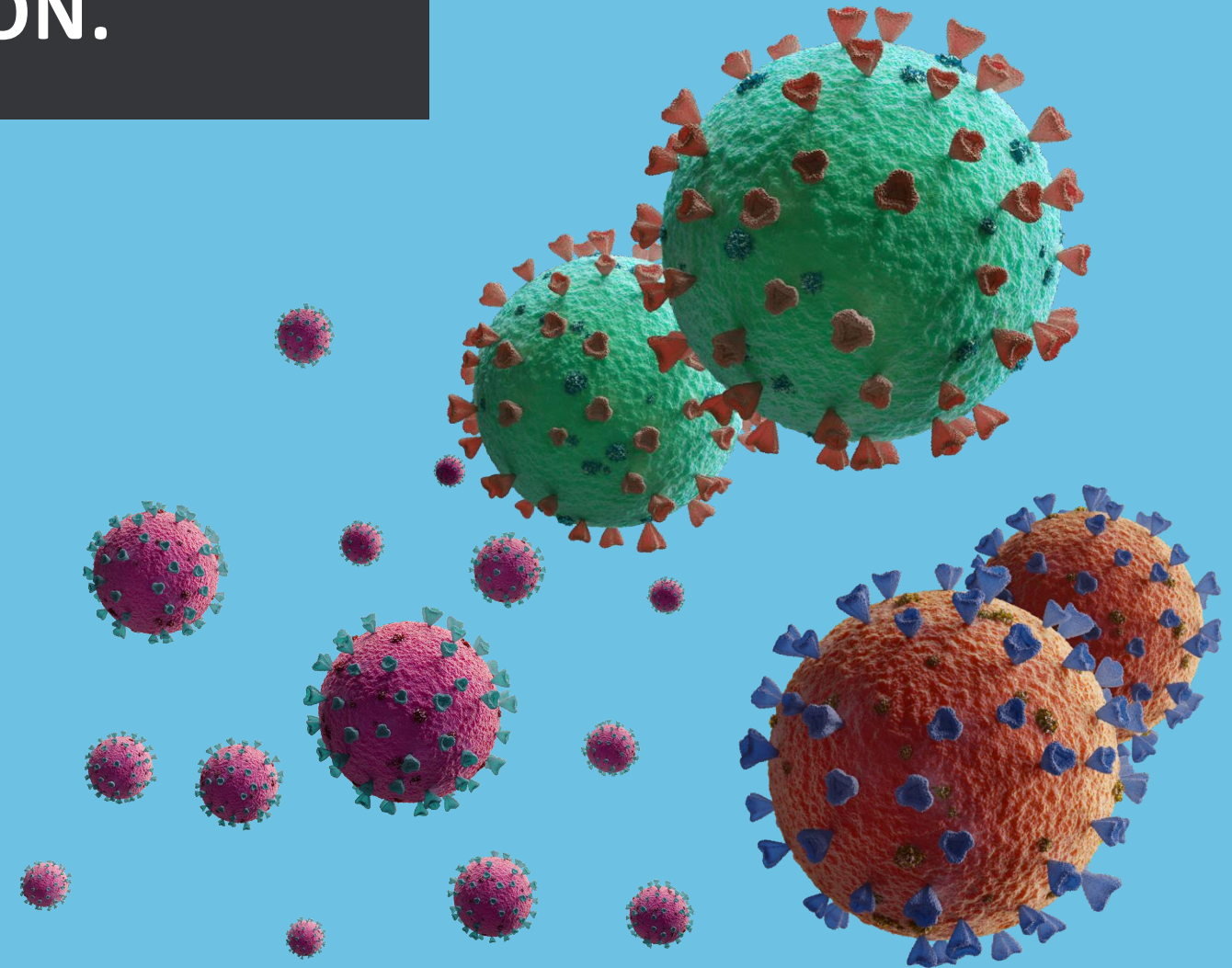
KNOW ABOUT VACCINES.
COMPARING INFORMATION.

FEDERAZIONE RINASCIMENTO ITALIA

Dr.
LORETTA BOLGAN



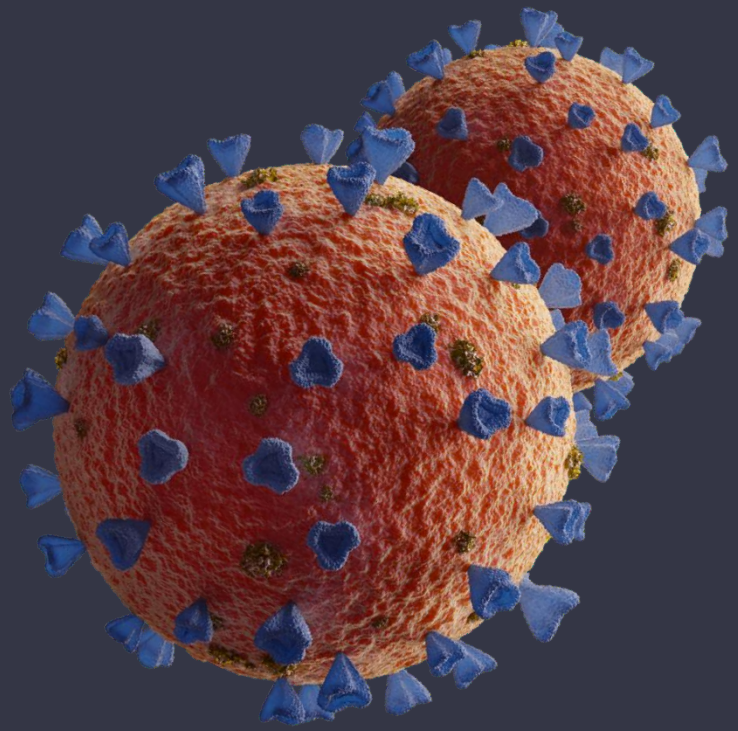
September 2020

IMMUNIZATION AND VACCINATION

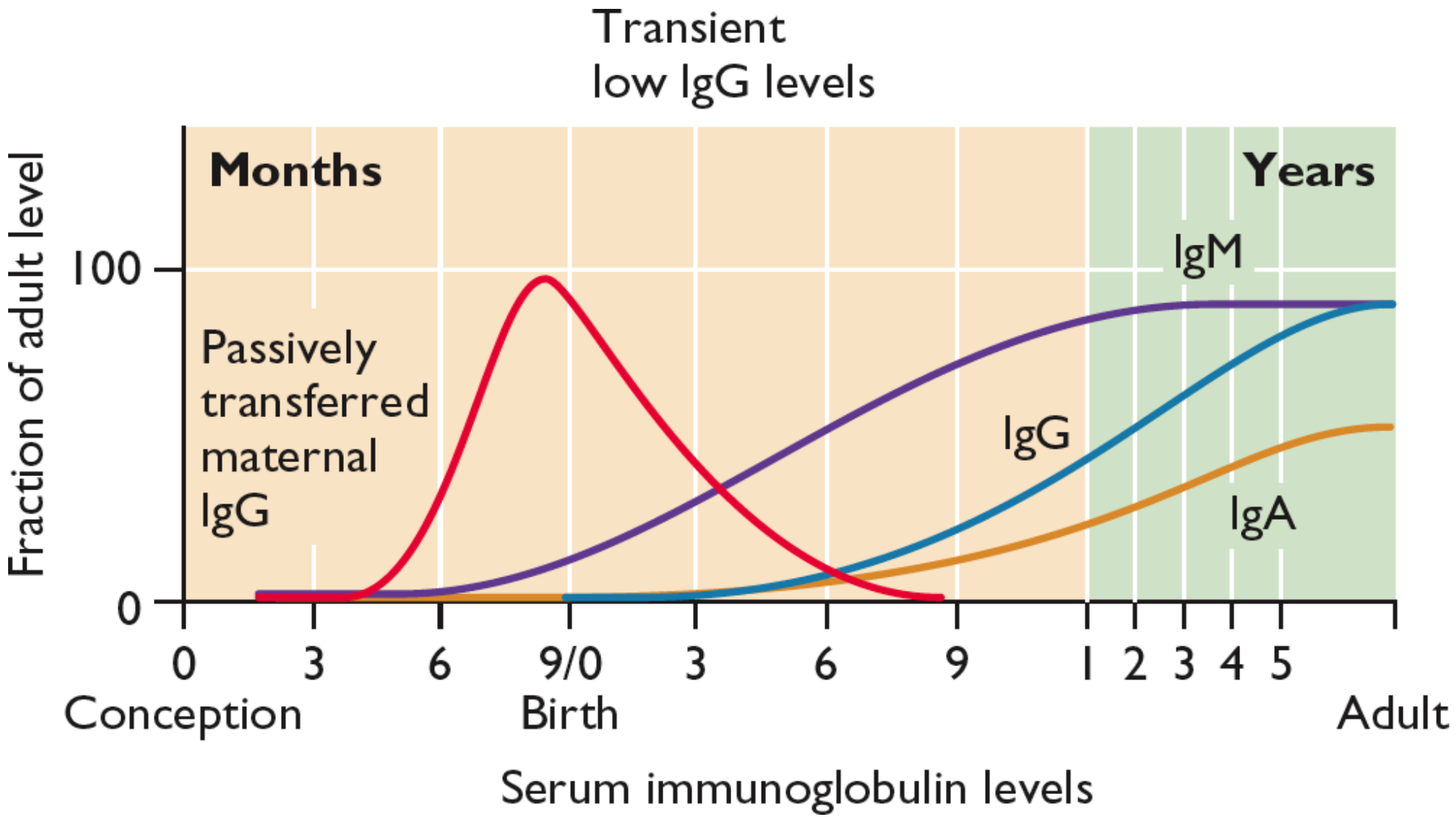
FEDERAZIONE RINASCIMENTO ITALIA

Immunization is the process by which a person is made immune or resistant to an infectious disease, normally through the administration of a vaccine. Vaccines stimulate the body's own immune system to protect the person from subsequent infections or diseases. (WHO definition)

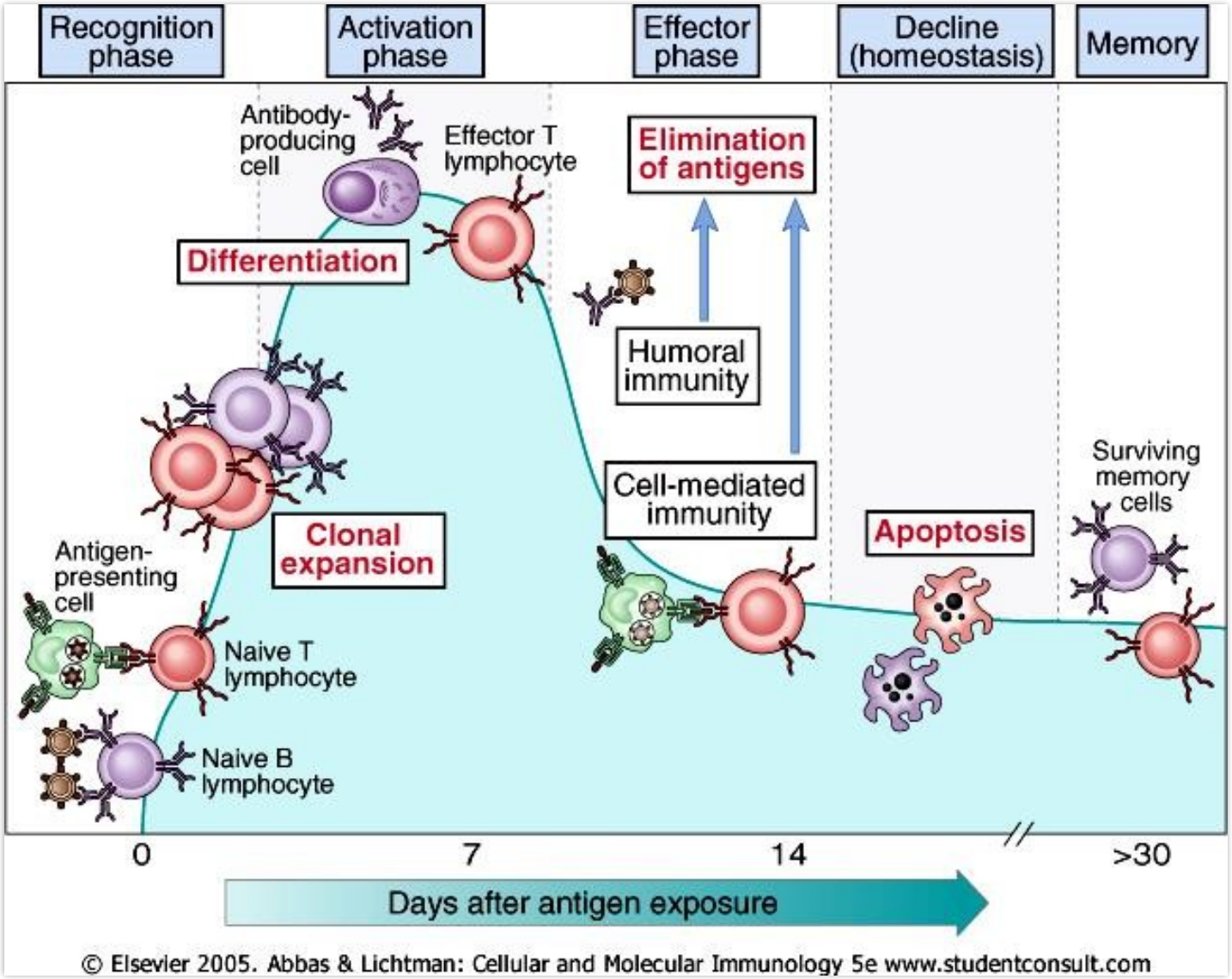
Vaccination: Injection of a killed infectious organism or mitigated with the aim of preventing the disease



IMMUNIZATION AND VACCINATION



IMMUNIZATION AND VACCINATION



DEFINITION OF VACCINE

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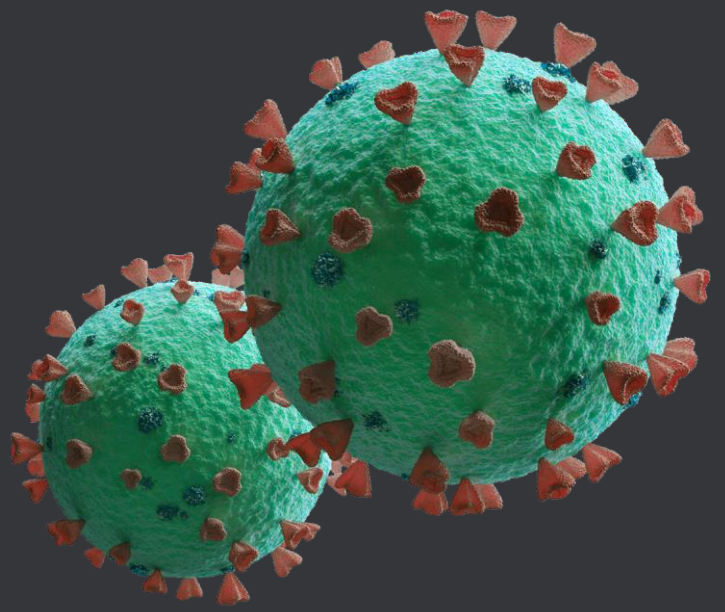
A vaccine is a biological preparation that enhances immunity Against a particular disease.

A vaccine typically contains an agent that resembles a disease-causing microorganism, and often consists of weakened or killed forms of the microbe, its toxins or surface proteins.

The agent stimulates the body's immune system so that it is able to recognize the agent as foreign, destroy it, and "remember" it, so the immune system will be able to more easily recognize and destroy any of these microorganisms it later encounters. (WHO definition)



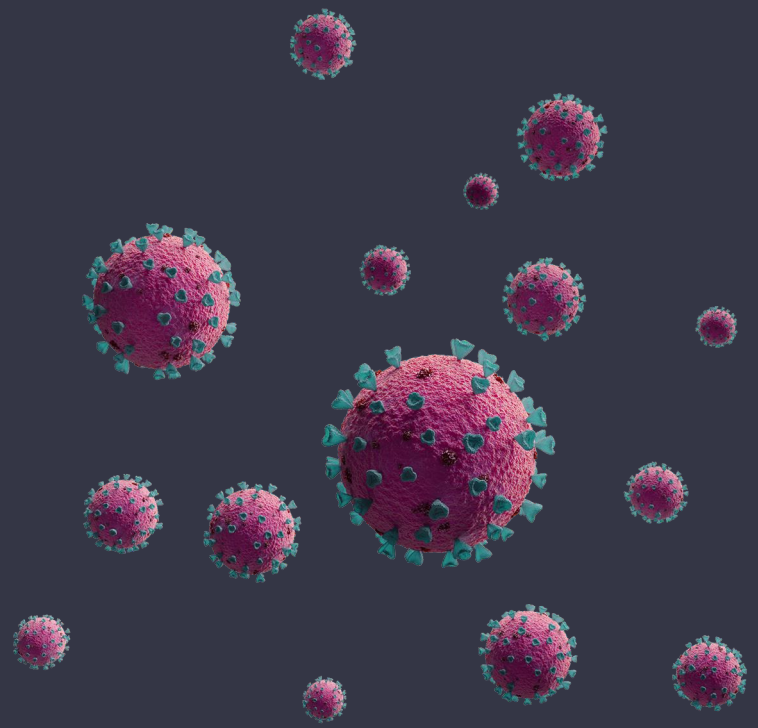
COMPOSITION OF A VACCINE

FEDERAZIONE RINASCIMENTO ITALIA

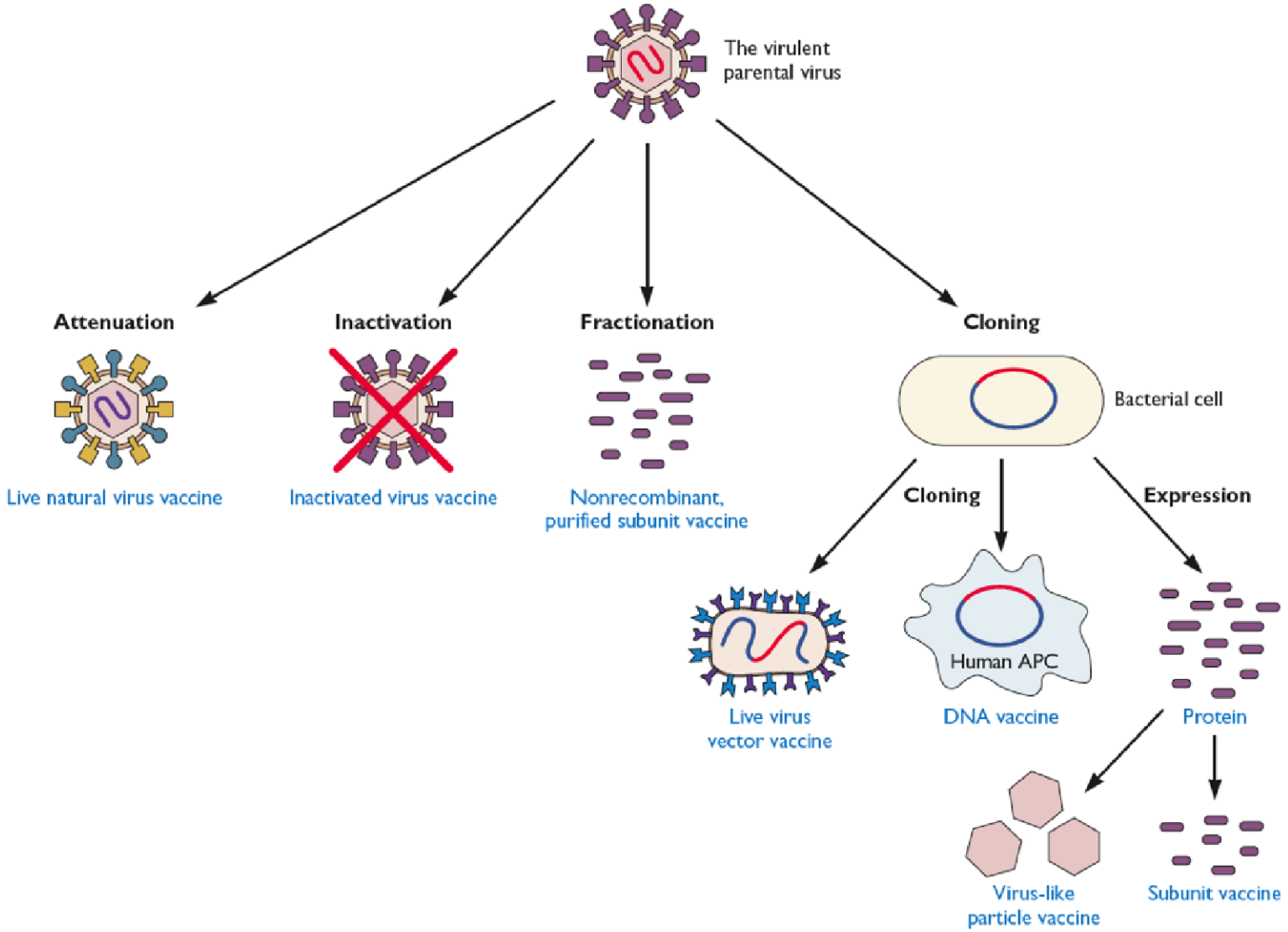
The vaccines consist of:

1. **Antigens**
2. **Adjuvants**
3. **Preservatives**
4. **Excipients**

May contain *impurities*
(processing residues and contamination) chemical, biological and elemental



ANTIGENES


The virulent parental virus
Attenuation
Inactivation
Fractionation
Cloning
Live natural virus vaccine
Inactivated virus vaccine
Nonrecombinant, purified subunit vaccine
Bacterial cell
Cloning
Expression
Live virus vector vaccine
Human APC
DNA vaccine
Protein
Virus-like particle vaccine
Subunit vaccine

ANTIGENES

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Adjuvant	Composition			Major Immune Effects
(vaccines where used)	**Component**	**Origin**	**Other Uses**	
Aluminum (D, T, pertussis, IPV, hepatitis A & B, HPV, meningococcal and pneumococcal)	Aluminum as salts mixed with antigen (adsorption)	Naturally occurring present in soil, water, air	Medicines, cosmetics, food industry	Increases local inflammation, improves antigen update by APCs. Acts to increase antibody production
Virosomes (Hepatitis and influenza)	Vesicles where influenza antigens in aqueous volume are enclosed within a standard phospholipid cell membrane bilayer	Natural phospholipids, Seasonal influenza glycoproteins	None	Increases uptake by APCs. May interact with B cells leading to T-cell activation.
AS04 (Hepatitis B, HPV)	(3-deacyl-monophosphoryl lipid A) derived from LPS from *Salmonella Minnesota*, *Aluminum salts*	Natural exposure to LPS from Gram-negative bacteria occurs frequently	None	Directly stimulates TLR-4 increasing APC maturation and Th1 responses.
MF59® (Influenza-seasonal and pandemic)	Squalene	Animal source (shark liver oil). Found naturally in human tissues: adipose tissues, skin, arterial walls, skeleton, muscles, lymph nodes	Cosmetics, moisturizers	Increases APC recruitment and activation. Promotes antigen uptake and migration of cells to lymph nodes.
AS03 (Influenza-pandemic)	• Vitamin E (α-Tocopherol) • Surfactant polysorbate 80 • Squalene	• Naturally occurring in humans. • Surfactant and emulsifier • Animal source (shark liver oil). See above	• Vitamin • Used in foods, eye drops & intravenous injections • Naturally occurring. See above	Promotes local production of cytokines and recruitment of innate cells.
Thermo-reversible oil-in-water (Influenza-pandemic)	Squalene	Animal source (shark liver oil). See above	Naturally occurring. See above	Not reported
ISA51 (therapeutic vaccine NSCLC)	Mineral oil DRAKEOL 6 VR Surfactant mannide-mono-oleate	Refined mineral oil of vegetable origin	Food industry	Strongly immunogenic

D = diphtheria, T = tetanus, IPV = inactivated poliomyelitis vaccine, HPV = human papilloma virus, LPS = lipopolysaccharide, APC = antigen presenting cells, TLR = toll-like receptor, NSCLC = non-small cell lung cancer, MPL = monophosphoryl lipid A.

ALUMINUM ADJUVANT

FEDERAZIONE RINASCIMENTO ITALIA

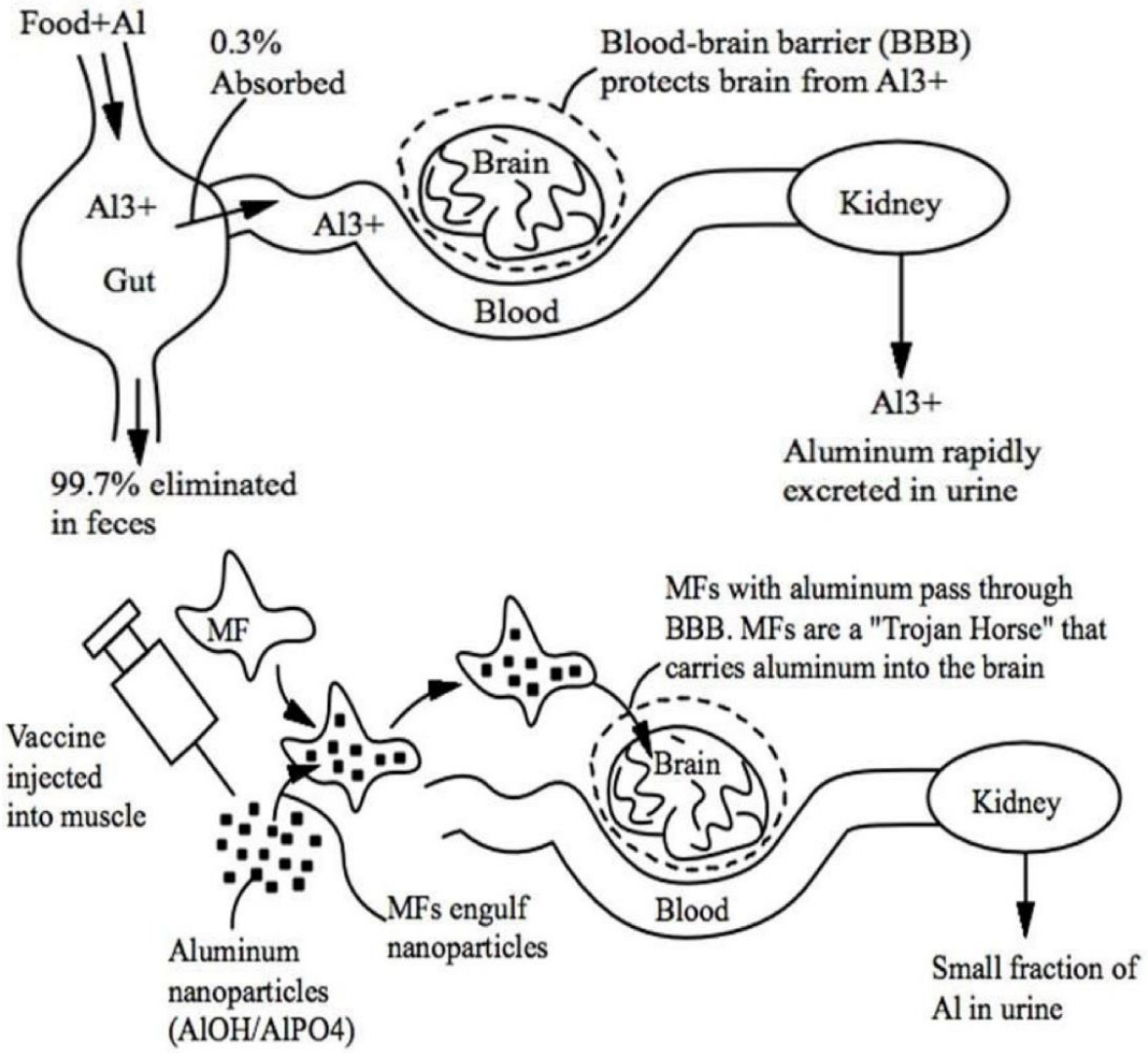
1 mg of Al^{+3} by injection (Infanrix hexa + Prevenar 13) is equivalent to about 300 mg orally

To date there is a lack of toxicological studies on adjuvant aluminum for injection administered in the pediatric age group. In vitro and animal studies confirm neurotoxicity, genotoxicity, and immunotoxicity
Of aluminum.

