produce VLPs with optimal quality, stability, and good immunogenicity at high yield
Live Attenuated Vaccine	• Induction of B and T cell immune response • No adjuvant required • Single administration • Often confers long term immunity	• Needs very detailed characterization withrespect to reversion. Storage at cooler temps

General Vs Emergency Vaccine Licensure Process



Analytical Tools for Quality Checking of Vaccines

- ELISA
- Enzyme-Linked Immunosorbent Spot
- Flow Cytometry
- Mass Cytometry
- Multiplexed bead-based SARS-CoV-2 serological assay
- BSL-3 sparing neutralization assays

Variants of SARS-CoV-2:

- D614G mutation: Mutation appeard to be at the 614th amino-acid position of the spike protein, the amino acid aspartate (D) was regularly being replaced by glycine (G) because of a copying fault that altered a single nucleotide in the virus's 29,903-letter RNA code
- N501Y or S:N501Y: The variant has a mutation in the receptor binding domain (RBD) of the spike protein at position 501, where amino acid asparagine (N) has been replaced with tyrosine (Y)
- 69/70 deletion: This double deletion has occurred spontaneously many times, and likely leads to a change in the shape of (i.e., a conformational change in) the spike protein
- P681H: Near the S1/S2 furin cleavage site, a site with high variability in coronaviruses. This mutation has also emerged spontaneously multiple times
- ORF8 stop codon (Q27stop): This mutation is not in the spike protein but in a different gene (in open reading frame 8), the function of which is unknown. Similar mutations have occurred in the past. In Singapore, one strain with this type of mutation emerged and disappeared

India’s Response to COVID

- Great work done by Government authorities in designing a draft guidance
- Proactive support by regulators- rolling submissions
- CEPI approved lab at Delhi
- Diagnostics kits
- Consistent research at NIV and ICMR making the viral sequences publically available
- Global supply of hydroxychloroquine when and where required
- First ever country to provide licensure for two vaccines; Oxford-Astrazeneca-SIIPL and Bharat Biotech-ICMR

Take home message

- COVID-19 pandemic has brought vaccines into focus
- New technologies being developed- new platforms
- Every platform has its own strengths and challenges
- Excellent partnerships and collaborations-way to vaccine development.
- Excellent support from academia in better understanding of COVID-19 disease.
- CROs and CMOs played an important role in supporting the industry.

Thank you