EMA ANSWER.
"the use of aluminum hydroxide and aluminum phosphate in vaccines as adjuvants (used to enhance immune response) has been well established for many years.
A final safety evaluation of the new vaccine formulation can only be conducted on the basis of clinical studies."

Study cited by EMA, FDA and WHO on toxicology
Of aluminum:

Vaccine. 2011 Nov 28;29(51):9538-43.
Updated aluminum pharmacokinetics following infant exposures through diet and vaccination.
Mitkus RJ1, King DB, Hess MA, Forshee RA, Walderhaug MO.



IMPURITIES IN THE VACCINES ANALYZED

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VACCINES EXAMINED: INFANRIX HEXA - HEXYON - GARDASIL 9 - PRIORIX TETRA

CHEMICAL-PROTEIN CONTAMINATIONS: presence of known and unknown chemical contaminants (70-80%) in non-residual amounts (contamination from raw materials and cross-contamination from other production processes, toxins derived from the production process: potentially toxic and/or carcinogenic). Proteins and peptides from cell lines (human, chicken, bacterial: allergenic and/or autoimmune)

BIOLOGICAL CONTAMINATIONS: adventitious viruses: endogenous retroviruses (potentially carcinogenic and autoimmune)

PROCESSING RESIDUES: human fetal DNA/RNA, chicken embryonic DNA/RNA, DNA/RNA from L1 fragments of papilloma virus

EMA ANSWERS.

FEDERAZIONE RINASCIMENTO ITALIA

CHEMICAL CONTAMINANTS

The dossier is part of the Common Technical Document (CTD) and manufacturing details are included in Module 3. It should be noted that most of the information in Form 3 is considered commercially confidential and the agency is unable to release this at the request of a third party.

Although specific uniform limits do not apply for most of the substances listed in the application, there are requirements for warnings on the product label under specific circumstances for a number of substances.

It is advisable to consult the Material Safety Data Sheets for these substances for information on their toxic levels. The website of the European Food Safety Authority (EFSA), can also provide useful information on the toxic levels of these substances when ingested orally.

IMPURITIES OF BIOLOGICAL MATERIAL

FEDERAZIONE RINASCIMENTO ITALIA

ADVENTITIOUS VIRUSES:

Priorix tetra: Human endogenous retrovirus K, Avian leukosis virus, HERV-H/env62

Hexyon: Tetanus phage, Vectors used for cloning

Gardasil 9: Molluscum contagious virus, Phages, Murine leukemia virus, Human endogenous retrovirus K, Yeast and its viruses

ICH Topic Q 5 A EMA

The risk of viral contamination is a common feature of all biotechnology products derived from cell lines. Such contamination could have serious clinical consequences and may result from contamination of the source cell lines themselves (cell substrates) or from accidental introduction of the virus during manufacturing.

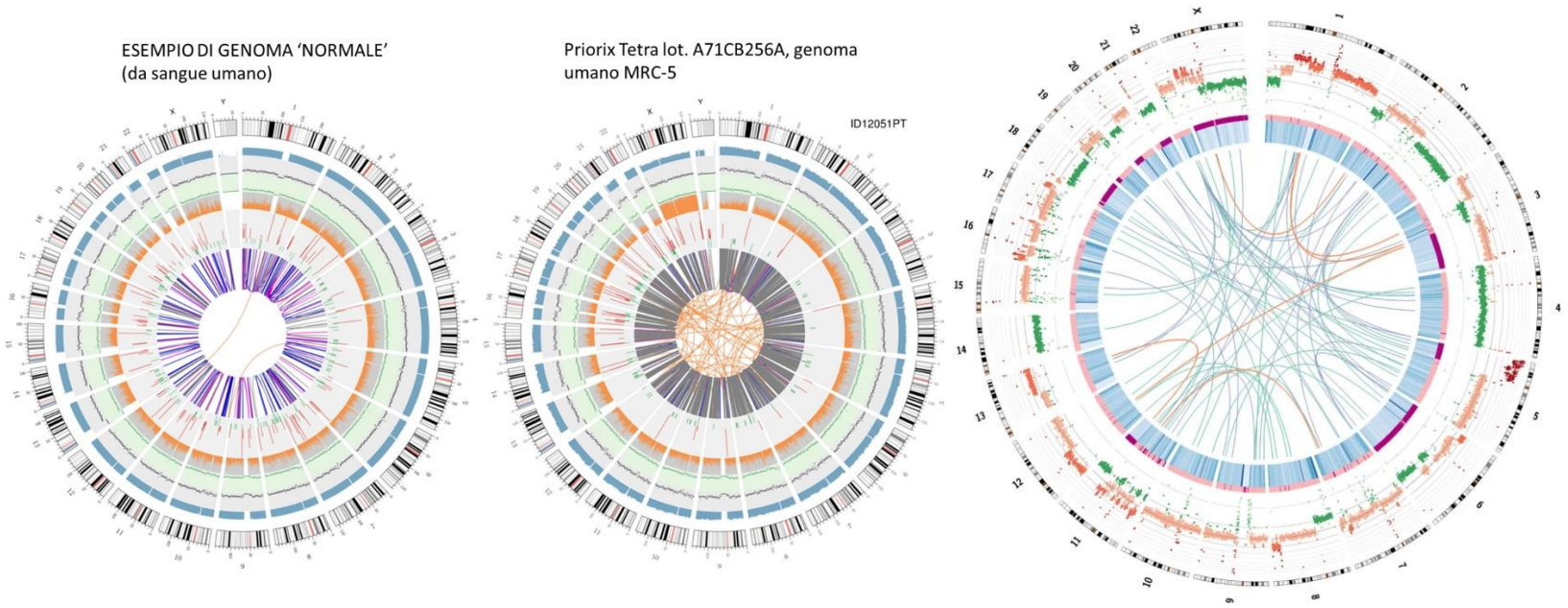
There must be evidence that any virus or viral particle known to be present in the culture broth was actually inactivated or removed during the downstream process

IMPURITIES OF BIOLOGICAL MATERIAL

FEDERAZIONE RINASCIMENTO ITALIA

PRIORIX TETRA: Total DNA: 1.7-3.7 µg/dose of which 80% human (human fetal DNA/RNA from MRC-5 cell line). Amount of DNA such that it is the main ingredient of the vaccine.

Cell line genomic sequencing: found complete individual genome, highly genetically modified and potentially carcinogenic



Approximate comparison between fetal DNA (left) and HeLa cell DNA (right)

The translocations of HeLa cells represented in the circos plot by the nucleus lines refer to the entire genome (thus coding and noncoding part), whereas in the case of fetal vaccine cells they refer only to coding genes

IMPURITIES OF BIOLOGICAL MATERIAL

FEDERAZIONE RINASCIMENTO ITALIA

DEFINITIONS

ONCOGENICITY: The ability of an acellular agent-such as a chemical agent, virus, viral nucleic acid, viral gene, or subcellular elements-to cause tumor formation in the normal cells of an animal.
Tumors that show up in a tumorigenicity test contain cells derived from inoculated cells

TUMORIGENICITY: the ability of an inoculated cell population in an animal model to produce a tumor by proliferation at the site of inoculation and/or at a distant site by metastasis
Tumors that show up in an oncogenicity test are host-derived.

IMPURITIES OF BIOLOGICAL MATERIAL

FEDERAZIONE RINASCIMENTO ITALIA

EMA ANSWER:

"according to the European Pharmacopoeia, MRC-5 diploid cell lines are not tumorigenic, as demonstrated by decades of use and control, and therefore no upper limit for MRC-5 cell DNA applies."

- Jacobs JP. Updated results on the karyology of the WI-38, MRC- 5 and MRC-9 cell strains. Developments in Biological Standardization, 1976, 37:155-156.
- Jacobs JP et al. Guidelines for the acceptability, management and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man. Journal of Biological Standardization, 1981, 9:331-342.
- Petricciani JC et al. Karyology standards for rhesus diploid cell line DBS-FRhL-2. Journal of Biological Standardization, 1976, 4:43-49.
- Schollmayer E et al. High resolution analysis and differential condensation in RBA-banded human chromosomes. Human Genetics, 1981, 59:187–193.

Rønne M. Chromosome preparation and high resolution banding techniques: a review. Journal of Dairy Science, 1989, 72:1363–1377.

The reference literature to claim that diploid cells used for vaccine production are safe from the point of view of genetic stability is outdated. As early as 40 years ago (1976), the first genetic abnormalities, which were considered negligible for vaccine safety, were found, and from what is reported in the WHO guideline since then no updates have been made with new sequencing technologies, particularly in NGS, which is moreover cheap and rapid, with the result that progressively more and more genetically modified DNA in uncontrolled amounts has been allowed by agencies in vaccines administered for decades.

IMPURITIES OF BIOLOGICAL MATERIAL

FEDERAZIONE RINASCIMENTO ITALIA

Dr. T. DEISHER (letter to housekeepers - April 8, 2019)

Injecting our babies with human fetal DNA contamination carries the risk of causing two established diseases:

- **Insertional mutagenesis: human fetal DNA incorporates into the child's DNA causing mutations. Gene therapy using homologous recombination of small fragments has demonstrated that amounts as small as 1.9 ng/mL of DNA fragments result in insertion into the genome of stem cells in 100% of injected mice. Levels of human fetal DNA fragments in our children after vaccination with MMR, VARIVAX (varicella) or hepatitis A vaccines reach levels above 1.9 ng/mL.**

- **Immunopathology: fetal human DNA stimulates the immune system's reaction to attack the baby's/girl's body.**

NOT BORN WITH IT

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60% showed improvement at 6 months with only a single umbilical cord blood infusion of their own banked cord blood, with no conditioning.

If the mutations in the children's blood occurred during in utero development, then the umbilical cord blood would have those mutations and would not have helped these children.

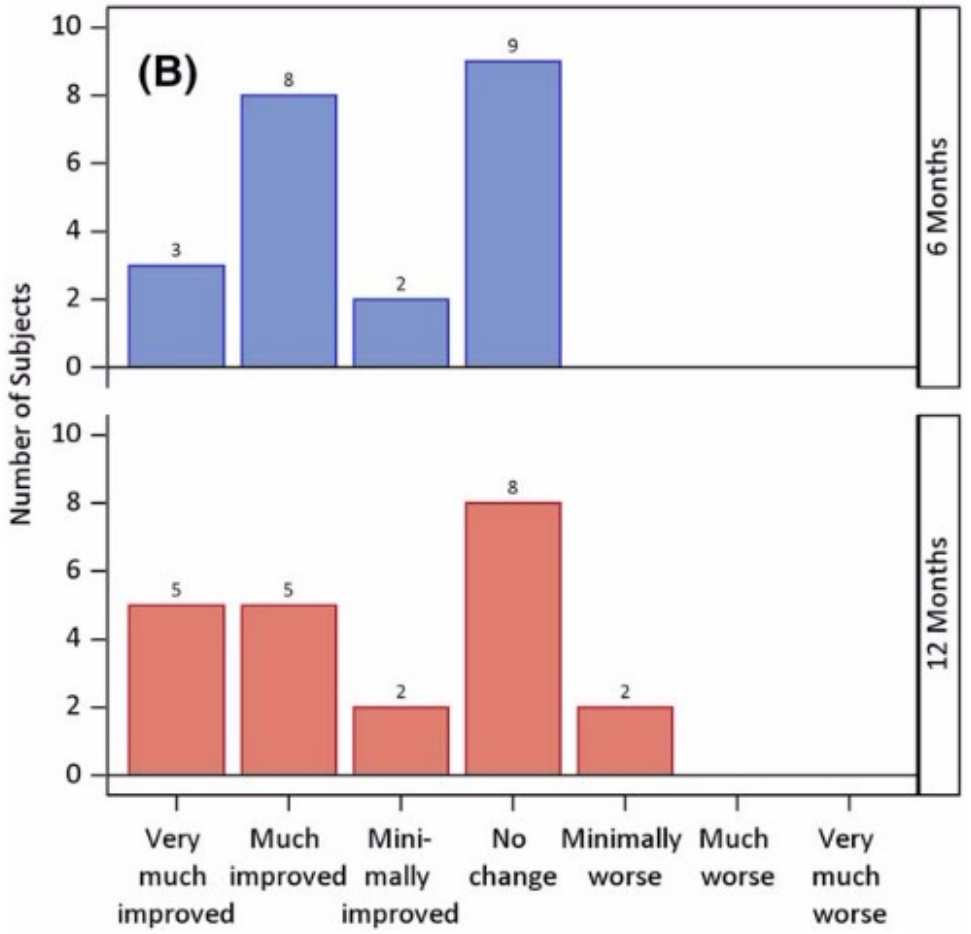

Figure 3. Global Impression Scale (GCI). **(A)**: CGI-Severity over time. **(B)**: CGI-Improvement over baseline as assessed at 6 and 12 months.

COULD FETAL MANUFACTURED VACCINES MUTATED HUMAN STEM CELLS?

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Not only do primitive human cells take up DNA into the nucleus, the DNA is also readily inserted into the genome.

Table 2: DNA uptake in Various Cell lines

	Spontaneous Cellular uptake	Spontaneous Nuclear uptake	Incorporation in Genomic DNA
HFF1	Yes	Yes	Not Done
NCCIT	Yes	Yes (variable)	0.0026pg per cell 24 hrs 0.04pg per cell 48 hrs

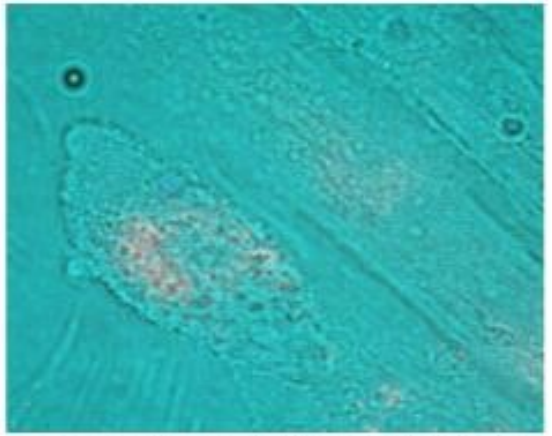

Fig 1. HFF1 spontaneous cellular and nuclear DNA uptake (bright field & Cy3 red combined).

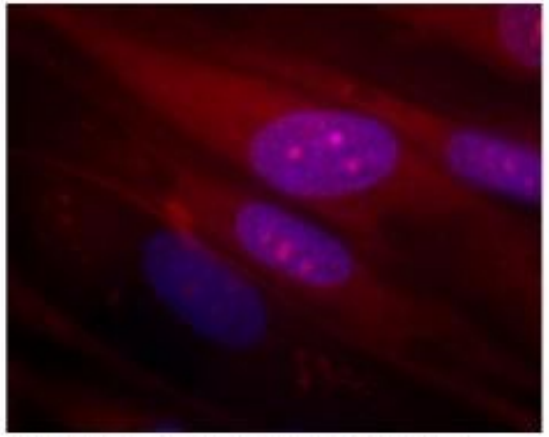

Fig 2. HFF1 cellular and nuclear DNA uptake after permeabilization with saponin. (Cy3 red & nucleus blue combined)

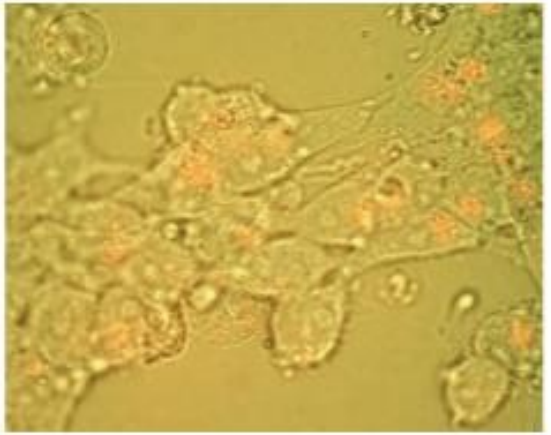

Fig 3. NCCIT spontaneous cellular DNA uptake (bright field & Cy3 red combined)

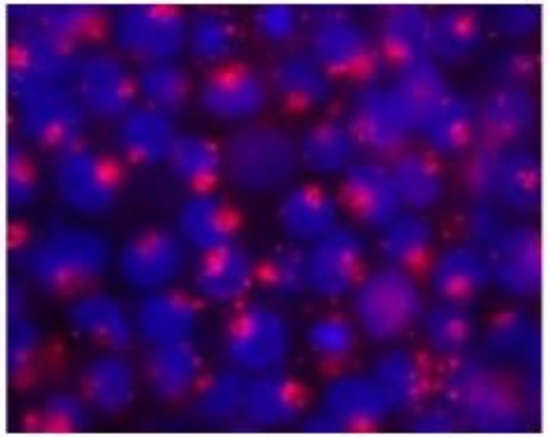

Fig 4. NCCIT cellular DNA uptake after lipopolysaccharide activation (Cy3 red & nucleus blue combined)

COULD FETAL MANUFACTURED VACCINES MUTATED HUMAN STEM CELLS?

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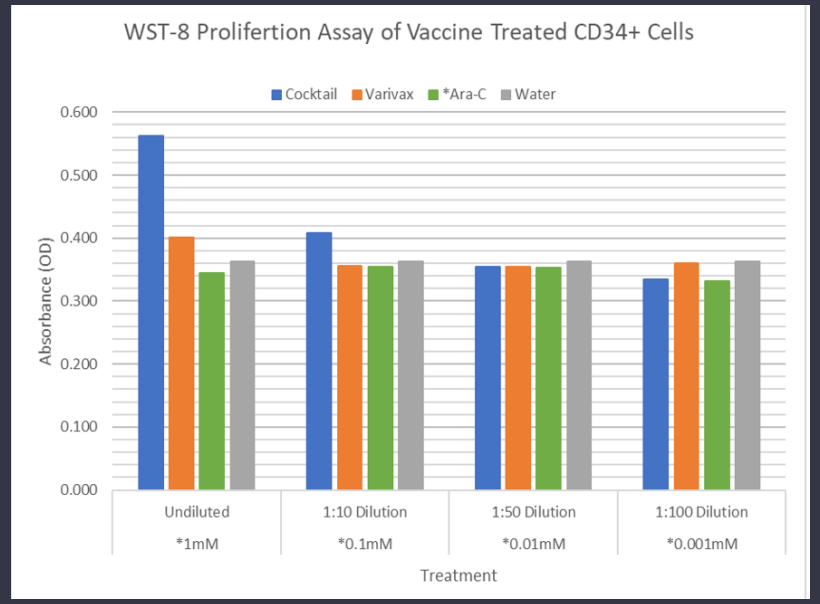
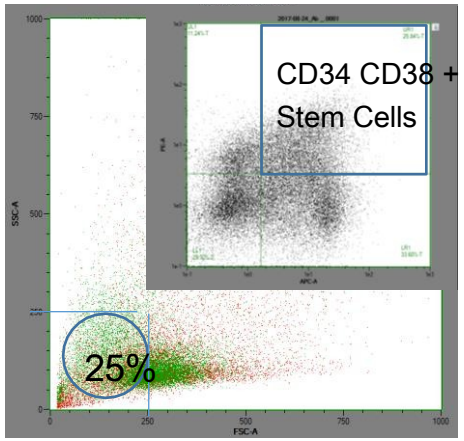
7 days after vaccine addition, undiluted vaccine cocktail treated wells have higher metabolic activity

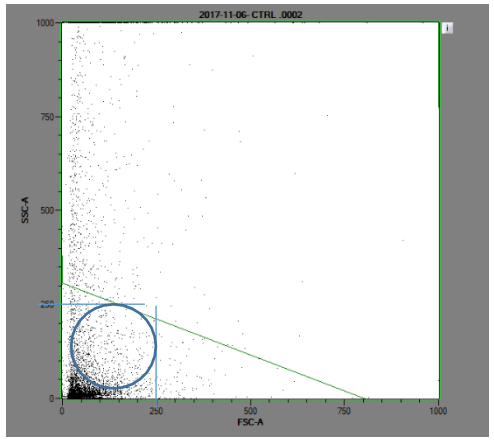


Human stem cells do not survive in culture Without supplements unless a mutation event occurs.

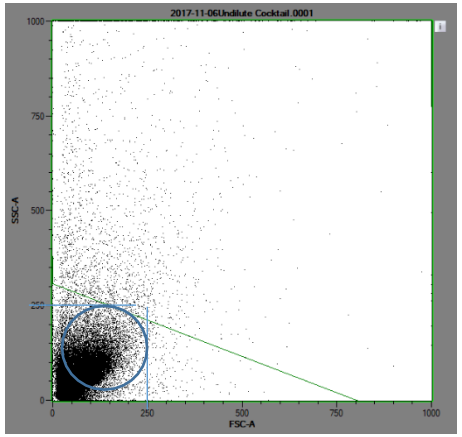
Starting Stem Cells



Day 80, stem cells die off



Day 80, Vaccines & stem cells



COULD FETAL MANUFACTURED VACCINES MUTATED HUMAN STEM CELLS?

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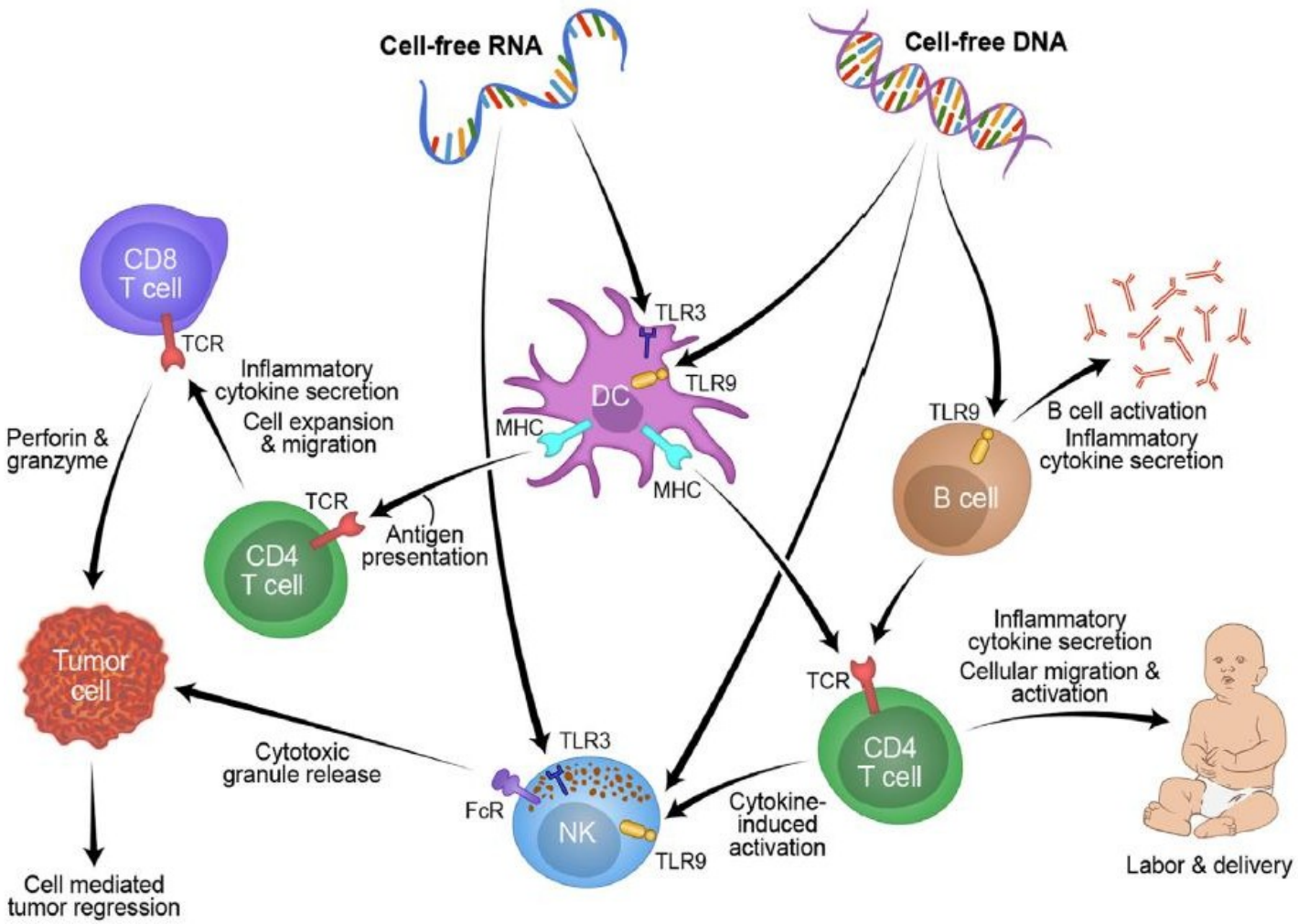
Immune reactivation by cell-free fetal DNA in healthy pregnancies re-purposed to target tumors: novel checkpoint inhibition in cancer therapeutics
E. Enniga et al Front. Immunol, Aug. 26, 2015

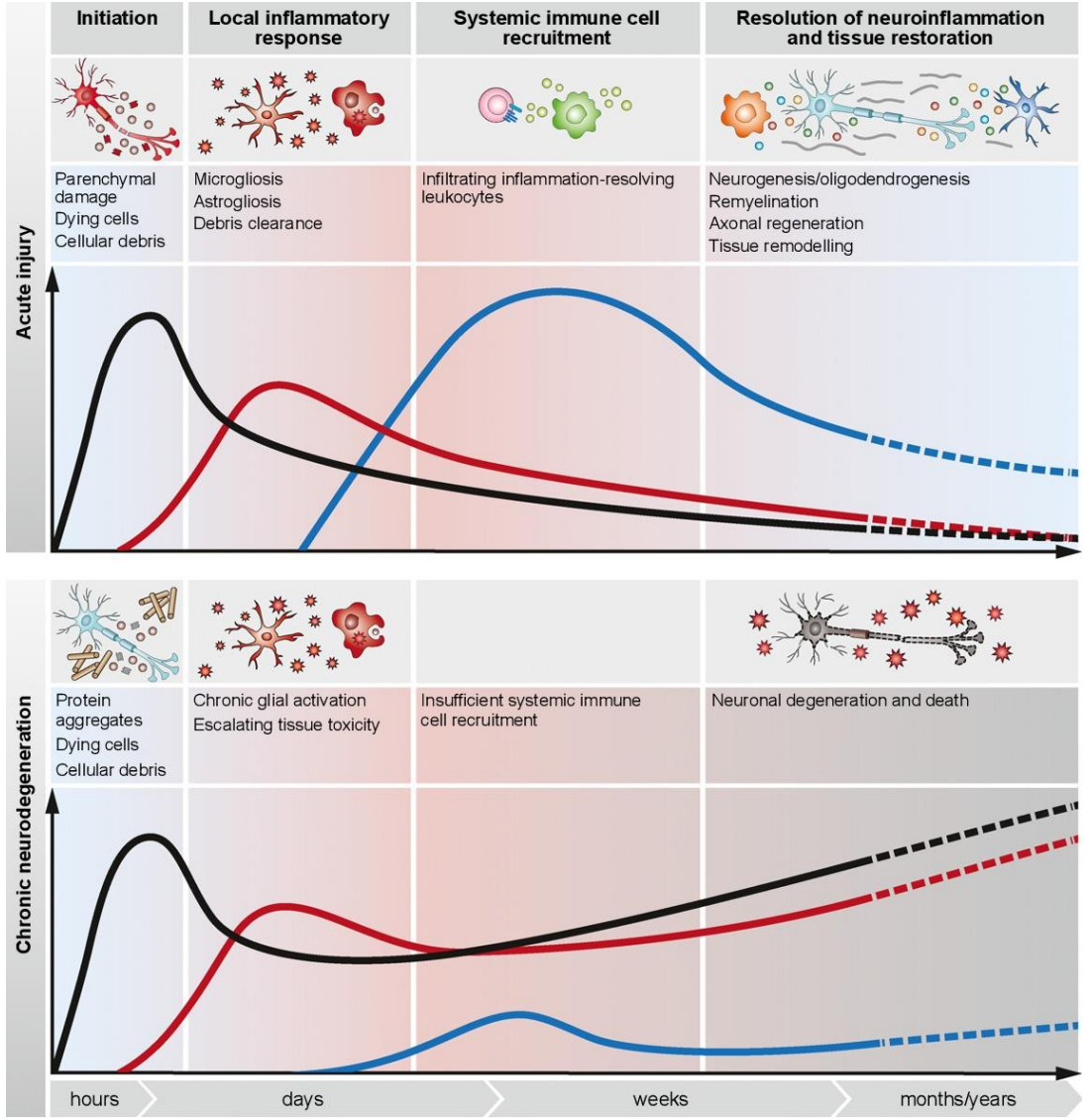
Dysregulation of TLR9 in neonates leads to fatal inflammatory disease driven by IFN-γ
Alison G. et al
PNAS February 11, 2020 117 (6) 3074-3082

The Role of Toll-Like Receptors in Autoimmune Diseases through Failure of the Self-Recognition Mechanism.
Farrugia M, Baron B.
Int J Inflam. 2017;2017:8391230



Local and systemic cell response immune to acute or chronic CNS damage.

In neuroinflammatory situations, both acute (top) and chronic (bottom), CNS parenchymal damage (black line) leads to glial cell activation and local inflammatory response (red line). In response to acute CNS damage, circulating leukocytes are recruited to the CNS (blue line) and participate in the resolution of the innate inflammatory response.
When this response is not resolved, it can lead to chronic neuroinflammation, associated with increasing toxicity and neuronal death, as in chronic neurodegenerative diseases; the lack of resolution reflects insufficient recruitment of immune cells that resolve systemic inflammation in the central nervous system.

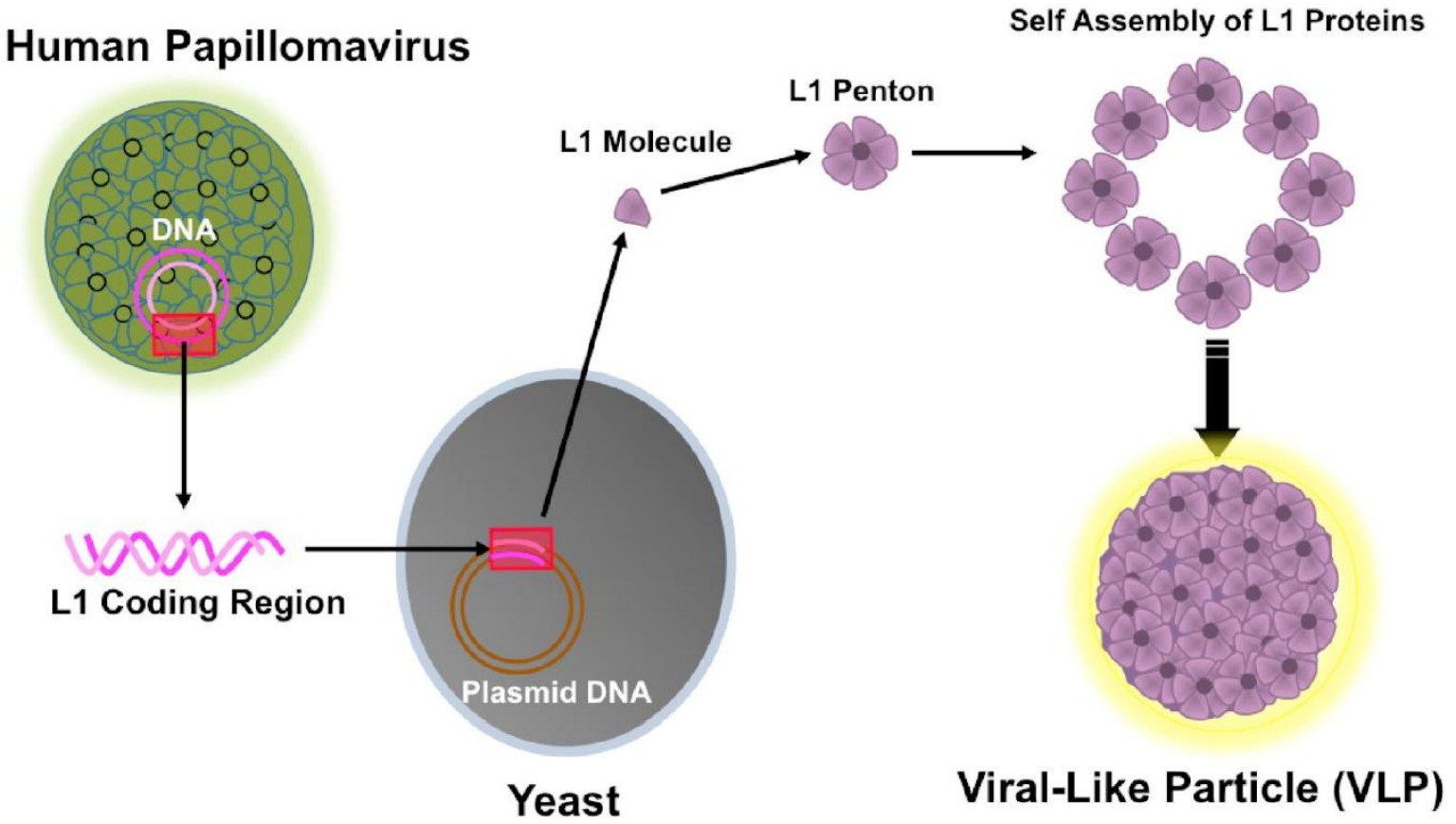


IMPURITIES OF BIOLOGICAL MATERIAL

FEDERAZIONE RINASCIMENTO ITALIA

GARDASIL 9: L1 fragments of the papilloma virus genome. L1 fragments 18, 16, 6 were sequenced.
The implications related to the presence of these fragments are those already reported by Prof. Lee in his publications, namely that the presence of aluminum stabilizes their degradation, enhancing their ability to activate a potent long-term inflammatory response and to be transported through the lymphatic system into macrophages in various districts of the body.

FDA RESPONSE: 10/21/2011
"The presence of these DNA fragments is expected, does not pose a risk to vaccine recipients, and is not a safety factor."



EFFECTIVENESS

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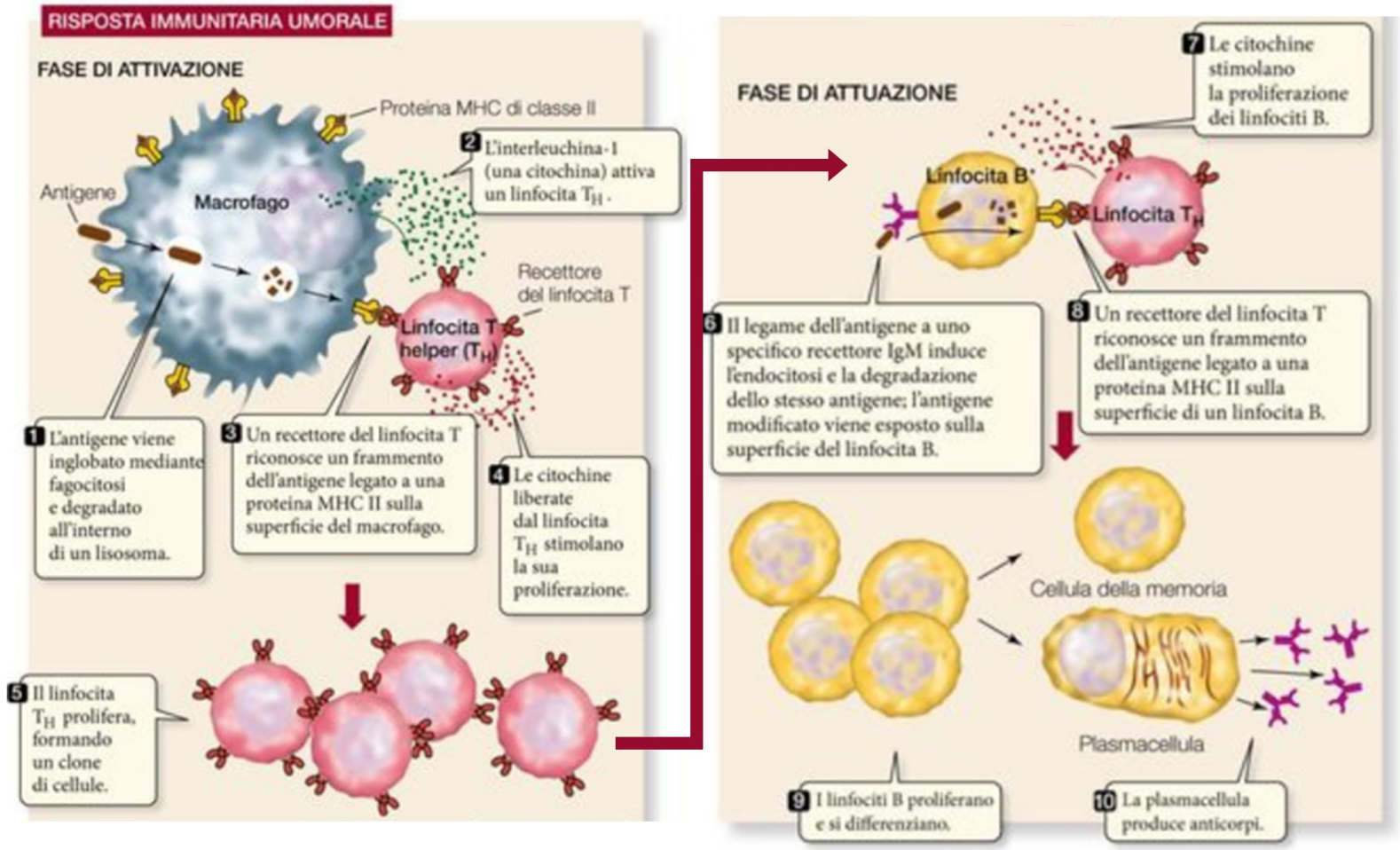
In vaccines containing aluminum adjuvant, the presence of insoluble and indigestible aggregates has been noted, particularly in Infanrix hexa. Since antigens must be digested by macrophages in order to stimulate specific antibody production, this poses an efficacy problem.

Security issue:
these aggregates could be Transported with aluminum through the lymphatic system to different districts of the body



EFFECTIVENESS

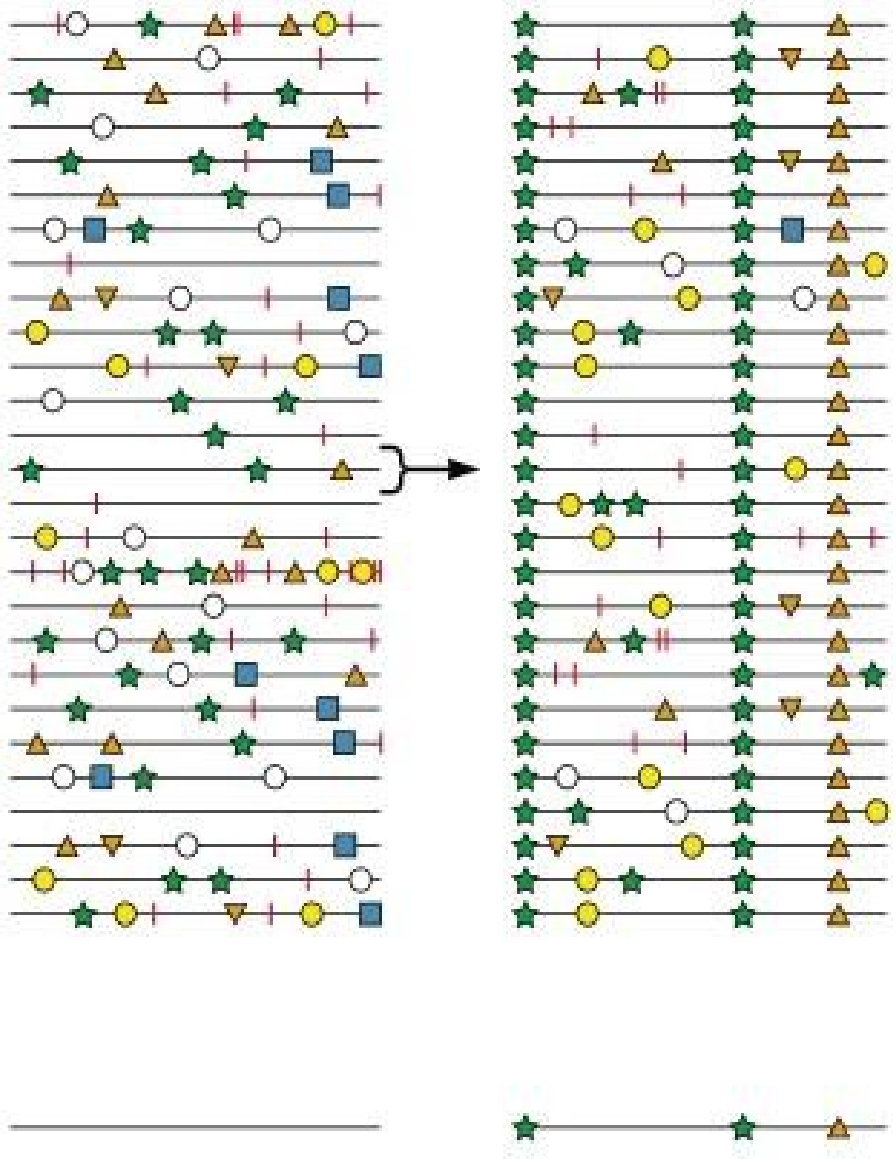
FEDERAZIONE RINASCIMENTO ITALIA

Forty minor variants have been identified in the vaccine mumps genome compared with the reference genome used for the analysis (Jeryl- Lynn vaccine genome), most located in the gene-NP (nucleocapsid protein) and having low impact on the protein (synonymous variants, i.e., leading to no amino acid change) or moderate impact ('missense' variants, i.e., leading to amino acid change, i.e., nonsynonymous mutations) in the protein
coded. Comparison of the minor variants of mumps found in the two batches reveals 4 differences

A total of 245 variants were identified in the vaccine varicella genome compared to the reference genome used for analysis (wild genome of the Dumas strain). Of these variants, 154 are 'major' variants from the wild-type virus supported by the totality of sequences spanning the polymorphic site, while the remaining 91 are minor variants

EMA ANSWER.
Changes in the genomic sequence of a virus do not necessarily impact the antigenic determinants required to stimulate the desired immune response in humans

efficacy: vaccine resistance
Safety: reversion of virulence and increased complications from infection



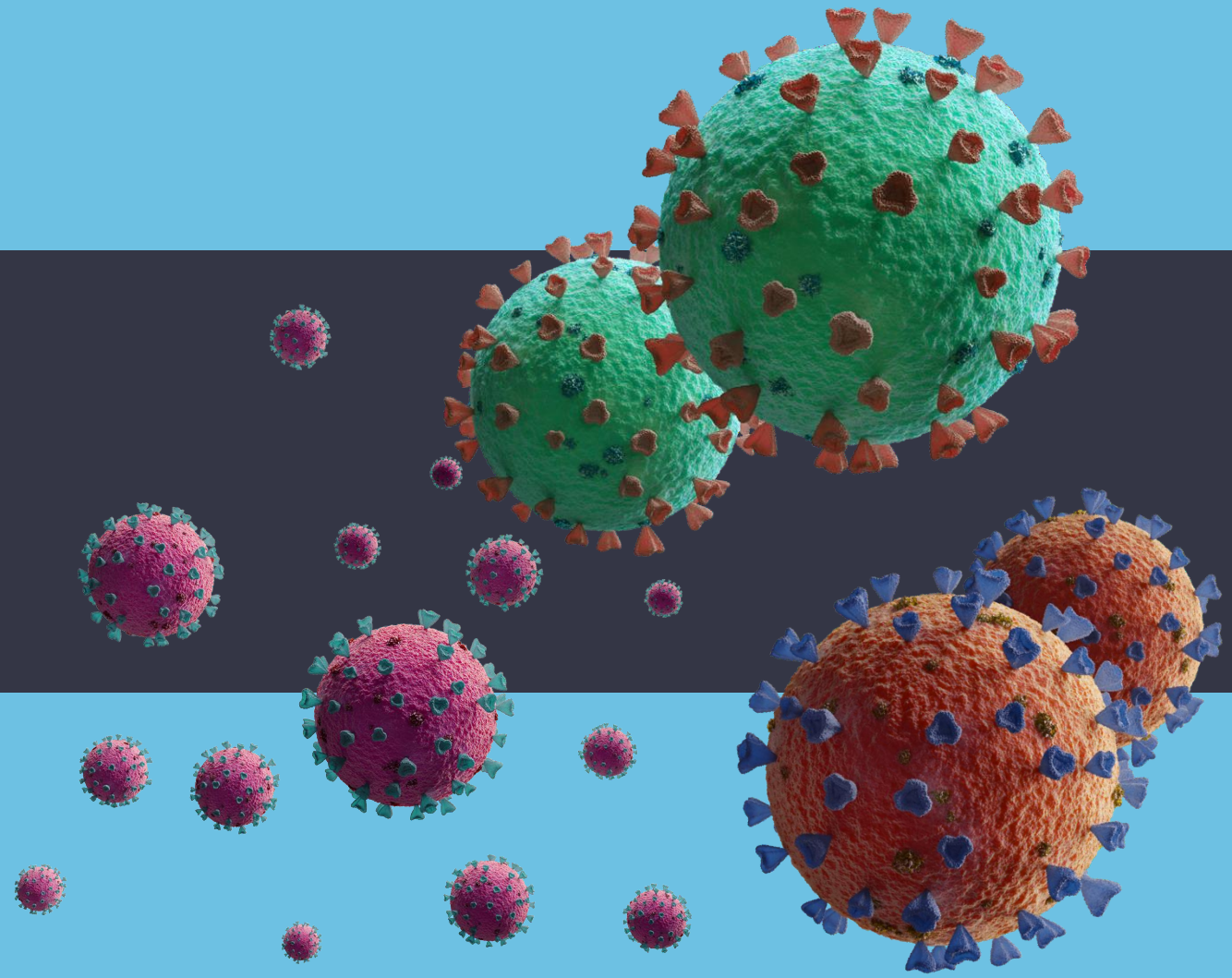
Results Analysis	Infanrix hexa	Hexyon	Priorix tetra	Gardasil 9
Antigens	Protein antigens (none of them) were not identified. Presence of an insoluble and indigestible macromolecule that could not be sequenced. MALDI-TOF analysis of the macromolecule: could not be studied because it encompassed the analysis matrix. "Prionic" behavior? by. deepen in the future.	Only 3 out of 6 protein antigens were identified and sequenced (tetanus toxoids, diphtheria and pertussis were identified). "Prionic" behavior? (antigens aggregate and precipitate over time becoming insoluble): to be further investigated in the future.	Only 3 of the 4 attenuated viruses were identified (and sequenced): rubella was detected in very low copy number (insufficient). From sequencing: the varicella, mumps and measles viruses have mutations that make the efficacy of the vaccine questionable. (quasi-species)	Only 7 of the 9 protein antigens were identified. (Strains 11 and 58 are missing) "Prionic" behavior? (antigens aggregate and precipitate over time becoming insoluble): to be further investigated in the future.
Candidate chemical contaminants (signals)	65 (35% known) PEG, formic acid	216 (30% known)	115-173 (29-43% known)	338 (22% known) Identification of APDB (illegal amphetamine) already notified to NAS on May 23, 2019
Candidate chemical toxins	8	16	no	10
Protein contaminants	no	no	Sarcoplasmin calcium-binding protein, Actin and Vimentin	no
Free peptide contaminants	16	no	no	no
Metals *	Aluminum equal to 723.5 µg ± 130 µg/dose Other metals under 5g/dose	Aluminum equal 546 µg ± 98.5 µg / dose Other metals under 5g/dose	Aluminum equal to 0.8 µg ± 0.15 µg / dose Other metals under 5g/dose	Aluminum equal to 416 µg ± 75 µg / dose Other metals under 5g/dose

**Aluminum, Hafnium, Antimony, Arsenic, Barium, Bismuth, Cadmium, Chromium, Chromium, Gallium, Indium, Iridium, Yttrium, Lanthanum, Mercury, Nickel, Palladium, Lead, Platinum, Copper, Rhodium, Ruthenium, Scandium,*
Tin, Strontium, Tantalum, Thallium, Tungsten, Vanadium

Results Analysis	Infanrix hexa	Hexyon	Priorix tetra	Gardasil 9
Residual DNA/RNA Coming from the culture cells.	Below the detectability limits of the instrument with standard fluorimetric methods (sensitivity limit 0.1 ng/µl) DNA of Monkey 4.69% (sensitivity DNA-seq library kit 10 pg)	DNA 6.88 ng; RNA 100.8 ng DNA and RNA from bacterial cultures. DNA/RNA from monkey (Vero cells) and from primates. Poliovirus 1 and 2 Editor's note: The material found was trace and degraded.	Total DNA: 1.7-3.7 µg/dose of which 80% human (human fetal DNA/RNA from MRC-5 cell line). Enough DNA to make it a main ingredient in the vaccine. Cell line genomic sequencing: found complete individual genome, highly genetically modified and potentially carcinogenic Other DNA: chicken	DNA Below the detectability limits of the instrument with standard fluorimetric methods (sensitivity limit 0.1 ng/µl) Human and mouse DNA (trace) RNA 8,732 ng
Adventitious viruses	no	Tetanus phage Vectors used for cloning	Human endogenous retrovirus K Avian leukosis virus HERV-H/env62	Molluscum contagious virus (Myoviridae) Beans Retrovirus (DNA): Murine leukemia virus Human endogenous retrovirus K Retrovirus (RNA): Murine leukemia virus
Other microbial contaminants	no	no	no	Yeast and its viruses
Processing residues of genetic material	no	no	no	DNA: L1 fragments (missing strain. 58; presence of strain 20) L1 fragments 18, 16, 6 (RNA) were sequenced.

FEDERAZIONE RINASCIMENTO ITALIA

INFECTION FROM SARS-COV-2



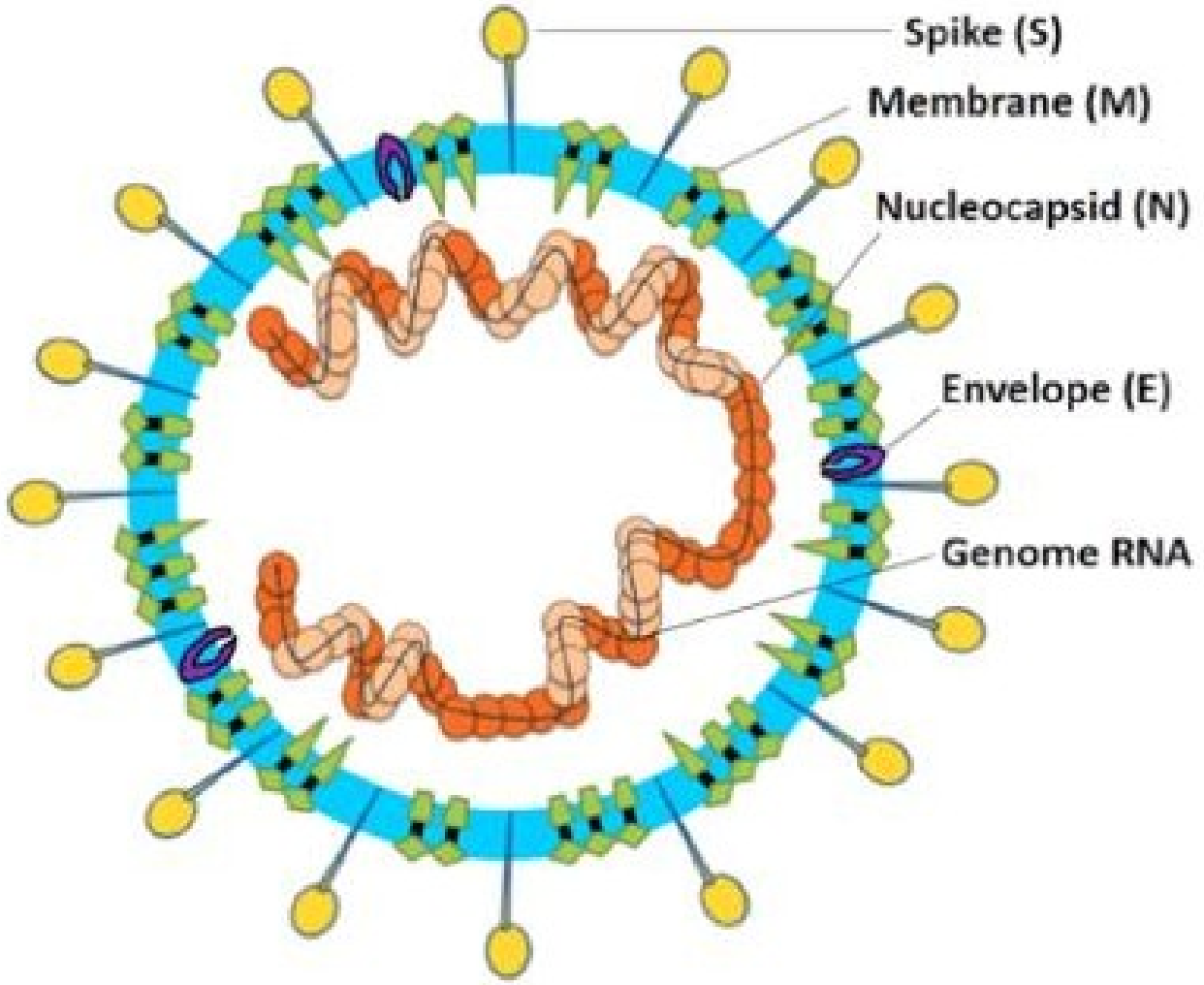
THE SARS-COV-2 VIRUS

SEVERE ACUTE RESPIRATORY SYNDROME BY CORONAVIRUS 2

FEDERAZIONE RINASCIMENTO ITALIA

SARS-Cov-2 is a beta-coronavirus consisting of single-stranded RNA with a lipid envelope containing four structural protein components, the most important of which is the S (Spike) protein

This protein determines the tropism of the virus and its pathogenicity, as it contains the binding site for the receptor for angiotensin-converting enzyme 2 (ACE2), which facilitates its entry into cells expressing it (particularly gut cells) and is the target of antibodies both produced following natural infection and vaccination.



STAGES OF INFECTION

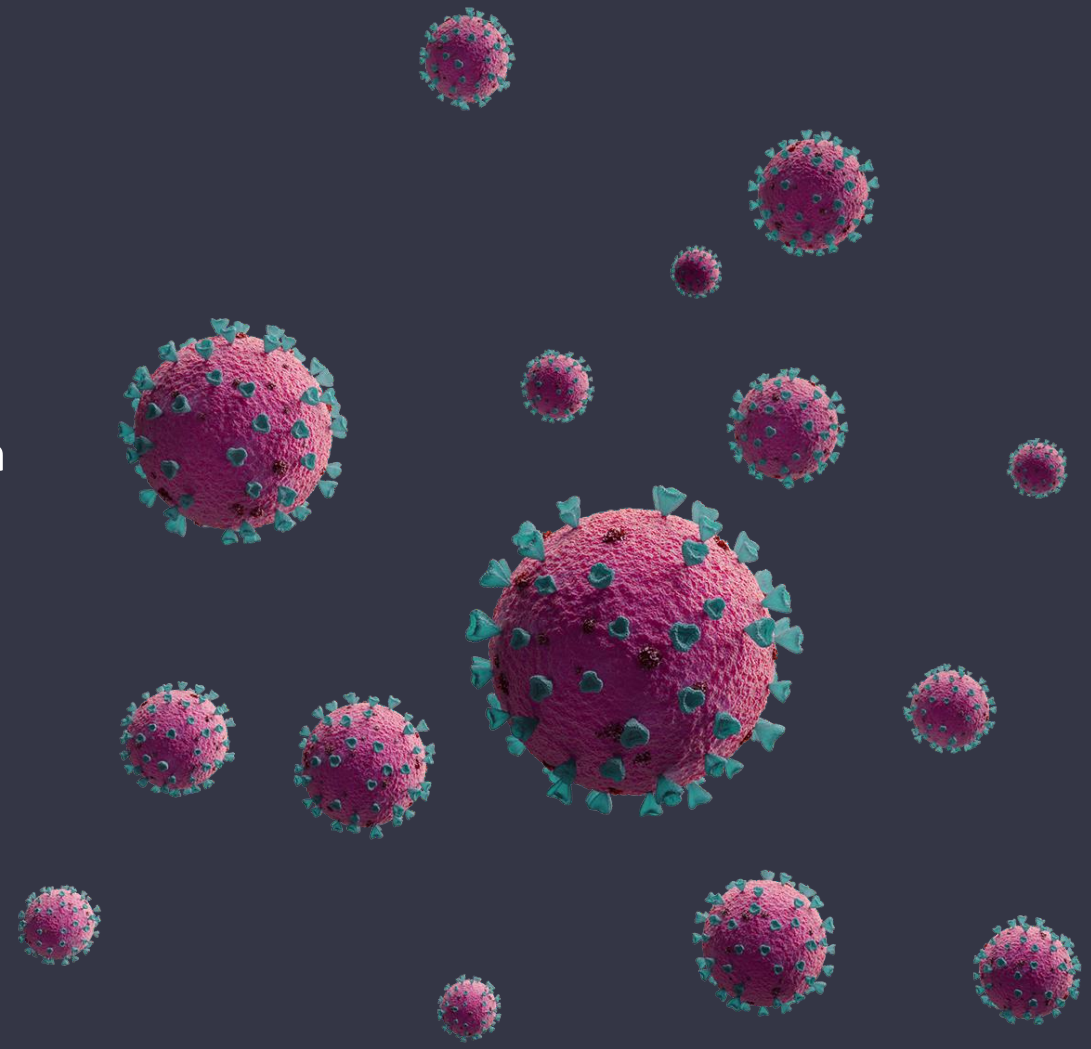
COVID-19 (CORONAVIRUS DISEASE 19)

FEDERAZIONE RINASCIMENTO ITALIA

VIRUS ENTRY

initiation of viral replication can cause death of infected cells, vascular leakage, and release of pro-inflammatory mediators with activation of an initial wave of inflammatory mediators

Depending on the intensity of this first inflammatory response the infection can be Asymptomatic or symptomatic.



STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)

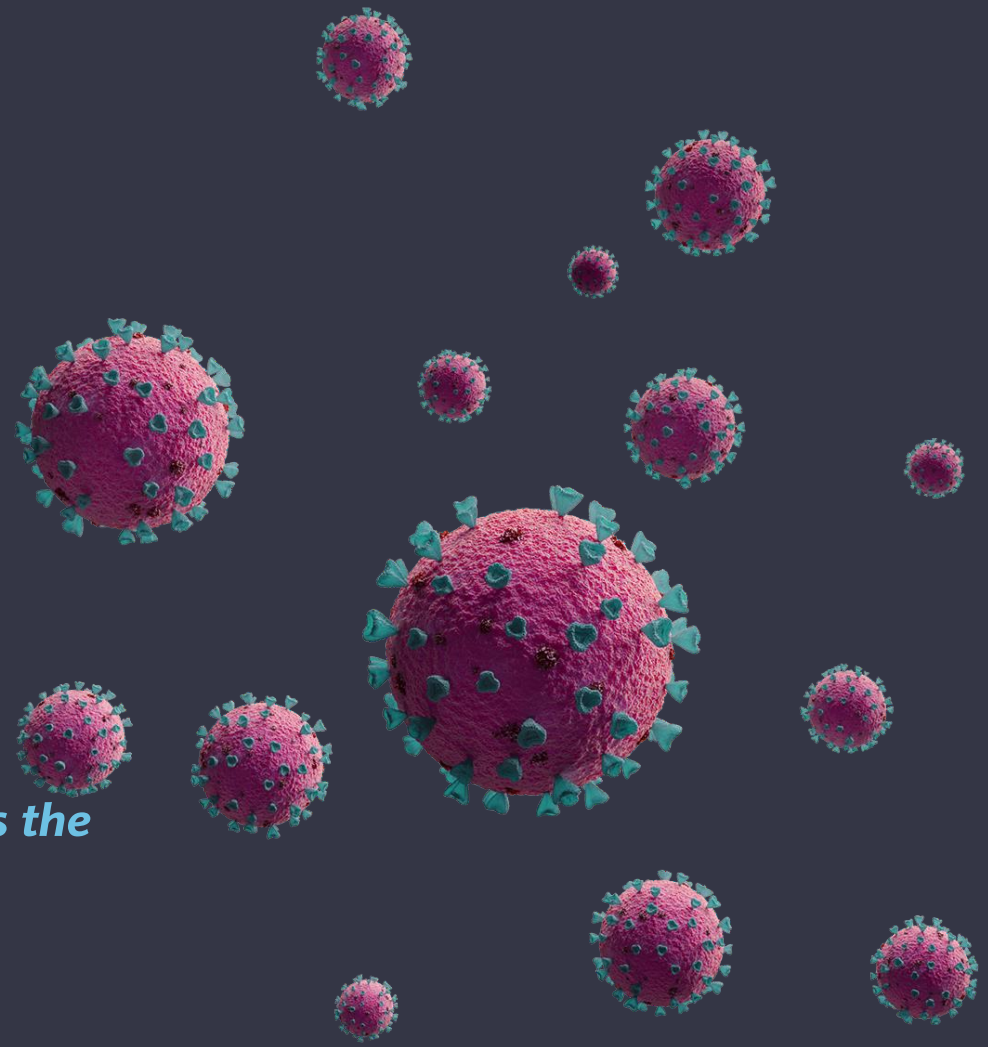
FEDERAZIONE RINASCIMENTO ITALIA

SYMPTOMATIC PHASE OF VIRAL INFECTION

SARS-CoV-2 infection results in a response lower antiviral characterized by low levels of Interferon-I (IFN-I) and IFN-III and by hyperinflammation due to elevated expression of inflammatory mediators and IL-6

Immunosuppression and lymphopenia may occur at this stage.

in a proportion of infected individuals, SARS-CoV2 evades the recognition by the immune system
Through the suppression of antiviral mechanisms, promoting the complication of the disease



STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)

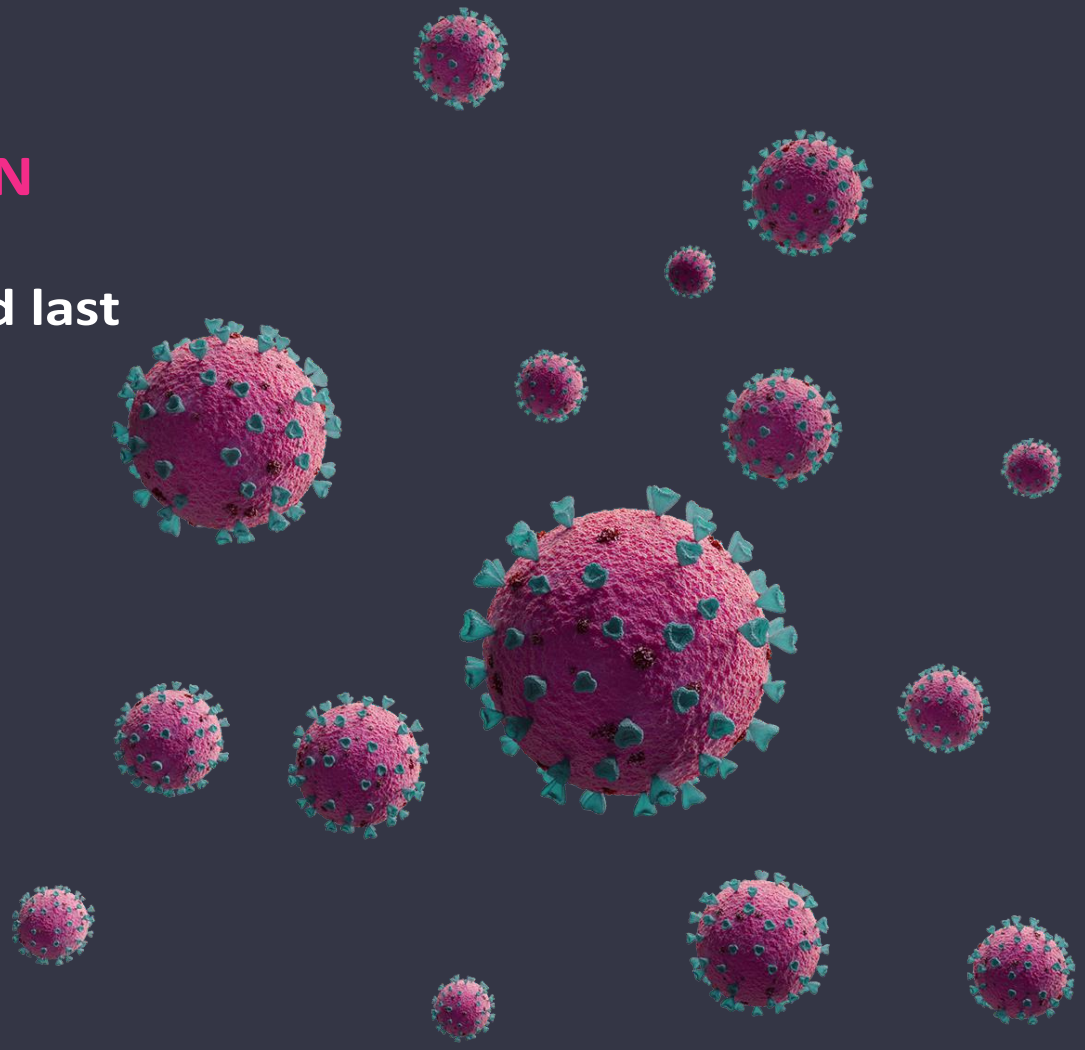
FEDERAZIONE RINASCIMENTO ITALIA

SYMPTOMATIC PHASE OF VIRAL INFECTION

Symptoms are similar to flu-like symptoms and last about 7-10 days.

The innate immune system reacts to Block the replication of the virus.

If the person has an efficient immune response the infection resolves without complications.



STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)

FEDERAZIONE RINASCIMENTO ITALIA

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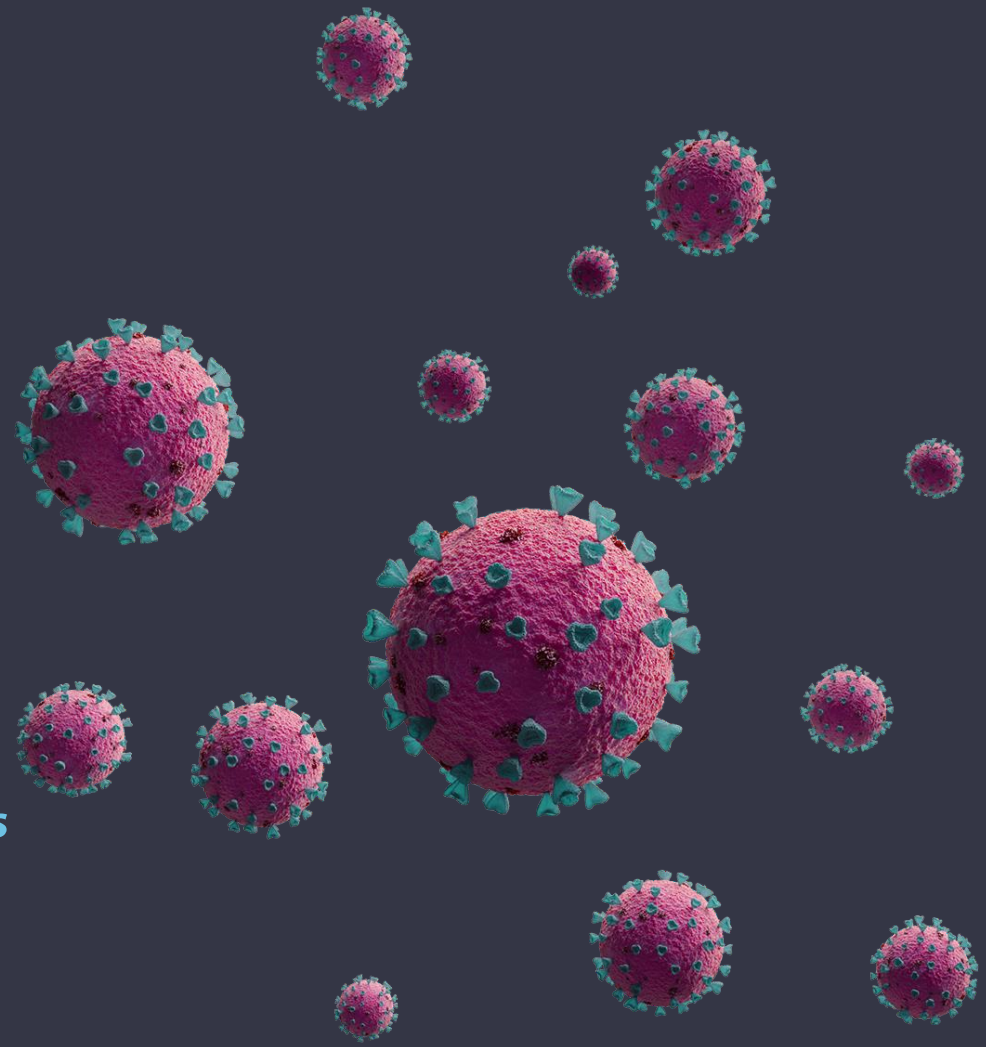


STAGES OF INFECTION

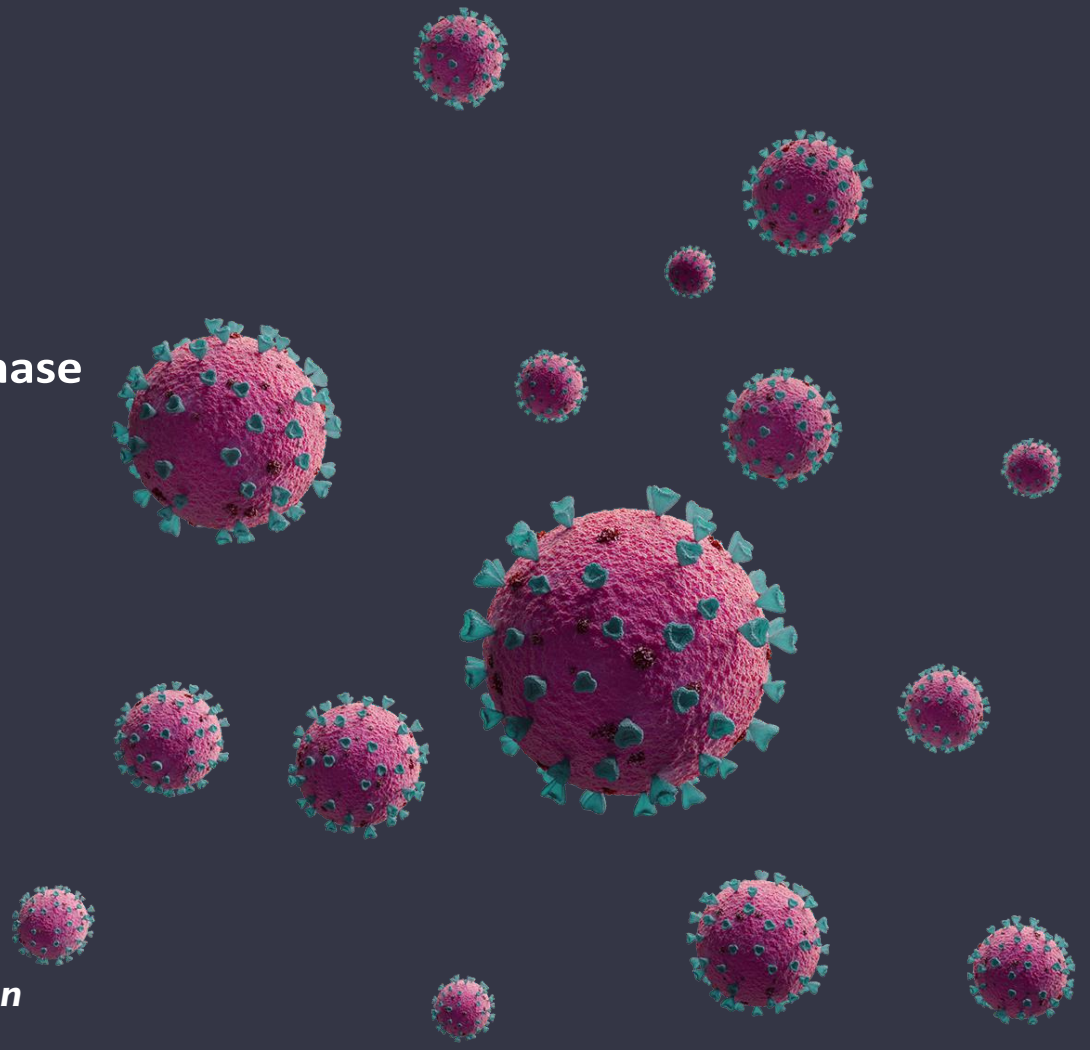
COVID-19 (CORONAVIRUS DISEASE 19)

FEDERAZIONE RINASCIMENTO ITALIA

COMPLICATION OF INFECTION

Onset of pulmonary symptoms. The immune system overreacts to uncontained infection during the first phase with the production of high amounts of inflammatory mediators.
Disseminated intravascular coagulation is one of the diseases that lead to patient death if not treated appropriately at an early stage.

If pharmacological intervention is not taken, the complication can rapidly progress to the most severe stage, with multi-organ damage.



** Only about 30-50% of patients are feverish at the time of admission*

STAGES OF INFECTION

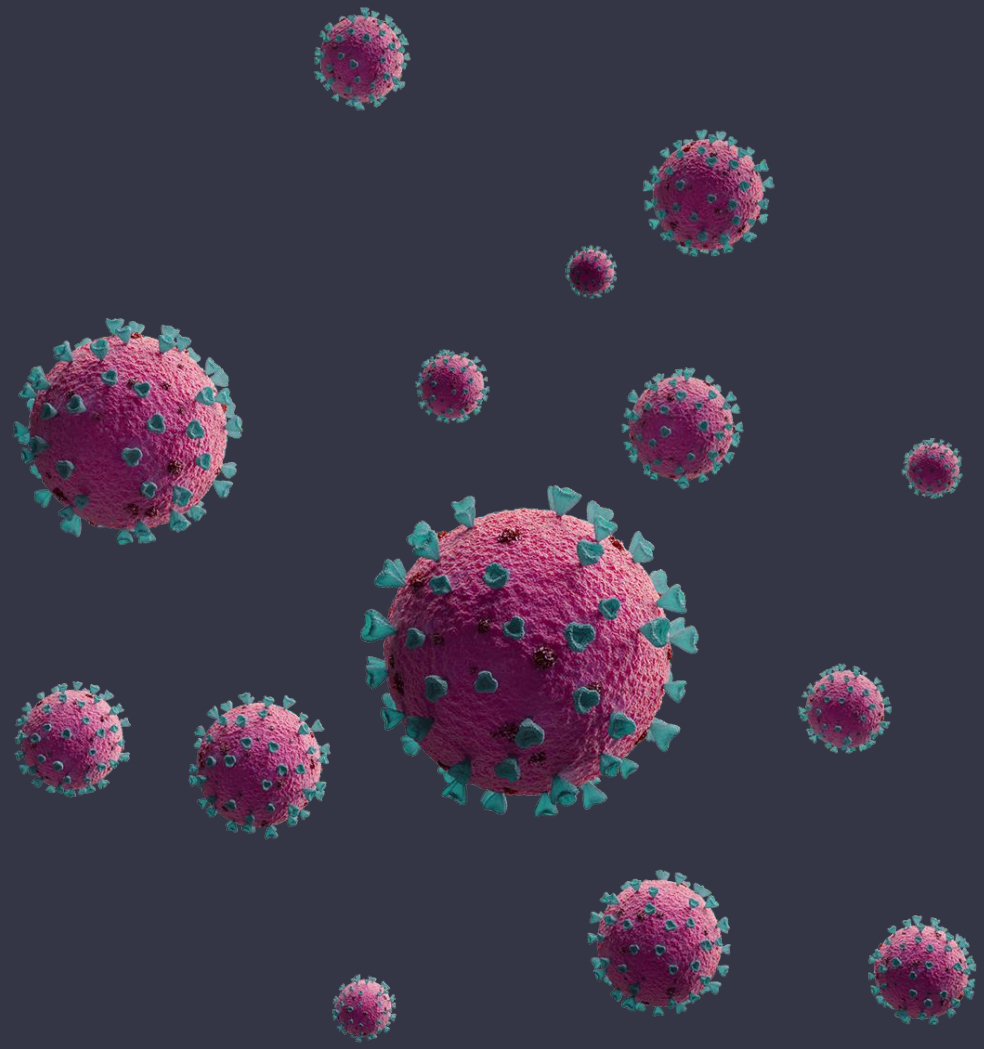
COVID-19 (CORONAVIRUS DISEASE 19)

FEDERAZIONE RINASCIMENTO ITALIA

VERY SEVERE/FATAL PHASE

When adaptive immune cells (T lymphocytes play a central role in this phase) become activated, they trigger a "second wave" of inflammation (cytokine storm syndrome and its subtypes), which can be seen in COVID-19 patients who have rapid deterioration after 7-10 days of infection, correlated with increased levels of acute phase markers (ESR, CRP, ferritin), coagulopathy (elevated D-dimer titers, disseminated intravascular coagulation), and cell lysis (CK, LDH)

The manifestations of multiorgan damage are related to the attack of the body's structures by the immune system.



STAGES OF INFECTION

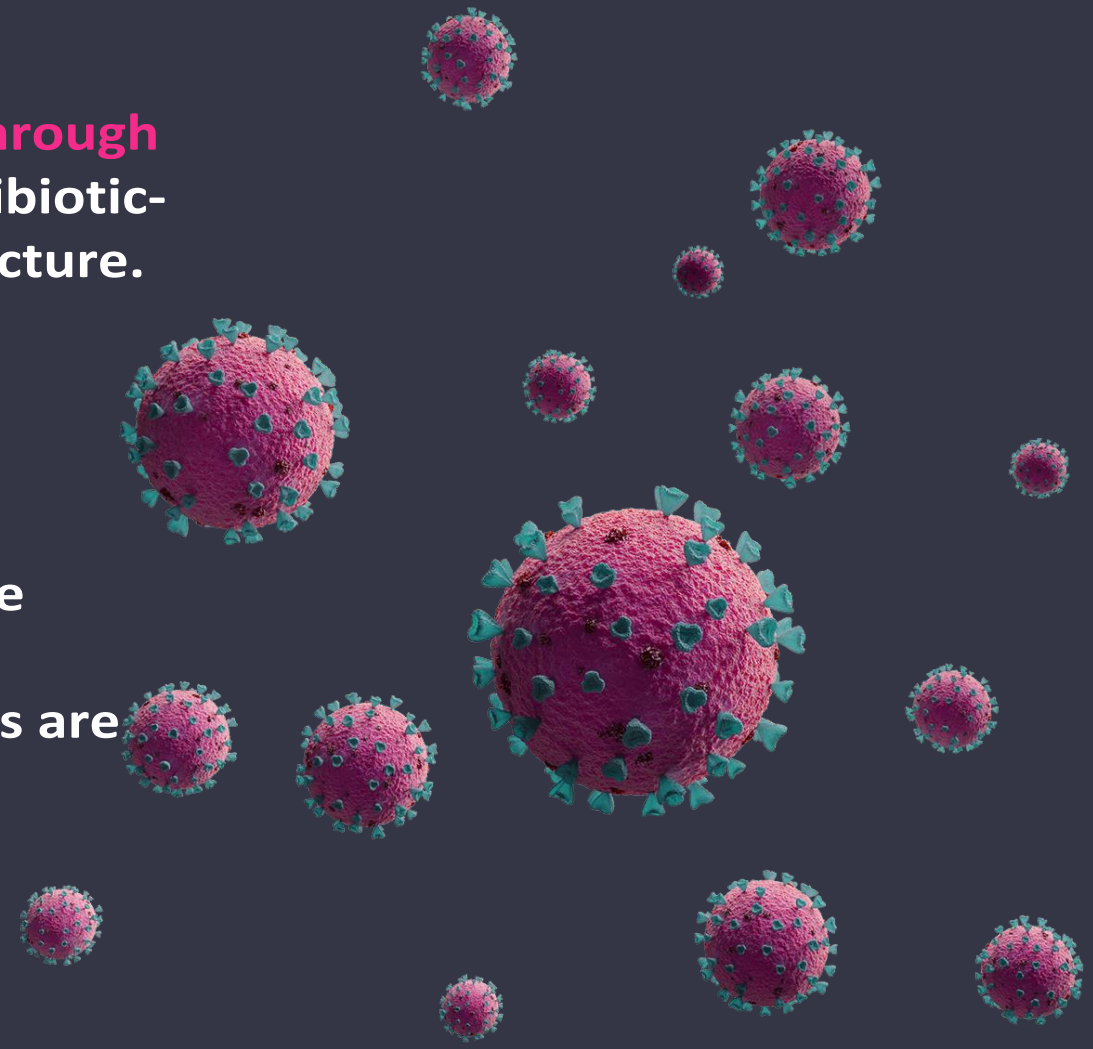
COVID-19 (CORONAVIRUS DISEASE 19)

FEDERAZIONE RINASCIMENTO ITALIA

During the complication, the person may go through bacterial co-infections (especially hospital antibiotic-resistant) that further aggravate the clinical picture.

POST-INFECTIOUS PHASE

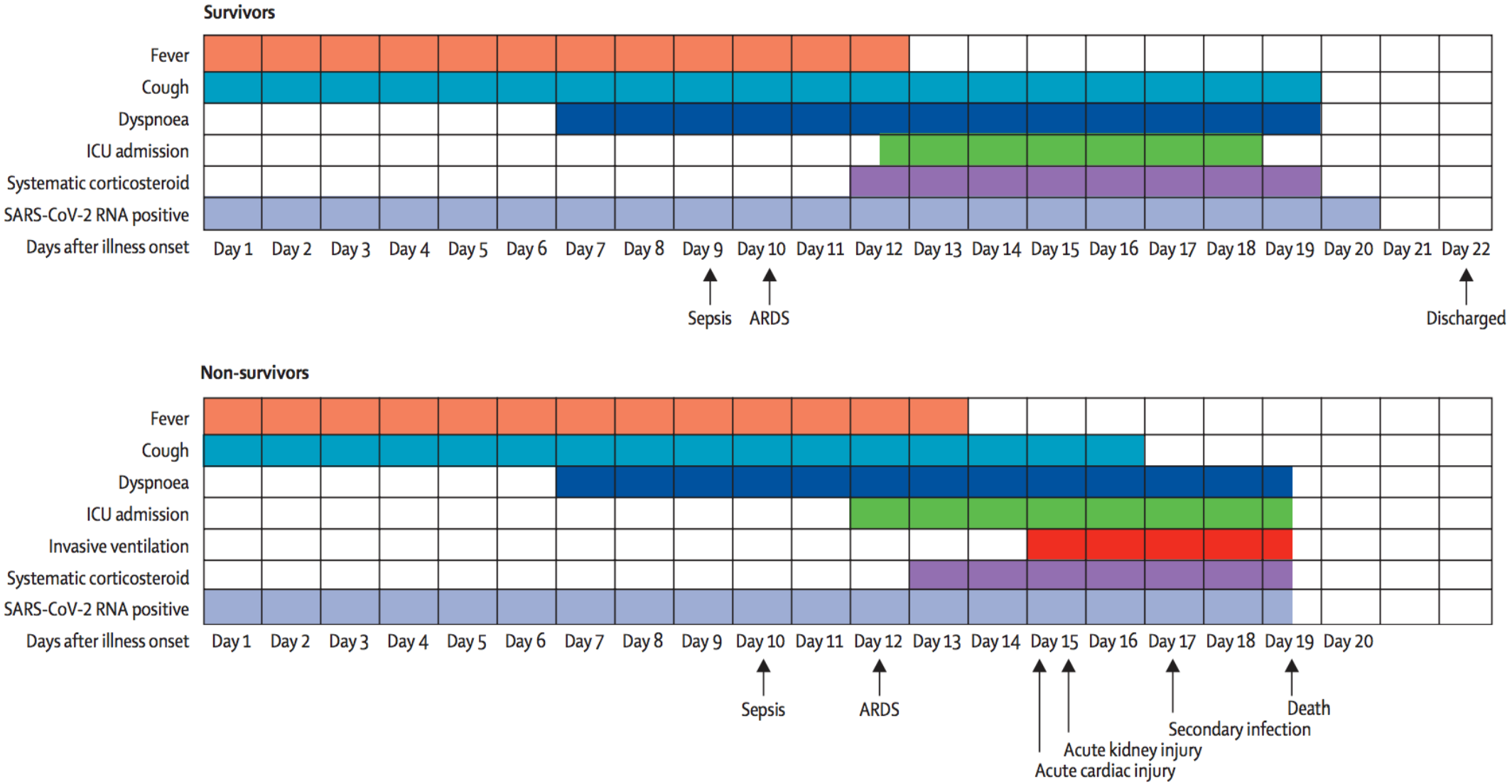
The existence of autoantibodies suggests an increased risk of autoimmune diseases in some patients with COVID-19, and further studies in larger groups of patients and long-term studies are certainly recommended to clarify these observations.



STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)

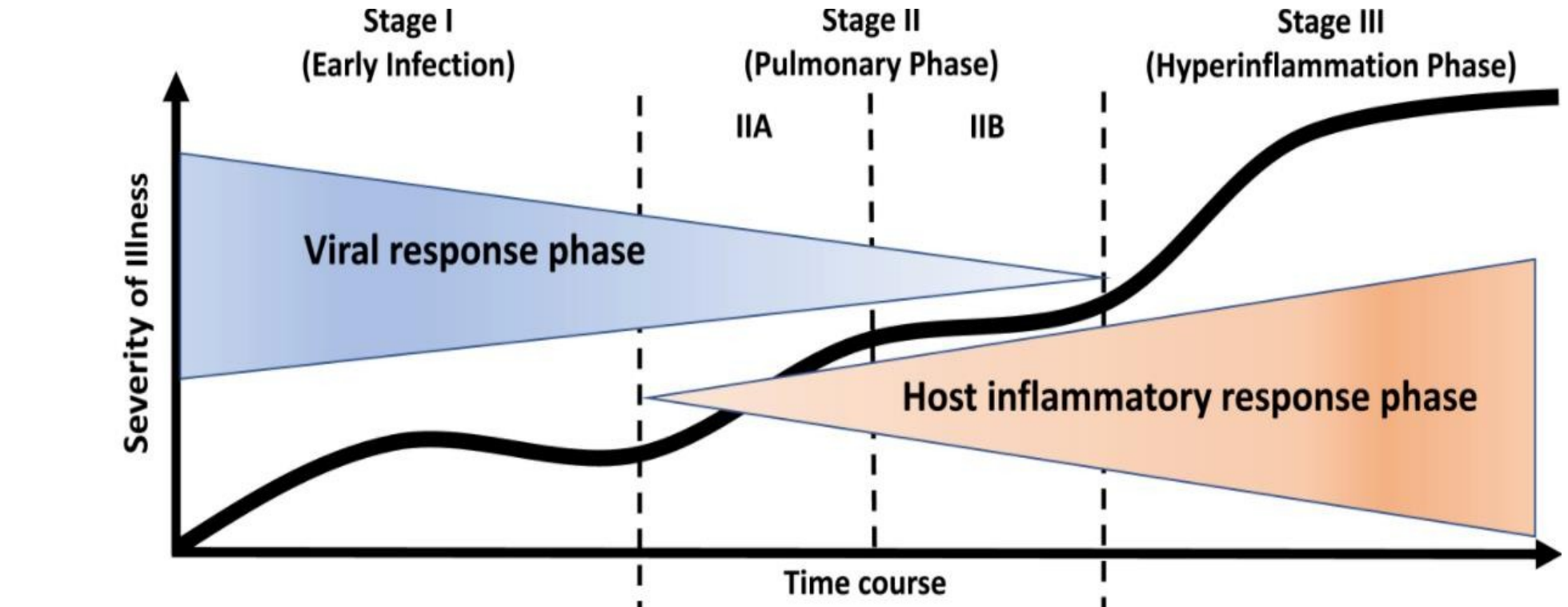
FEDERAZIONE RINASCIMENTO ITALIA



STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)

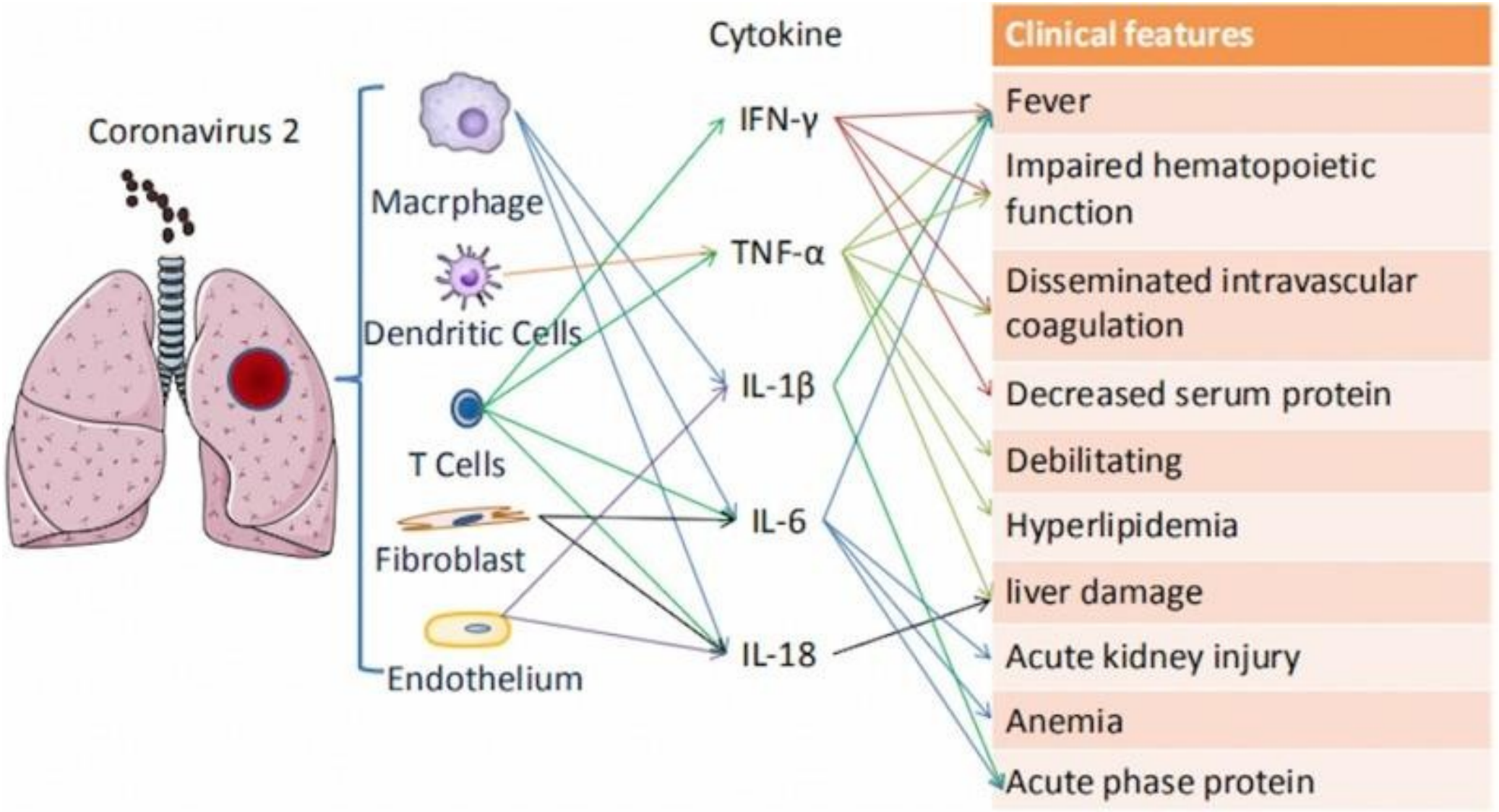
FEDERAZIONE RINASCIMENTO ITALIA



Clinical Symptoms	Mild constitutional symptoms Fever >99.6˚F Dry Cough, diarrhea, headache	Shortness of Breath Hypoxia (PaO2/FiO2≤300mmHg)	ARDS SIRS/Shock Cardiac Failure
Clinical Signs	Lymphopenia, increased prothrombin time, increased D-Dimer and LDH (mild)	Abnormal chest imaging Transaminitis Low-normal procalcitonin	Elevated inflammatory markers (CRP, LDH, IL-6, D-dimer, ferritin) Troponin, NT-proBNP elevation

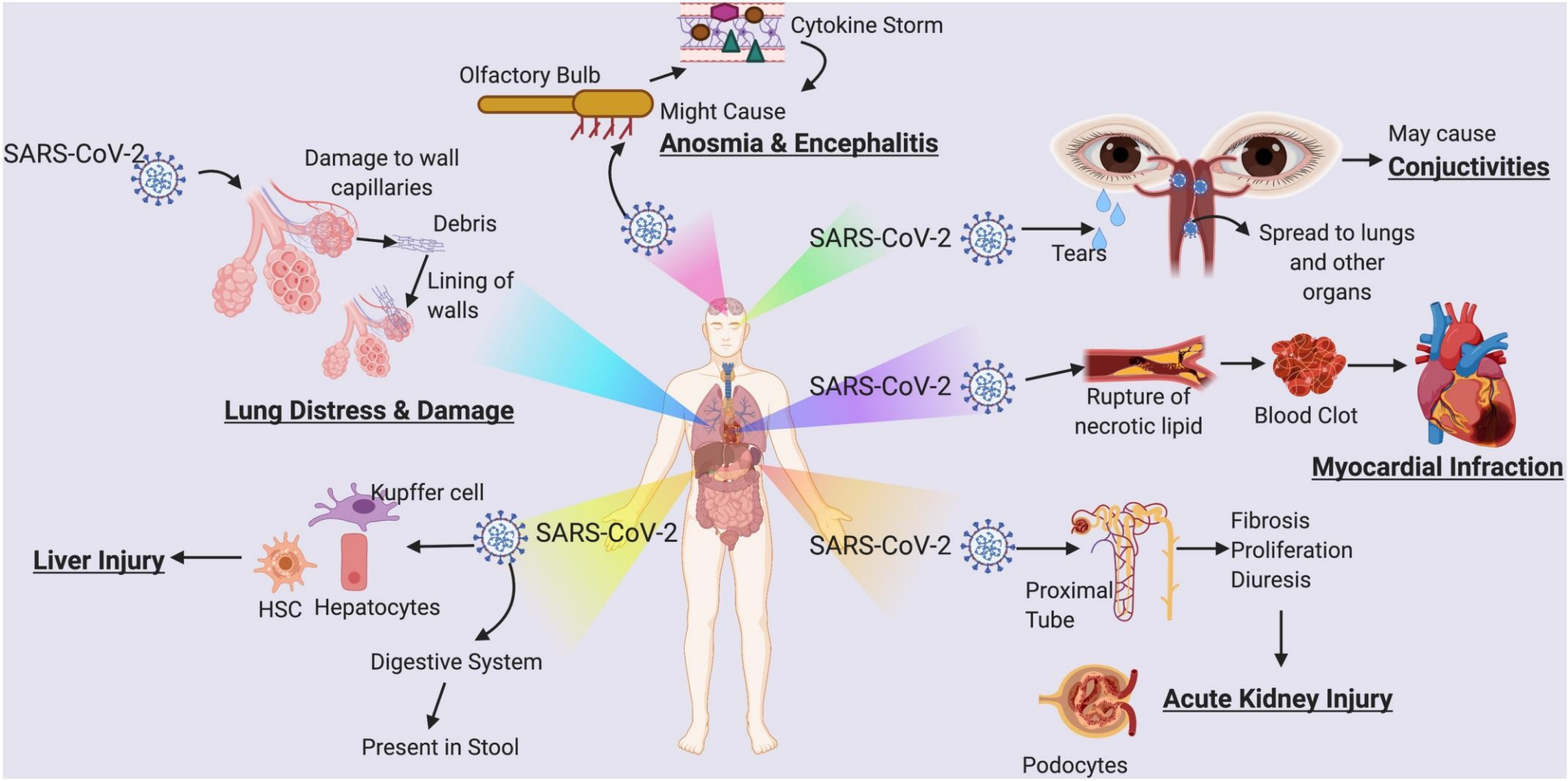
STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)



STAGES OF INFECTION

COVID-19 (CORONAVIRUS DISEASE 19)



Balachandar V, Mahalaxmi I, Subramaniam M, et al.
Follow-up studies in COVID-19 recovered patients - is it mandatory?
Sci Total Environ. 2020;729:139021. doi:10.1016/j.scitotenv.2020.139021

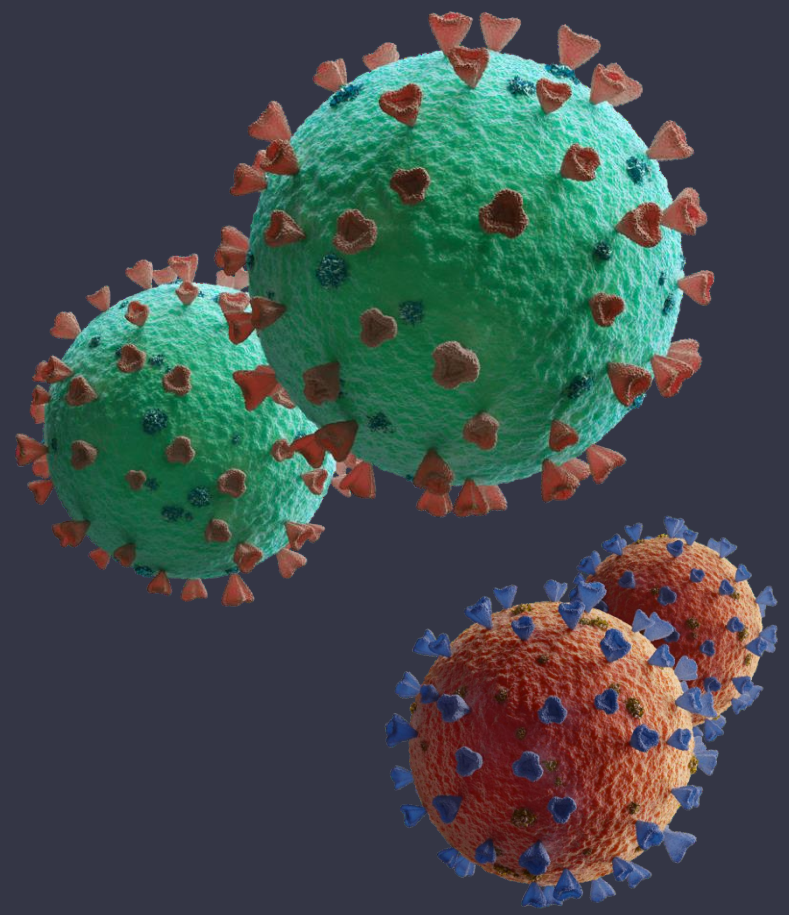
VIRAL EVASION MECHANISMS

FEDERAZIONE RINASCIMENTO ITALIA

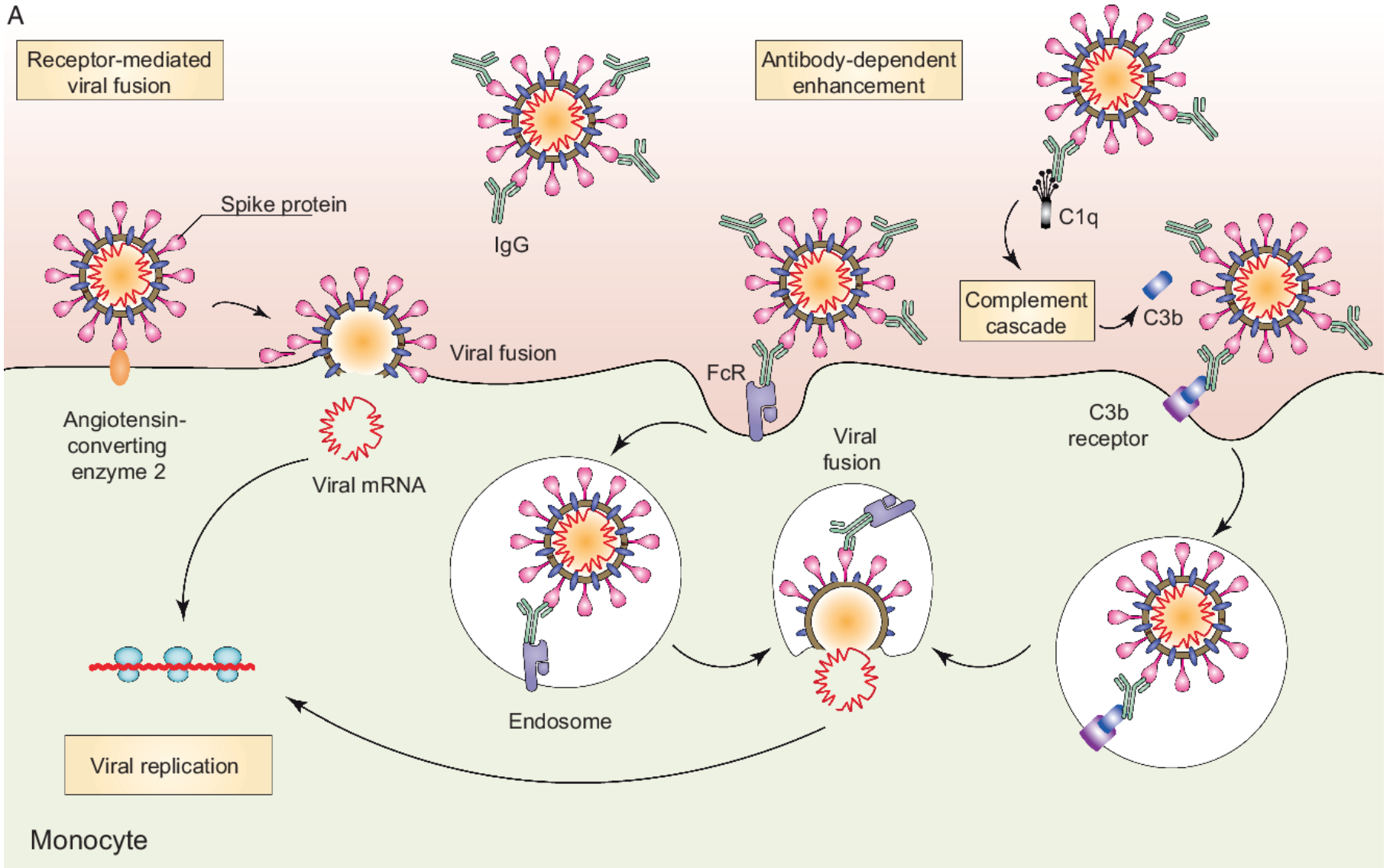
When the virus infects epithelial cells bearing the surface ACE2 receptor, it is able to evade the antiviral immune response by blocking the production of type I interferons and inflammatory cytokines (IL-1, IL-6 and TNF-α).

Excessive viral replication and epithelial cell death summon cells of the innate immune system from the bloodstream that internalize the virus and activate the inflammatory response, with the aim of blocking virus replication, which, however, leads to more tissue damage.

When the virus infects immune system cells (monocytes and tissue macrophages) it enters as a complex with antibodies (immunocomplex) through binding to the Fcy receptor present on the surface of these cells, blocks the production of interferons and other antiviral mediators but stimulates high production of proinflammatory cytokines leading to cytokine storm and severe tissue damage (antibody-mediated disease enhancement - ADE)



VIRAL EVASION MECHANISMS



VIRAL EVASION MECHANISMS

FEDERAZIONE RINASCIMENTO ITALIA

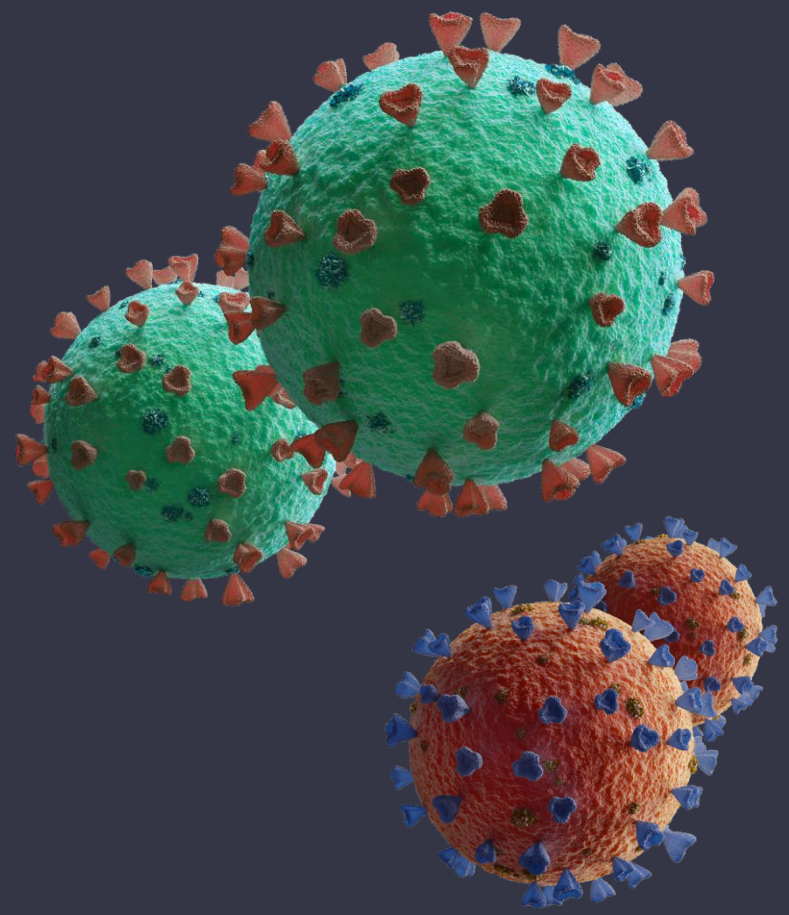
From the perspective of the mechanism of damage induction, severe/fatal complications associated with SARS-Cov-2 infection can be considered a consequence of ADE.

The ADE explains why the elderly are at greater risk than healthy children and adults, as they possess a greater amount of nonneutralizing antibodies from coronavirus infections or from infrequent vaccinations, and have an underperforming immune system in fighting infections.

Pregnant women are also susceptible to the enhancement of the disease, and Infants under one year of age, in case of reinfection.

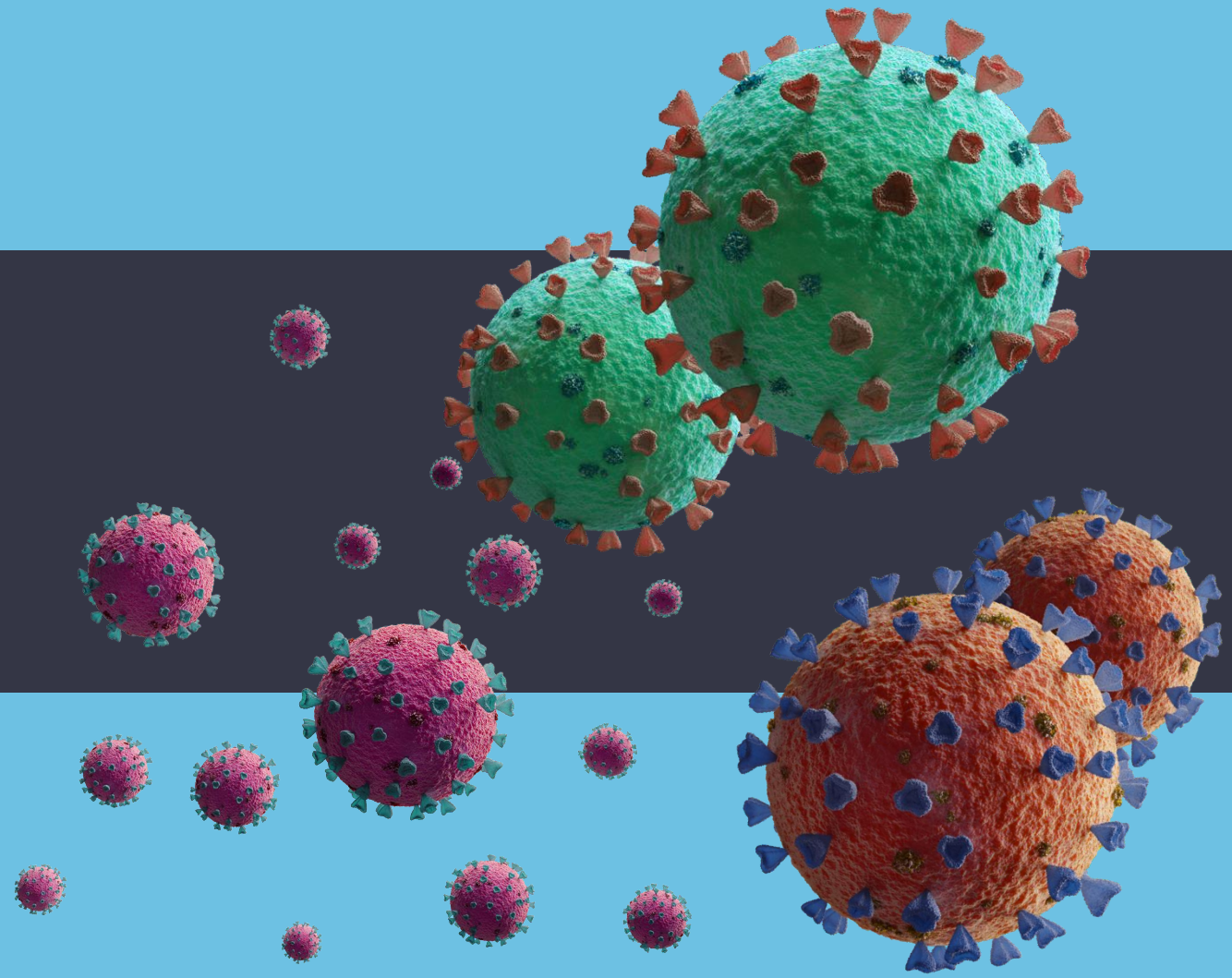
Hyperimmune serum and IVIGs are effective in treating COVId-19 patients because the transfused antibodies are able to block viral immune complexes from entering immune system cells.

ADE is an important risk factor for vaccination against COVID-19 and influenza because of the high variability of viruses that may predispose to the production of nonneutralizing antibodies.



FEDERAZIONE RINASCIMENTO ITALIA

VACCINES FOR. SARS-COV-2



WHO - DRAFT LANDSCAPE OF COVID-19 CANDIDATE VACCINES
25 september 2020

FEDERAZIONE RINASCIMENTO ITALIA

https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

40 vaccine candidates in clinical trials

Non-Replicating Viral Vector	ChAdOx1-S	University of Oxford/AstraZeneca	SARS-CoV2	Phase 3 ISRCTN89951424 Phase 2b/3 2020-001228-32 Phase ½ PACTR202006922165132 2020-001072-15	MERS, influenza, TB, Chikunguya, Zika, MenB, plague

149 candidate vaccines in preclinical trials

AUTHORIZATION PROCEDURES - EMA

HTTPS://WWW.EMA.EUROPA.EU/EN/AUTHORISATION-PROCEDURE

FEDERAZIONE RINASCIMENTO ITALIA

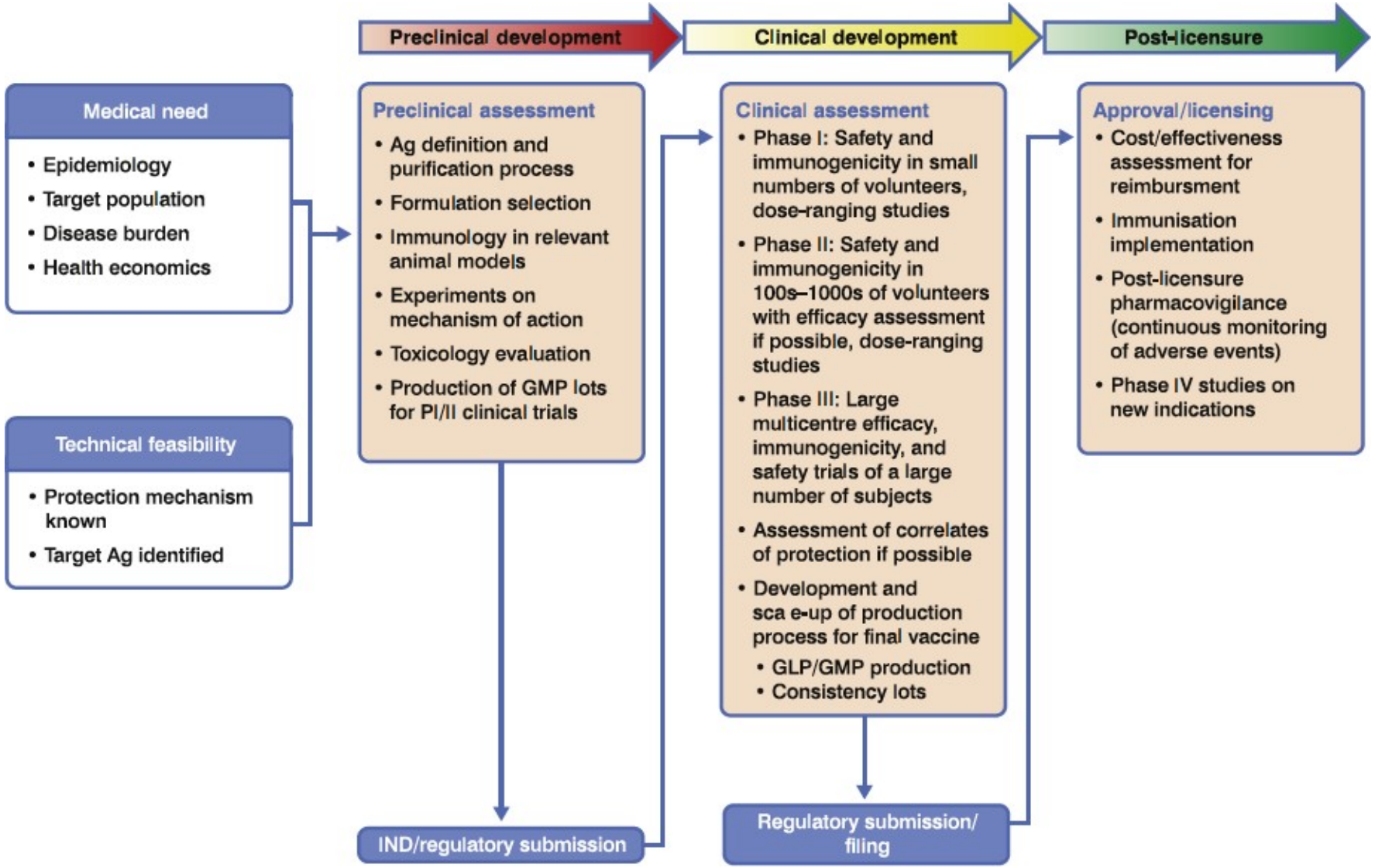
The two main procedures for the licensing of pandemic influenza vaccines are:

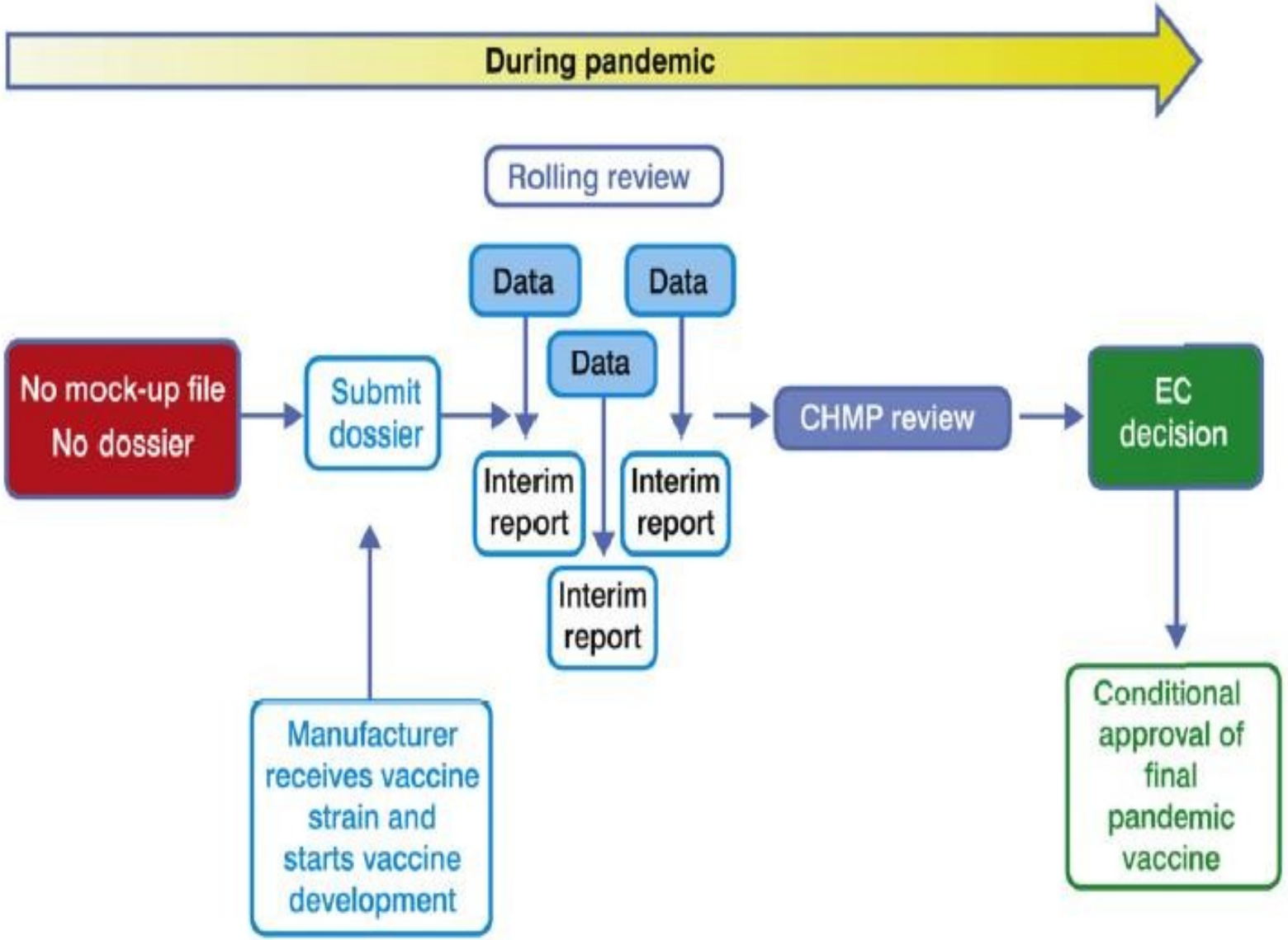
the "mock-up procedure" [mock-up procedure], which allows a vaccine to be developed and licensed prior to a pandemic, based on information generated with a viral strain that could potentially cause a pandemic. Once the actual pandemic-causing viral strain is identified, the manufacturer can include this strain in the mock-up vaccine and request that it be licensed as the "final" pandemic vaccine;

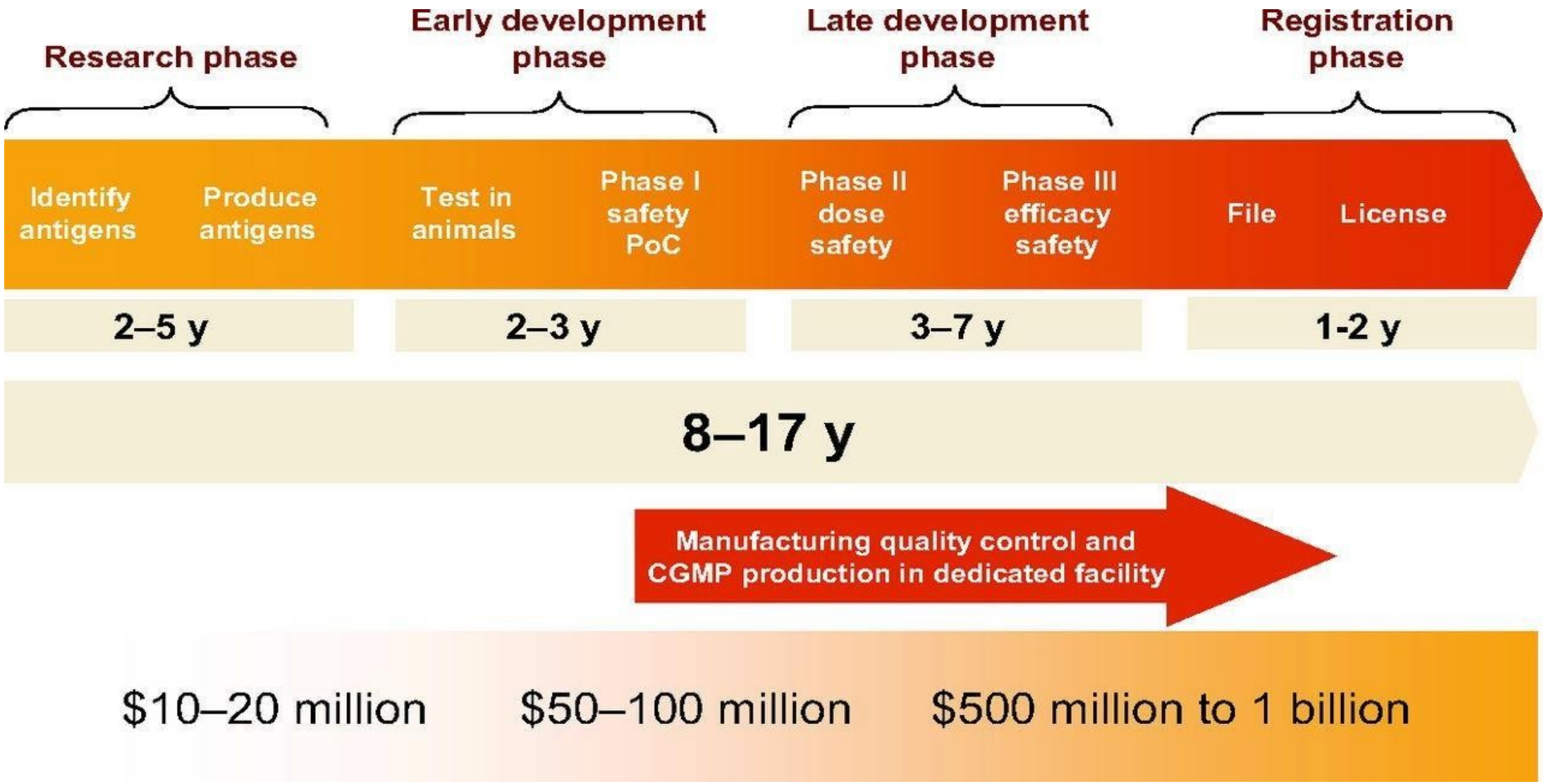
The "emergency procedure," which allows

The accelerated approval of a new vaccine developed after a pandemic has already been declared. The approval of these pandemic vaccines is faster than for a normal vaccine because the information provided by the manufacturer is evaluated in an accelerated time frame (about 70 days instead of 210 days).

A third procedure allows licensed vaccines against nonpandemic influenza, "seasonal influenza," to be modified to offer protection against pandemic influenza


Preclinical development
Clinical development
Post-licensure
Medical need
• Epidemiology
• Target population
• Disease burden
• Health economics
Technical feasibility
• Protection mechanism known
• Target Ag identified
Preclinical assessment
• Ag definition and purification process
• Formulation selection
• Immunology in relevant animal models
• Experiments on mechanism of action
• Toxicology evaluation
• Production of GMP lots for PI/II clinical trials
IND/regulatory submission
Clinical assessment
• Phase I: Safety and immunogenicity in small numbers of volunteers, dose-ranging studies
• Phase II: Safety and immunogenicity in 100s–1000s of volunteers with efficacy assessment if possible, dose-ranging studies
• Phase III: Large multicentre efficacy, immunogenicity, and safety trials of a large number of subjects
• Assessment of correlates of protection if possible
• Development and sca e-up of production process for final vaccine
• GLP/GMP production
• Consistency lots
Regulatory submission/ filing
Approval/licensing
• Cost/effectiveness assessment for reimbursment
• Immunisation implementation
• Post-licensure pharmacovigilance (continuous monitoring of adverse events)
• Phase IV studies on new indications


During pandemic
Rolling review
Data
Data
Data
No mock-up file
No dossier
Submit dossier
Interim report
Interim report
Interim report
CHMP review
EC decision
Manufacturer receives vaccine strain and starts vaccine development
Conditional approval of final pandemic vaccine


Research phase
Early development phase
Late development phase
Registration phase
Identify antigens
Produce antigens
Test in animals
Phase I safety PoC
Phase II dose safety
Phase III efficacy safety
File
License
2–5 y
2–3 y
3–7 y
1-2 y
8–17 y
Manufacturing quality control and CGMP production in dedicated facility
$10–20 million
$50–100 million
$500 million to 1 billion

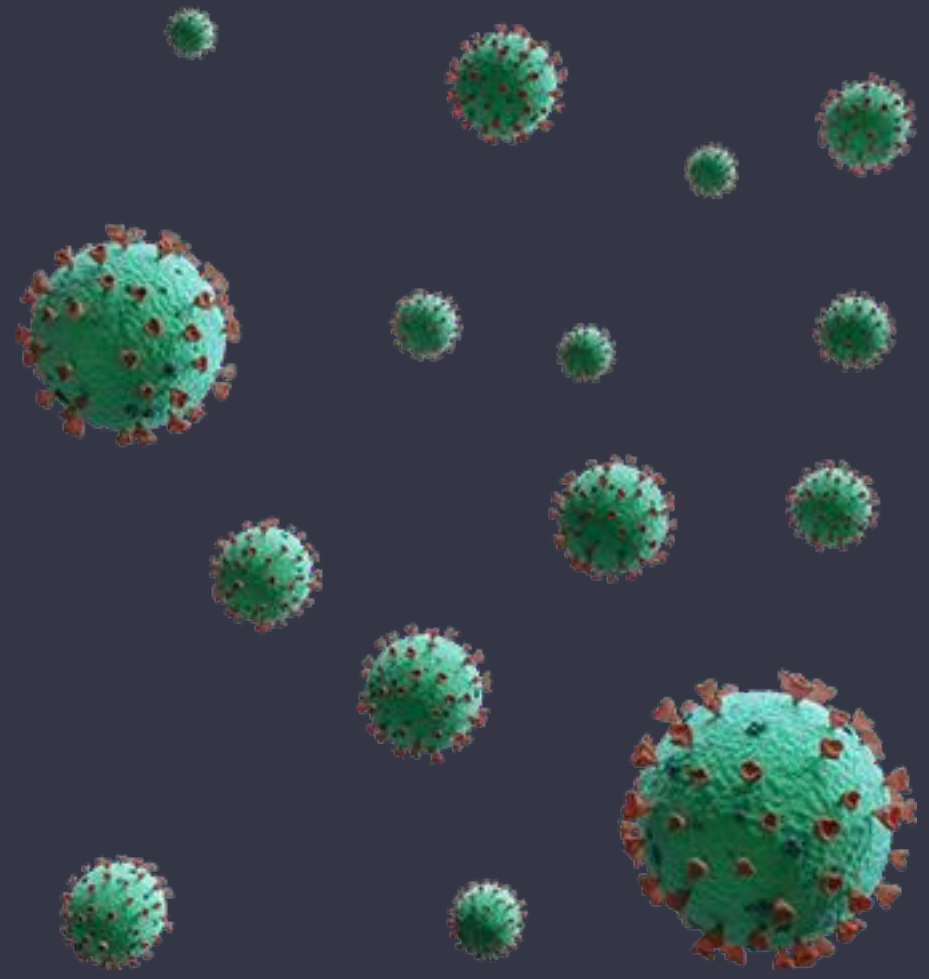
EFFECTIVENESS

QUASISPECIES

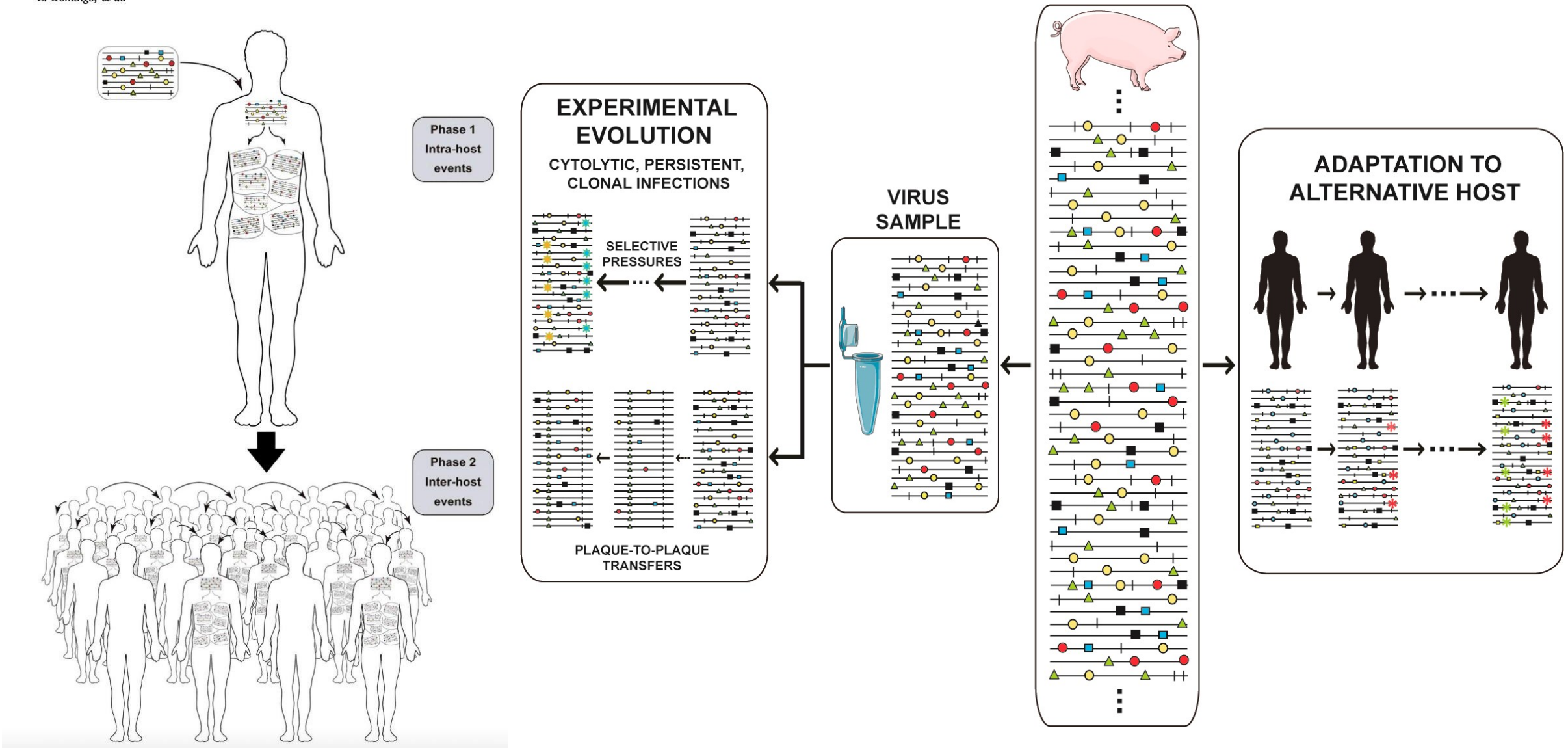
FEDERAZIONE RINASCIMENTO ITALIA

Viral quasispecies are defined as 'collections' of closely related viral genomes undergoing a continuous process of genetic variation, competition among the generated variants, and selection of the most suitable distributions in a given environment

Error rates for RNA viruses, retroviruses, and some DNA viruses are in the range of 10^{-3} to 10^{-5} mutations introduced per copied nucleotide, 10^{4} to 10^{6} times higher than those that occur during normal cellular chromosomal DNA replication.



E. Domingo, et al.



TOXICITY.

THE ORIGINAL ANTIGENIC SIN

FEDERAZIONE RINASCIMENTO ITALIA

When the vaccinated person becomes infected with a mutant of the virus wild different than the vaccine antigen, its adaptive immune system responds in a nonspecific and weak manner because it is programmed to respond effectively only to the vaccine antigen.

The virus continues to replicate in the vaccinated leading to A chronic infection with the risk of developing a long-term inflammatory-autoimmune state and recurrence of the infection in an atypical, drug-resistant form

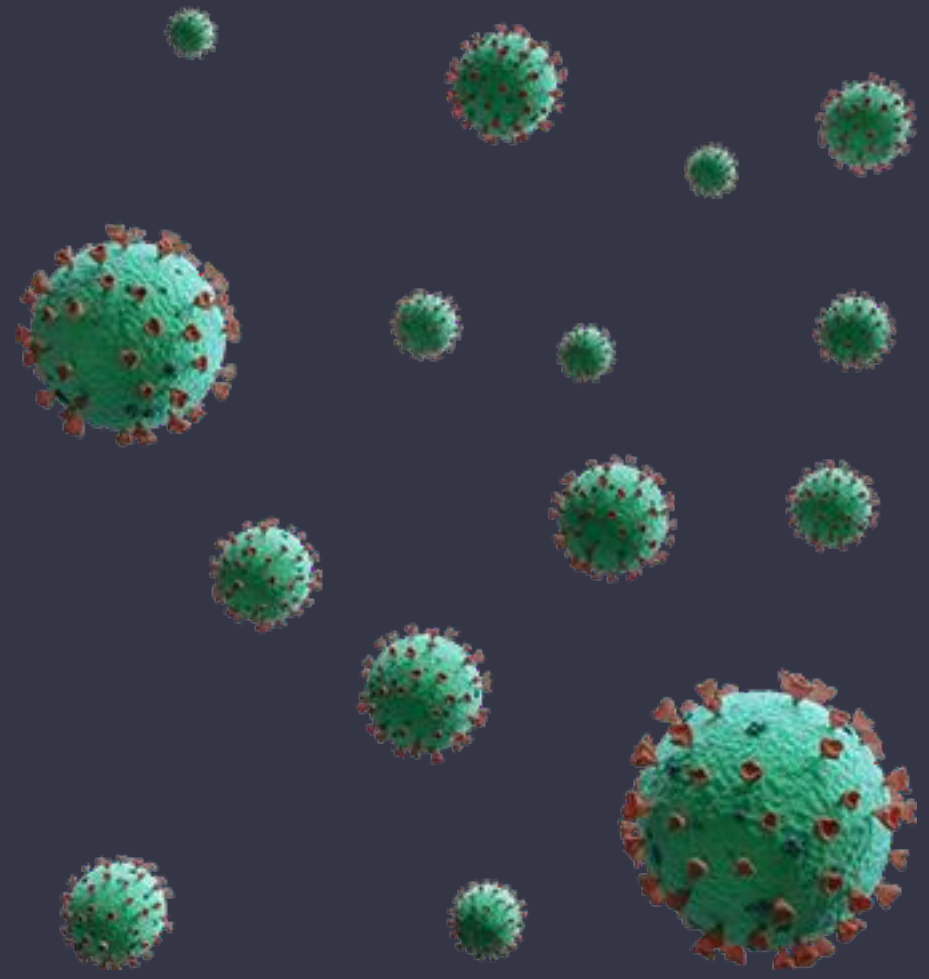
TOXICITY.

THE ANTIBODY-DEPENDENT ENHANCEMENT (ADE)

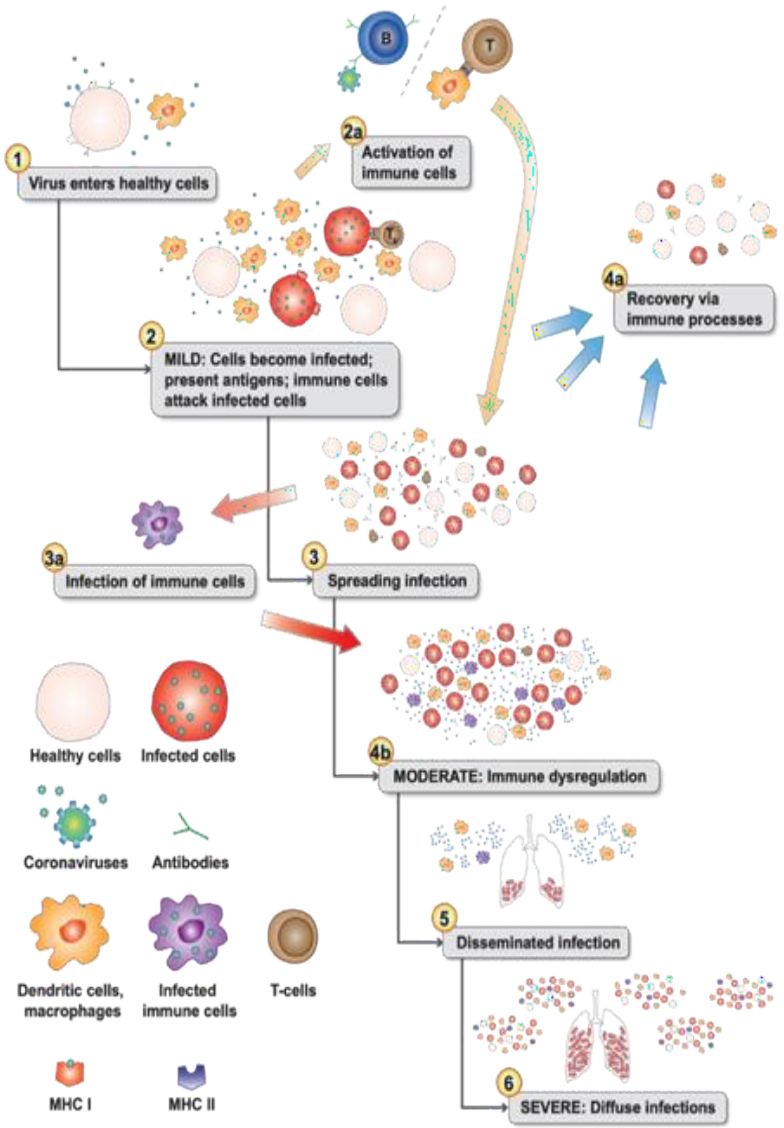
FEDERAZIONE RINASCIMENTO ITALIA

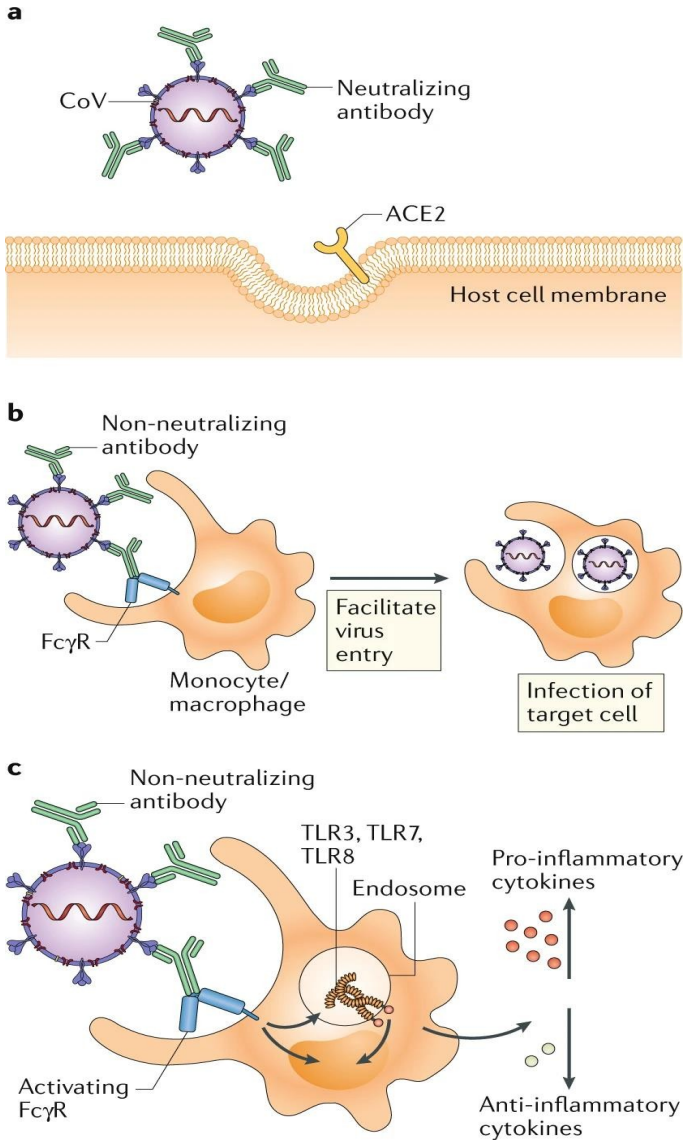
When a subject with a suboptimal antibody level (as a result of primary infection or vaccination) comes in contact with a similar virus and becomes infected, its immune system promotes infection and fatal complications of the disease.

In other words, a proportion of the vaccinated are predisposed by vaccination precisely to manifest the serious and fatal complications of the disease from which they want to protect themselves.



TOXICITY.


B
T
1
Virus enters healthy cells
2a
Activation of immune cells
4a
Recovery via immune processes
2
MILD: Cells become infected; present antigens; immune cells attack infected cells
3a
Infection of immune cells
3
Spreading infection
Healthy cells
Infected cells
4b
MODERATE: Immune dysregulation
Coronaviruses
Antibodies
5
Disseminated infection
Dendritic cells, macrophages
Infected immune cells
T-cells
MHC I
MHC II
6
SEVERE: Diffuse infections


a
CoV
Neutralizing antibody
ACE2
Host cell membrane
b
Non-neutralizing antibody
FcγR
Monocyte/ macrophage
Facilitate virus entry
Infection of target cell
c
Non-neutralizing antibody
TLR3, TLR7, TLR8
Endosome
Pro-inflammatory cytokines
Activating FcγR
Anti-inflammatory cytokines

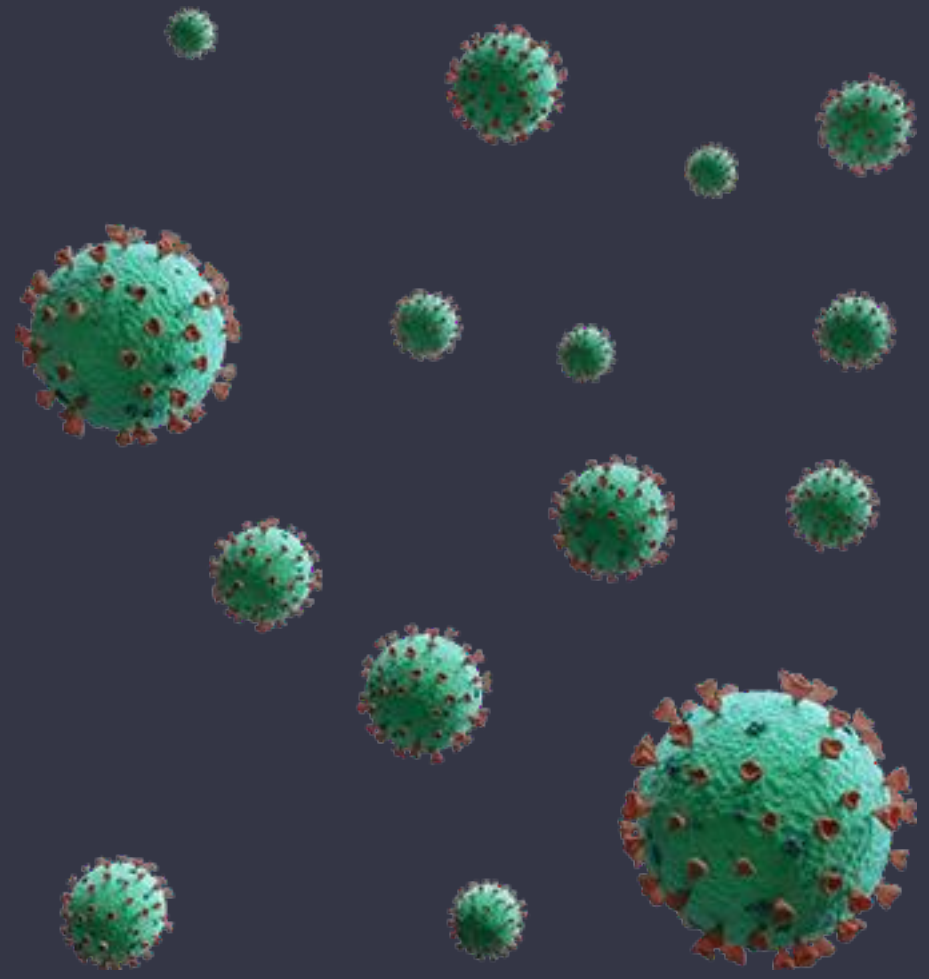
TOXICITY.

THE AUTOIMMUNE/INFLAMMATORY SYNDROME

FEDERAZIONE RINASCIMENTO ITALIA

Antibodies produced as a result of vaccination, by molecular mimicry attack both viral and human proteins, triggering autoimmune-inflammatory reactions against the body's structures.

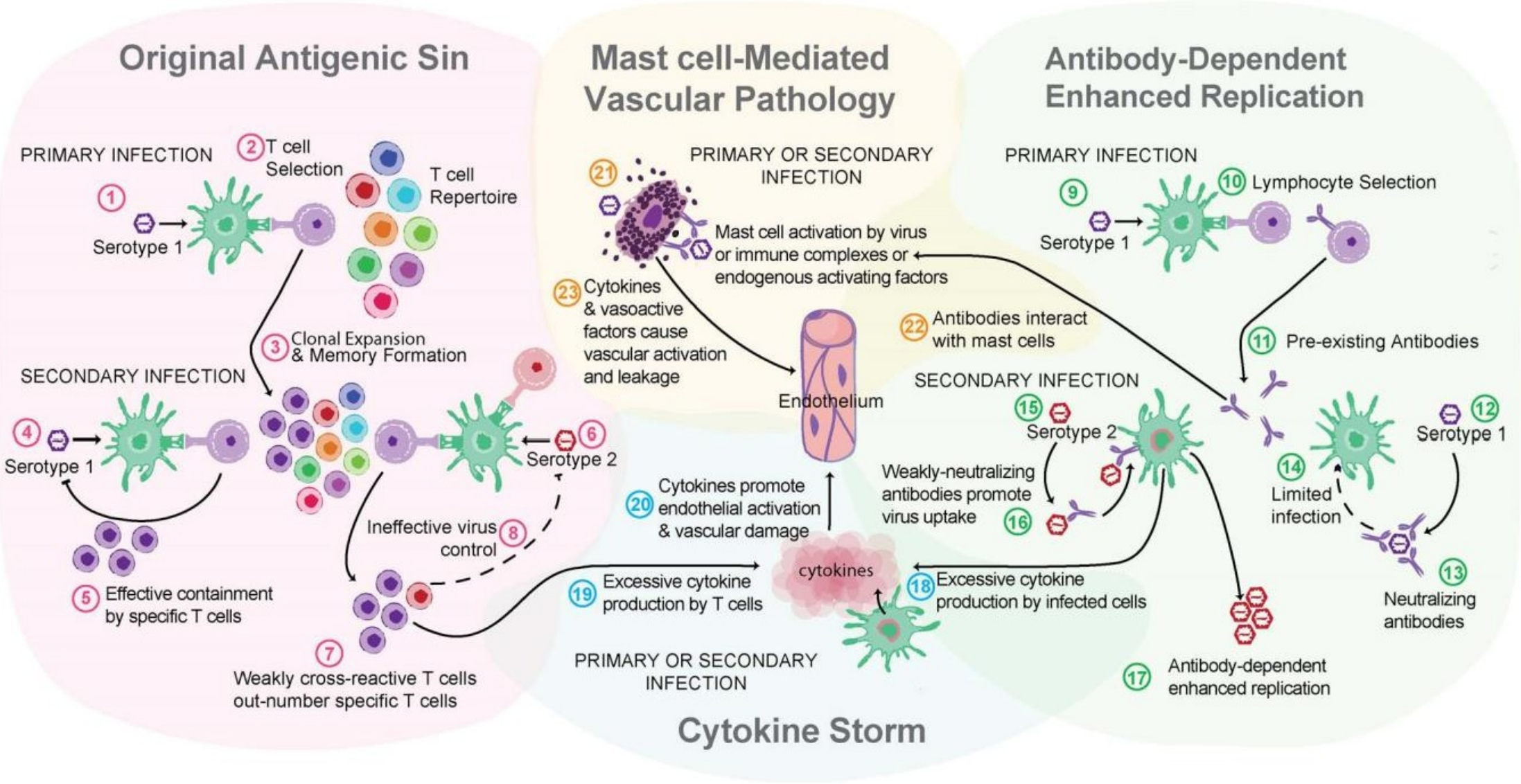
Autoimmunity then is an adverse reaction that can occur either as a result of excessive cytokine storm inflammation leading to tissue destruction or as a result of molecular mimicry between the vaccine antigen and human proteins.



TOXICITY.

THE AUTOIMMUNE/INFLAMMATORY SYNDROME

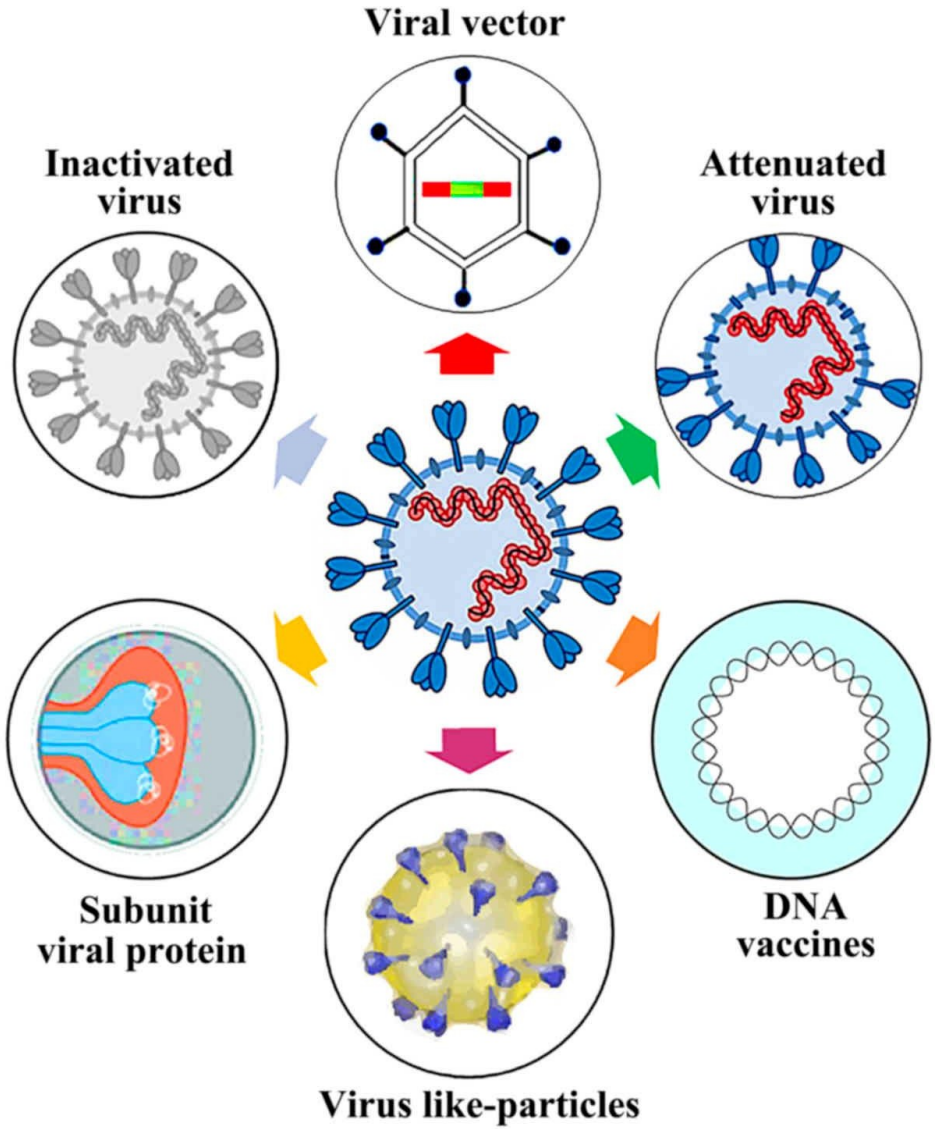
FEDERAZIONE RINASCIMENTO ITALIA



TOXICITY.

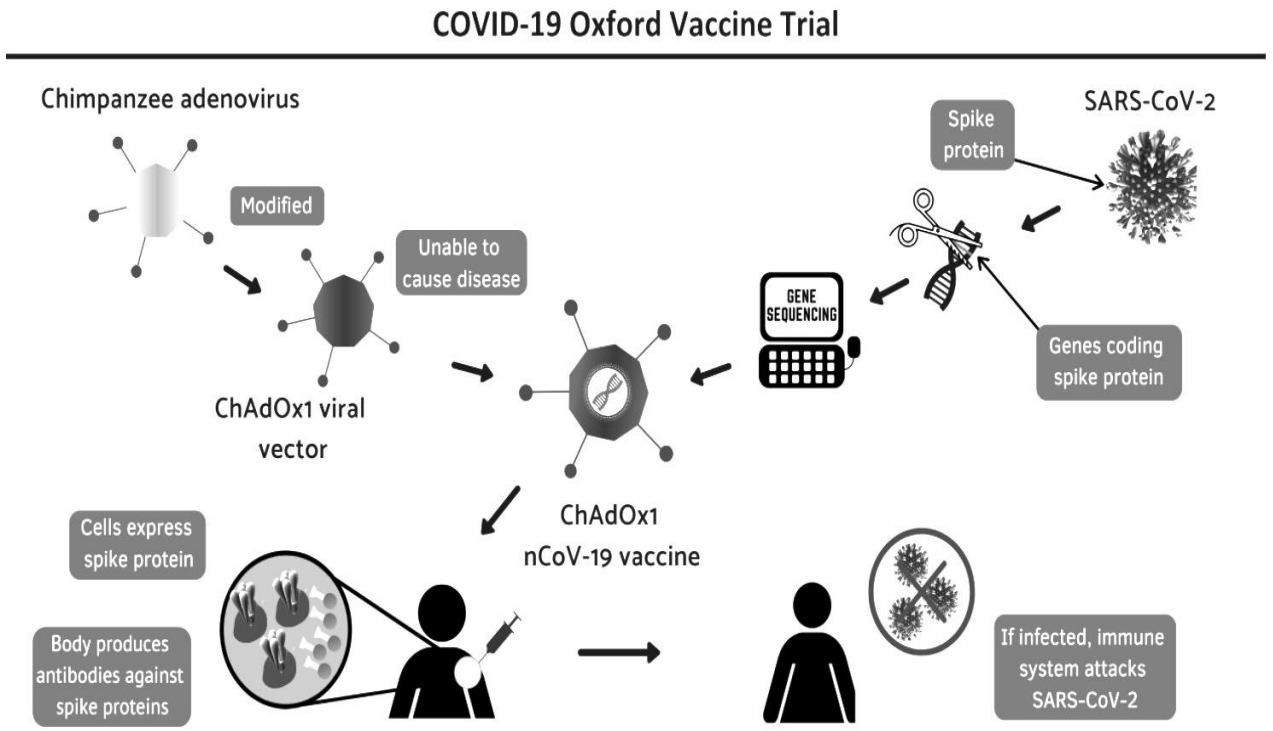
THE AUTOIMMUNE/INFLAMMATORY SYNDROME

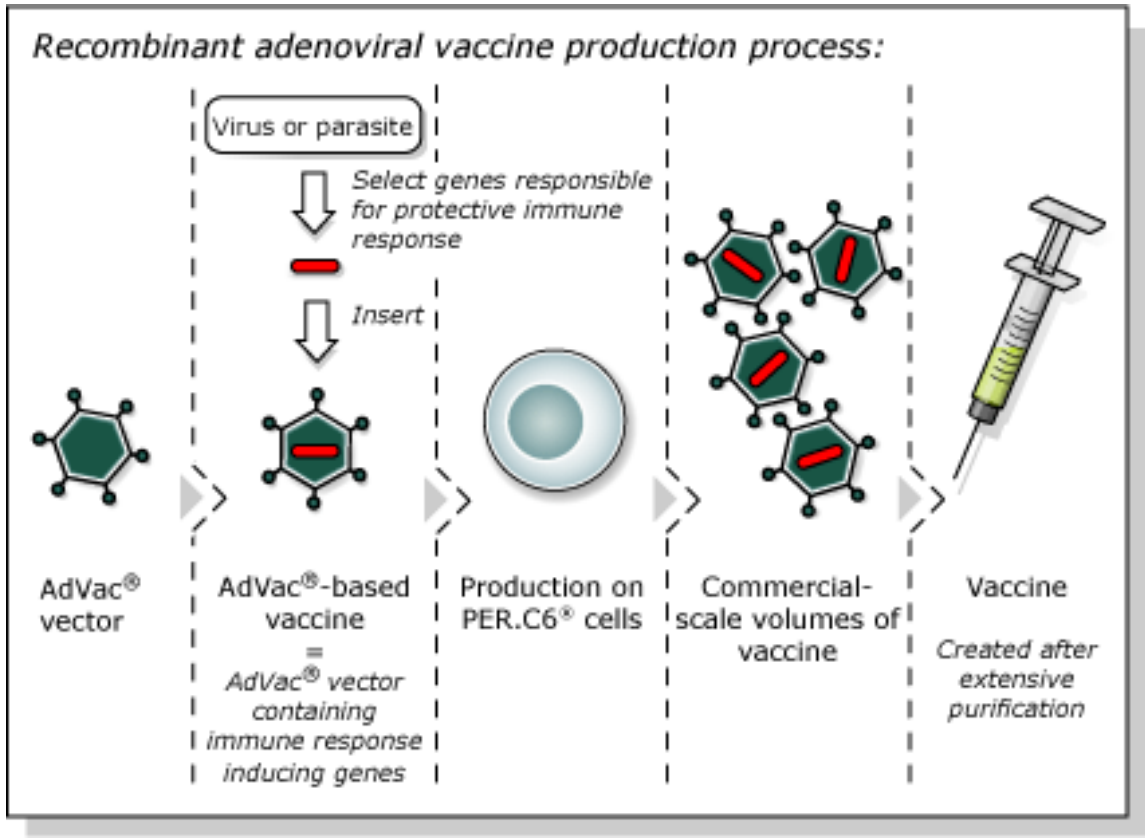
FEDERAZIONE RINASCIMENTO ITALIA



MODE OF PREPARATION
OF THE VIRAL ANTIGEN VACCINE CHADOX1 NCOV-19

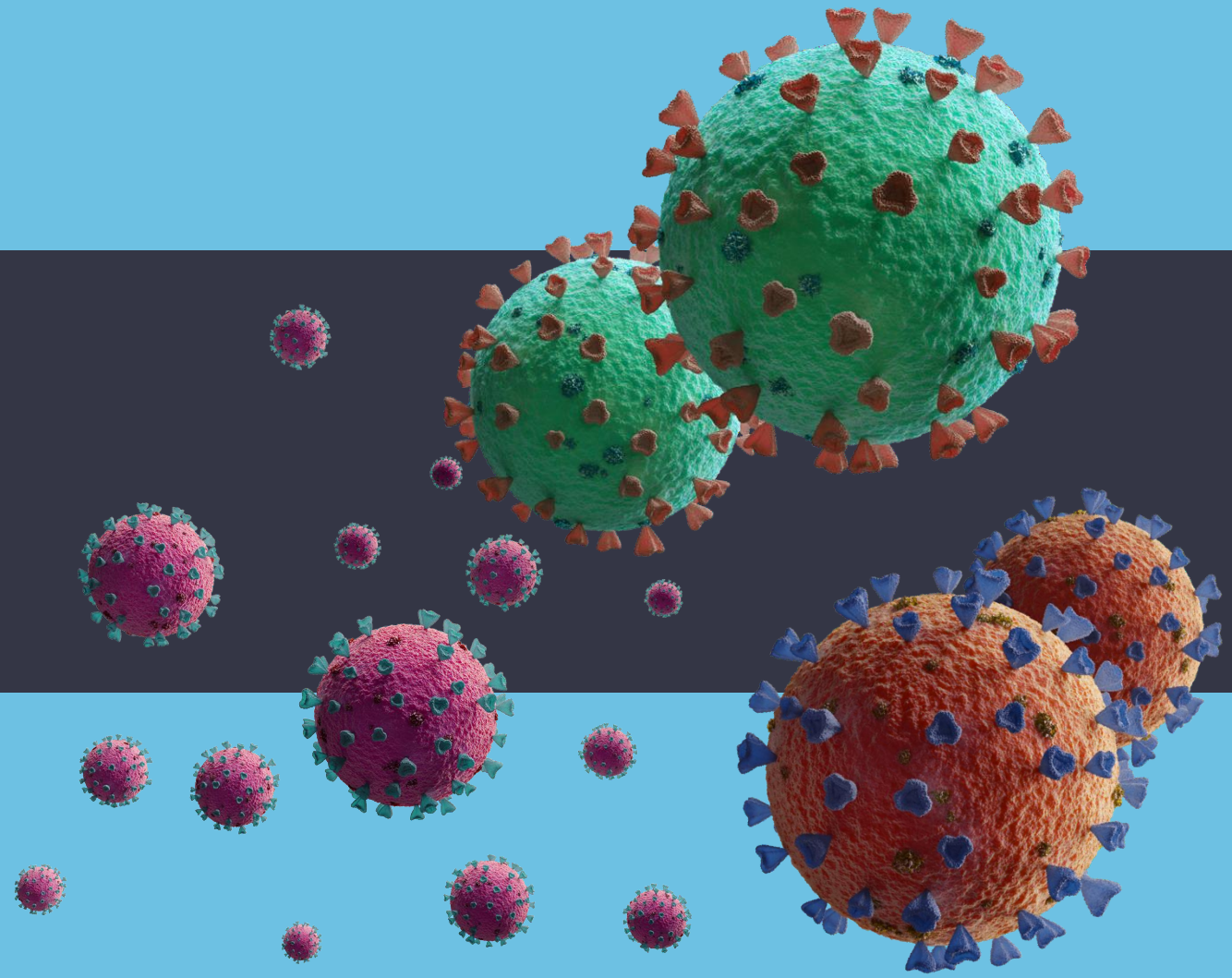
FEDERAZIONE RINASCIMENTO ITALIA





FEDERAZIONE RINASCIMENTO ITALIA

THE FLU SHOT



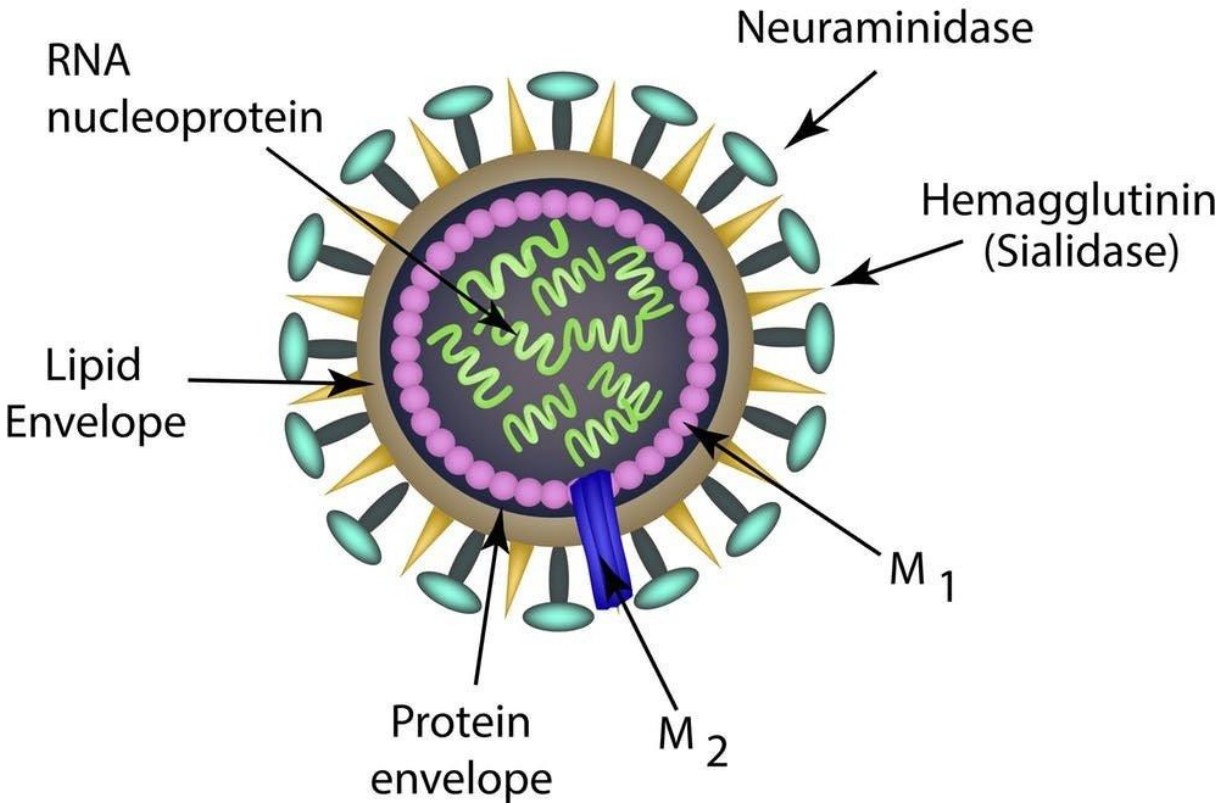
THE INFLUENZA VIRUS

FEDERAZIONE RINASCIMENTO ITALIA

The causative agents of seasonal influenza are of three types, constituting the genus Orthomixovirus: type A and type B viruses, responsible for classic influenza symptoms, and type C virus, of little clinical significance (often asymptomatic).

Capsular proteins, which are responsible for attaching the virus to the receptors of respiratory cells, are distinguished into: H (hemagglutinin) and N (neuraminidase).

The antigenic properties of hemagglutinins of type A influenza viruses allow 16 molecular species, H1 to H16, to be distinguished. These, combined with 9 molecular species of neuraminidases, allow type A influenza viruses to be classified into "HxNy" form subtypes (e.g., H5N1). H1N1, H2N2, H3N2 are pandemic viruses.



Orthomyxoviruses are a family of negative polarity single-stranded RNA viruses ((-)ssRNA)

THE INFLUENZA VIRUS

https://www.aifa.gov.it/web/guest/-/aifa-vaccini-influenzali-per-la-stagione-2020-2021

The quadrivalent influenza vaccine contains 2 strains of type A virus (H1N1 and H3N2) and 2 strains of type B virus, belonging to the two lineages B/Victoria and B/Yamagata.

The mismatch between vaccine strains and circulating viruses is called a mismatch: a mismatch occurs when the viral strain recommended and contained in the vaccine does not match the strain predominantly circulating in that flu season.

Because trivalent vaccines contain only one B strain, it is evident that the likelihood of a B mismatch is particularly high

Vaccini anti-influenzali autorizzati per la stagione 2020-2021

Nome commerciale del vaccino	Modalità di somministrazione
Agrippal S1 - Trivalente	uso intramuscolare o sottocutaneo profondo
Fluad - Trivalente	uso intramuscolare
Fluarix tetra - Tetravalente	uso intramuscolare
Flucelvax tetra - Tetravalente	uso intramuscolare
Fluenz tetra - Tetravalente	uso nasale
Influpozzi subunità - Trivalente	uso intramuscolare o sottocutaneo profondo
Influvac S - Trivalente	uso intramuscolare o sottocutaneo profondo
Influvac S tetra - Tetravalente	uso intramuscolare o sottocutaneo profondo
Vaxigrip tetra - Tetravalente	uso intramuscolare o sottocutaneo

NOTA:
EFLUELDA (uso intramuscolare o sottocutaneo) e FLUAD TETRA (uso intramuscolare) sebbene abbiano ricevuto l'Autorizzazione all'Immissione in Commercio nel 2020, non sono autorizzati per la stagione influenzale 2020/2021.

THE AVAILABLE VACCINES

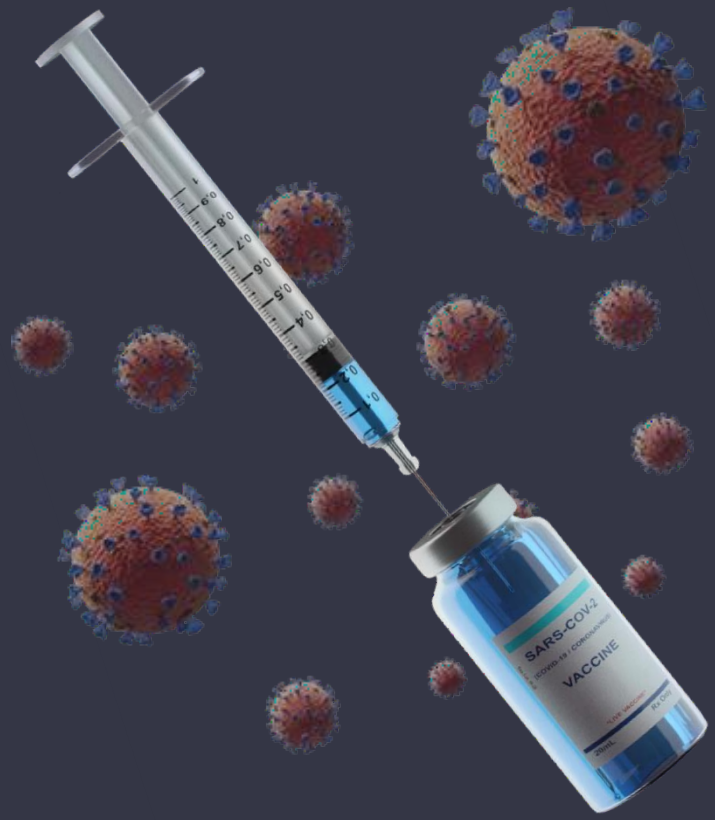
FEDERAZIONE RINASCIMENTO ITALIA

Inactivated vaccines (VII) Inactivated influenza vaccines currently licensed for use in Italy are a mix of split and subunit virus vaccines. In split vaccines, the virus has been rendered nonpathogenic by treatment with a detergent. In subunit vaccines, the hemagglutinin (HA) and neuraminidase (NA) antigens were further purified by removal of other viral components.

Currently available in Italy are trivalent influenza vaccines (TIV) containing 2 type A viruses (H1N1 and H3N2) and one type B virus and quadrivalent vaccines (QIV) containing 2 type A viruses (H1N1 and H3N2) and 2 type B viruses

Inactivated adjuvanted vaccine (VIIa) One of the trivalent products (Fluad) contains MF59 adjuvant, an oil-in-water emulsion composed of squalene as the oil phase.

** activation of very potent inflammation due to the adjuvant could aggravate the Cytokine storm syndrome observed in COVID-19 patients*



THE AVAILABLE VACCINES

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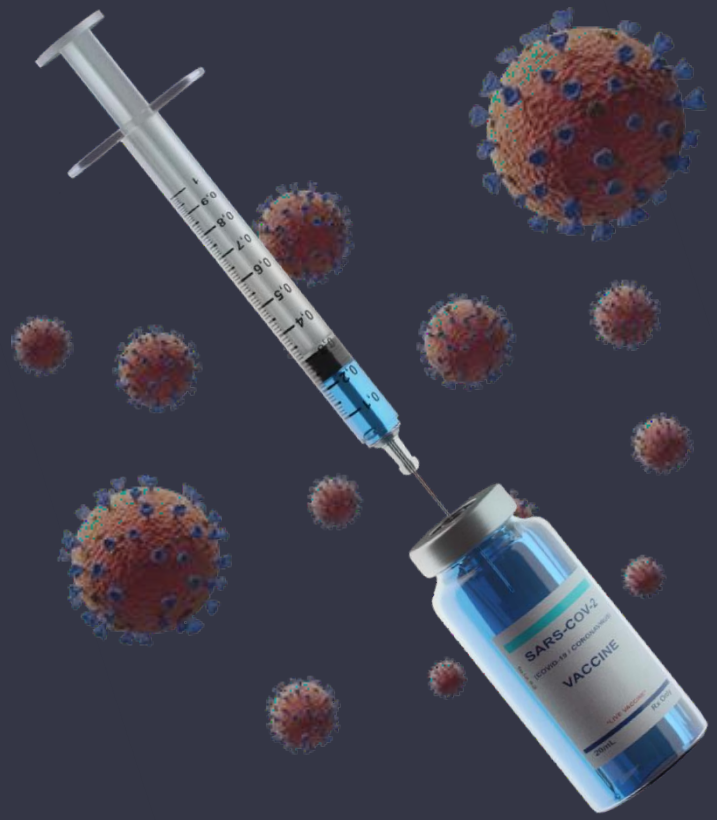


THE AVAILABLE VACCINES

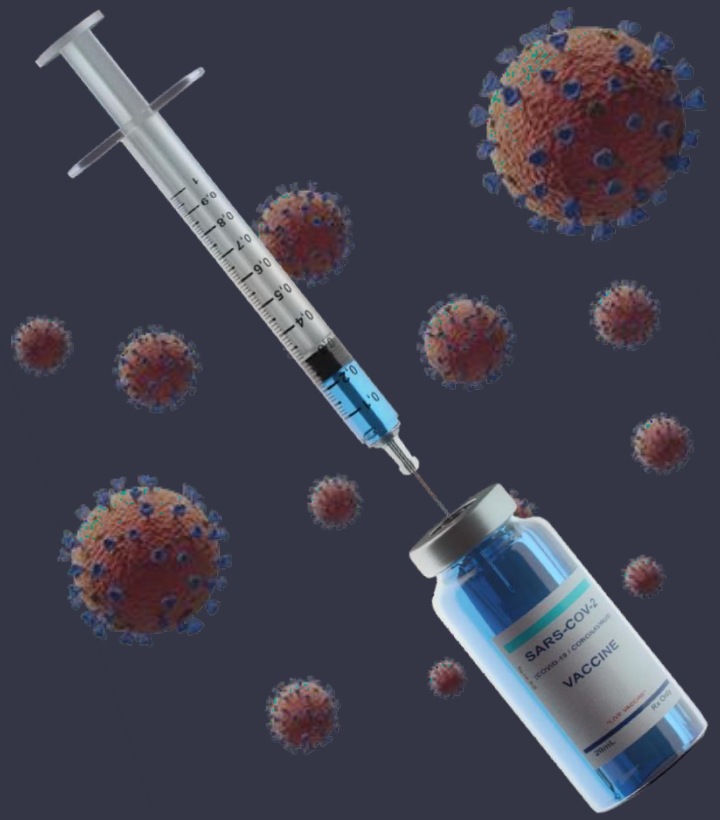
FEDERAZIONE RINASCIMENTO ITALIA

Live attenuated vaccine (LAIV) Quadrivalent LAIV vaccine is a live attenuated influenza vaccine administered by intranasal spray and licensed for use in people aged 2-59 years. The vaccine is not currently available in Italy

** Nervous system disorders: Guillain-Barré syndrome, Bell's Palsy, meningitis, eosinophilic meningitis, vaccine-associated encephalitis*
Congenital, familial, and genetic disorders: Exacerbation of symptoms of mitochondrial encephalomyopathy (Leigh syndrome)
https://www.fda.gov/media/83072/download (RCP FluMist)

Inactivated quadrivalent vaccine on cell culture (VIQCC) The VIQCC vaccine is a quadrivalent influenza vaccine that contains 2 type A viruses (H1N1 and H3N2) and 2 type B viruses grown on cell cultures (Madin Darby Canine Kidney (MDCK) cells* - Flucelvax Tetra)

** Immortalized cells. Risk of contamination of culture cell residues, adventitious cell viruses*



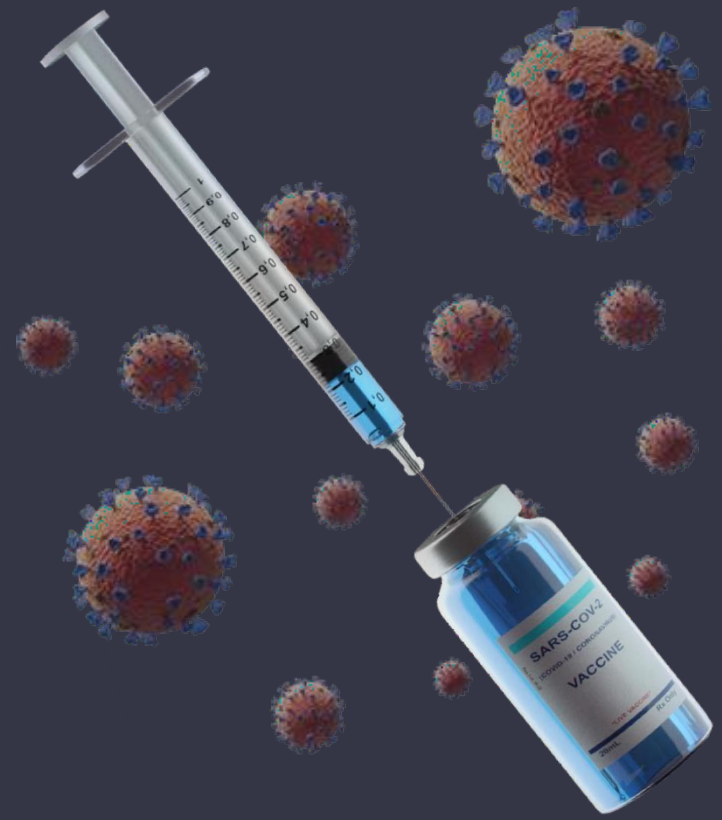
THE AVAILABLE VACCINES

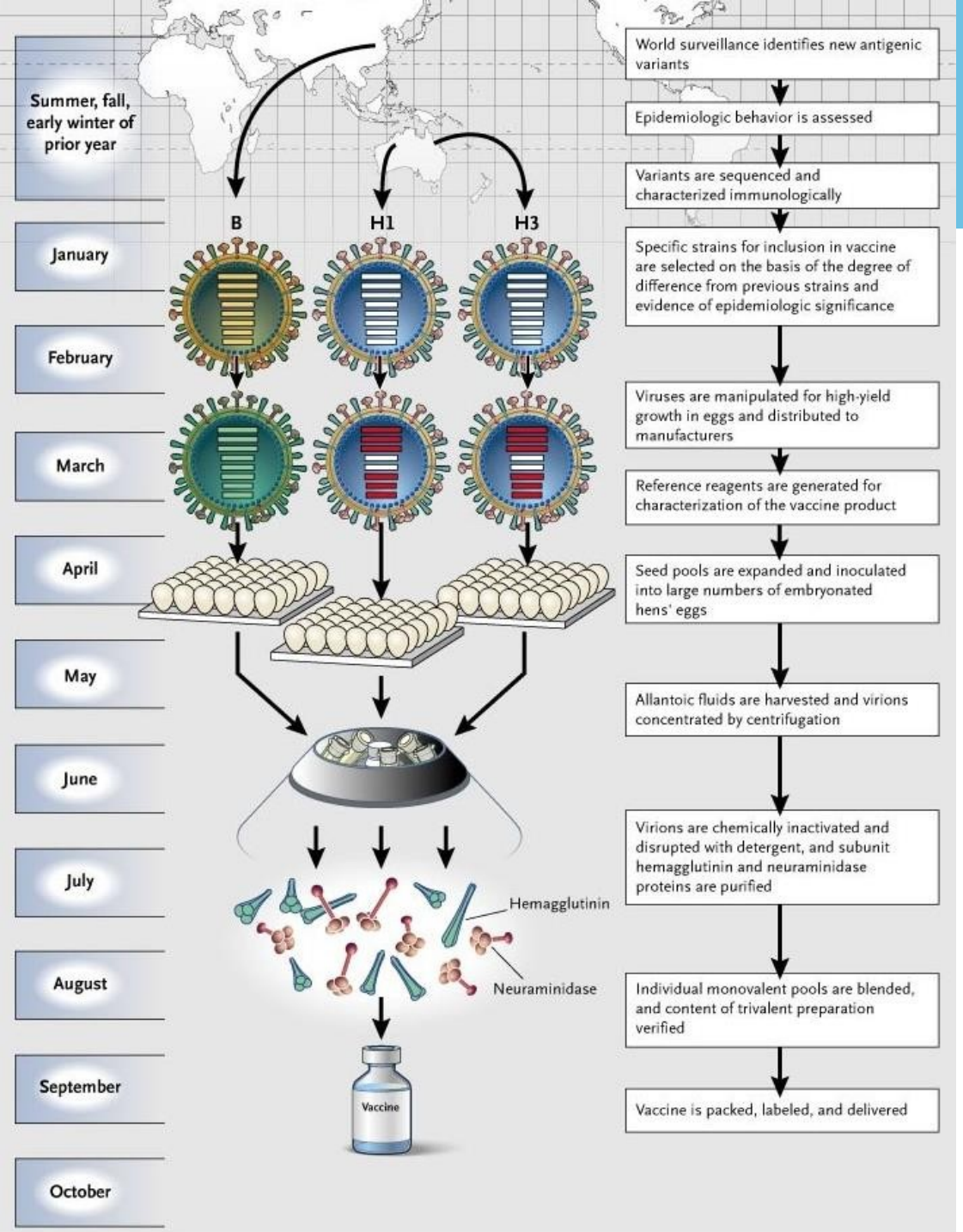
FEDERAZIONE RINASCIMENTO ITALIA

High-dose (HD) Vaccine The high-dose vaccine is a quadrivalent split vaccine containing two type A viruses (H1N1 and H3N2) and two type B viruses containing 60 mcg of hemagglutinin (HA) for each viral strain to ensure a greater immune response and thus greater efficacy, indicated in individuals aged 65 years and older

They are grown in **embryonated chicken eggs.**
Agrippal; Fluad; Fluarix tetra; Fluenz Tetra nasal spray; Influpozzi subunit; Influvac S; Influvac S tetra; Vaxigrip Tetra

** Risk of adventitious virus contamination from the environment and the line cellular, and fetal cell remnants*





THE TYPES OF INFLUENZA VACCINE

FEDERAZIONE RINASCIMENTO ITALIA

1958 — virus intero inattivato

1968 — particelle virali disgregate (vaccini split)

1976 — solo antigeni virali di superficie H e N (vaccini a subunità)

1997 — adiuvati con MF59

2002 — virosomale

Recenti Novità
- **Intradermico** – *split con nuovo delivery system*
- **Intransale** – *vaccino vivo attenuato*

FLU VACCINES
EFFECTIVENESS AND SAFETY

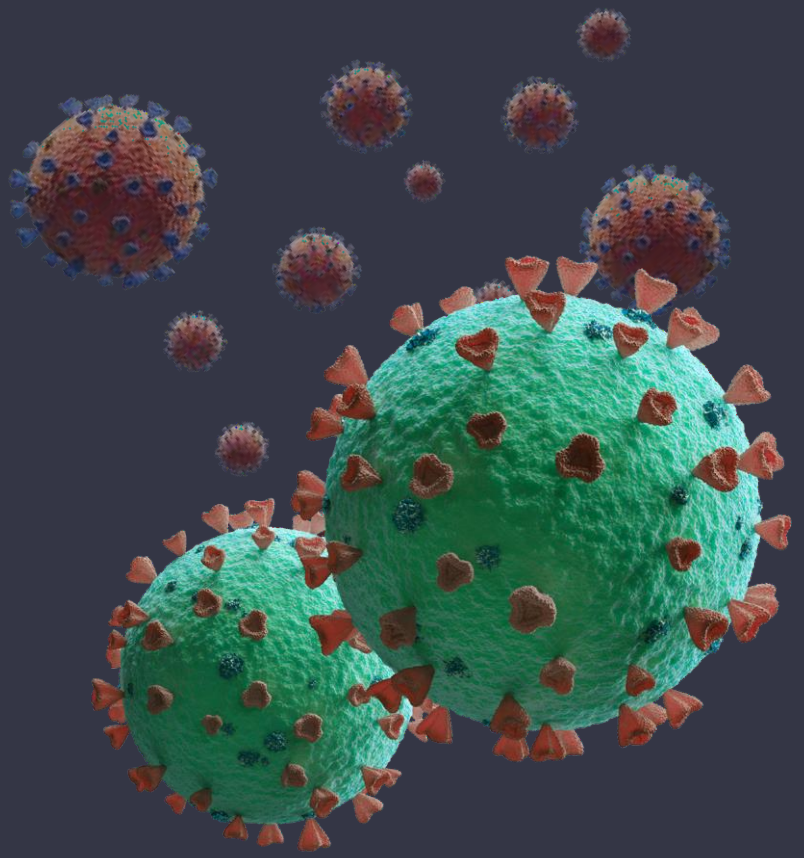
FEDERAZIONE RINASCIMENTO ITALIA

Featured Review: Three updated Cochrane Reviews assessing the effectiveness of influenza vaccines (February 2018)

**"From what is reported in this review, it can be concluded that:
the reviewed studies were unable to provide sufficient data to define the incidence of adverse reactions and complications and, in the case of the elderly, the reduction in mortality from the consequences of influenza.**

Overall, efficacy for the three age groups is rather modest and attributable to the vaccine's low protective capacity in vaccinees; even for efficacy much of the information needed to quantify it is lacking or absent.

Thus, the reviewed data can be considered inconclusive about the true benefit of influenza vaccination or, at best, seem to indicate a very small protective effect.



https://www.cochrane.org/news/featured-review-three-updated-cochrane-reviews-assessing-effectiveness-influenza-vaccines

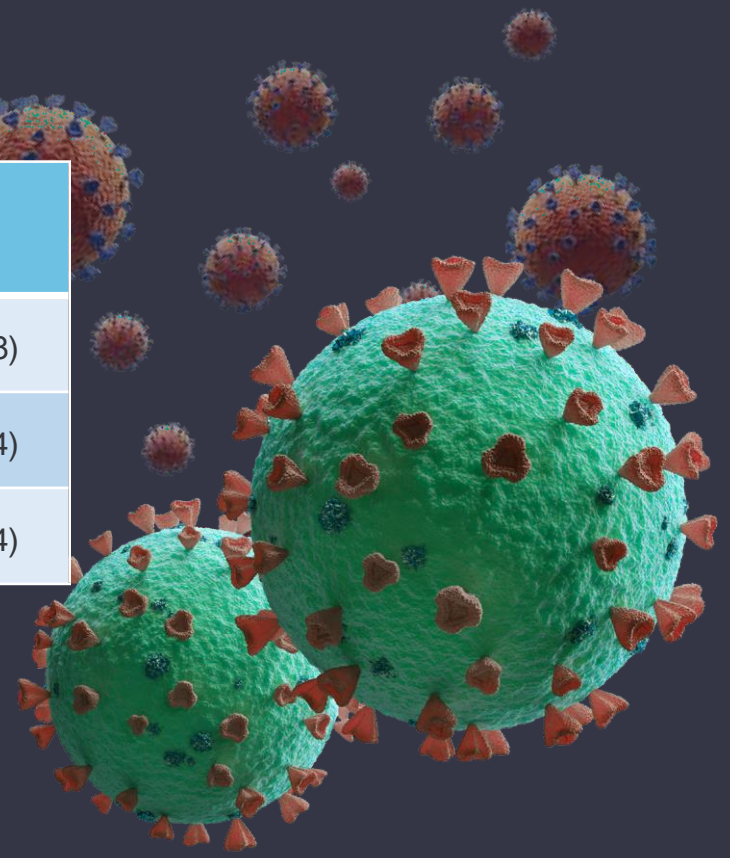
FLU VACCINES
EFFECTIVENESS AND SAFETY

FEDERAZIONE RINASCIMENTO ITALIA

Influenza vaccine effectiveness in an Italian elderly population during the 2016-2017 season

Ann Ist Super Sanità 2018 | Vol. 54, No. 1: 67-71 Francesca Valent and Tolinda Gallo

	ED visit HR1 (95% CI)	Outcome Hospitalization HR1 (95%CI)	Death HR1 (95%CI)
Influenza vaccination (any vs no vaccination)	1.13 (0.91-1.40)	1.11 (0.93-1.33)	1.05 (0.70-1.58)
Influenza vaccination (intradermal vs. no vaccination)	1.11 (0.95-1.48)	1.11 (0.92-1.34)	1.02 (0.67-1.54)
Influenza vaccination (tetravalent vs no vaccination)	0.81 (0.46-1.41)	1.47 (1.00-2.15)	1.12 (1.03-1.54)



tetravalent vaccination has the highest risk, with an average 47% increase (from 0% to as much as 215%) in hospitalizations for complications (influenza and pneumonia) and 12% increase in deaths (from 3% to 54%).

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