



Biotecnologia

ACH5525 Microbiologia, Imunologia e Parasitologia

1º semestre 2024

Docente:

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Data: Quinta-feira 14 – 18 h / **Sala:** 102, Prédio I1

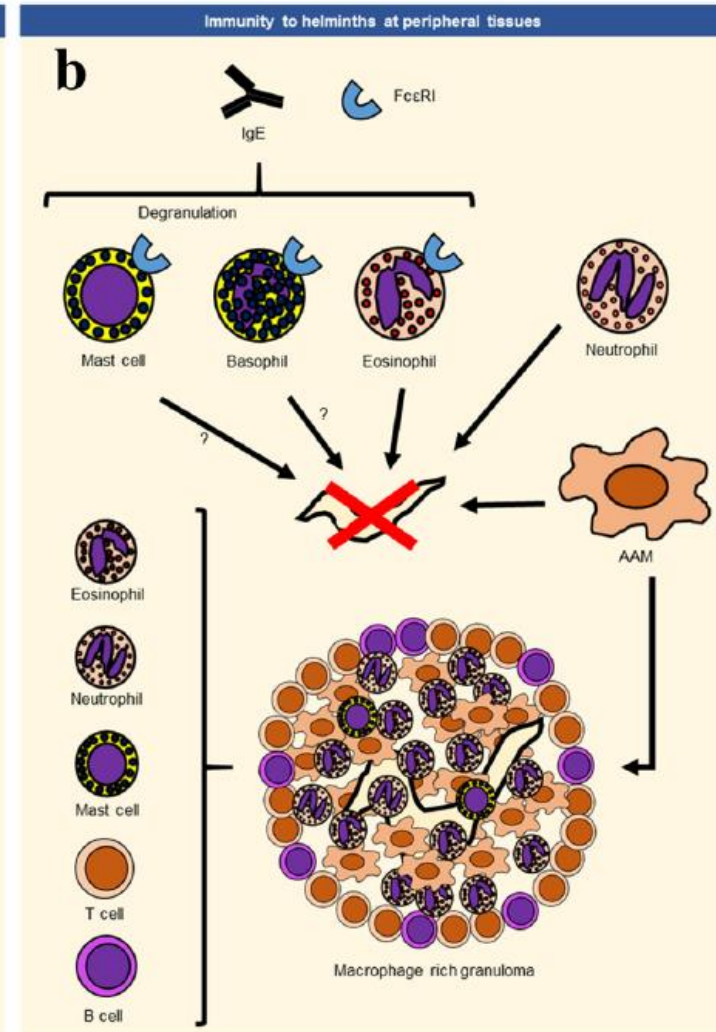
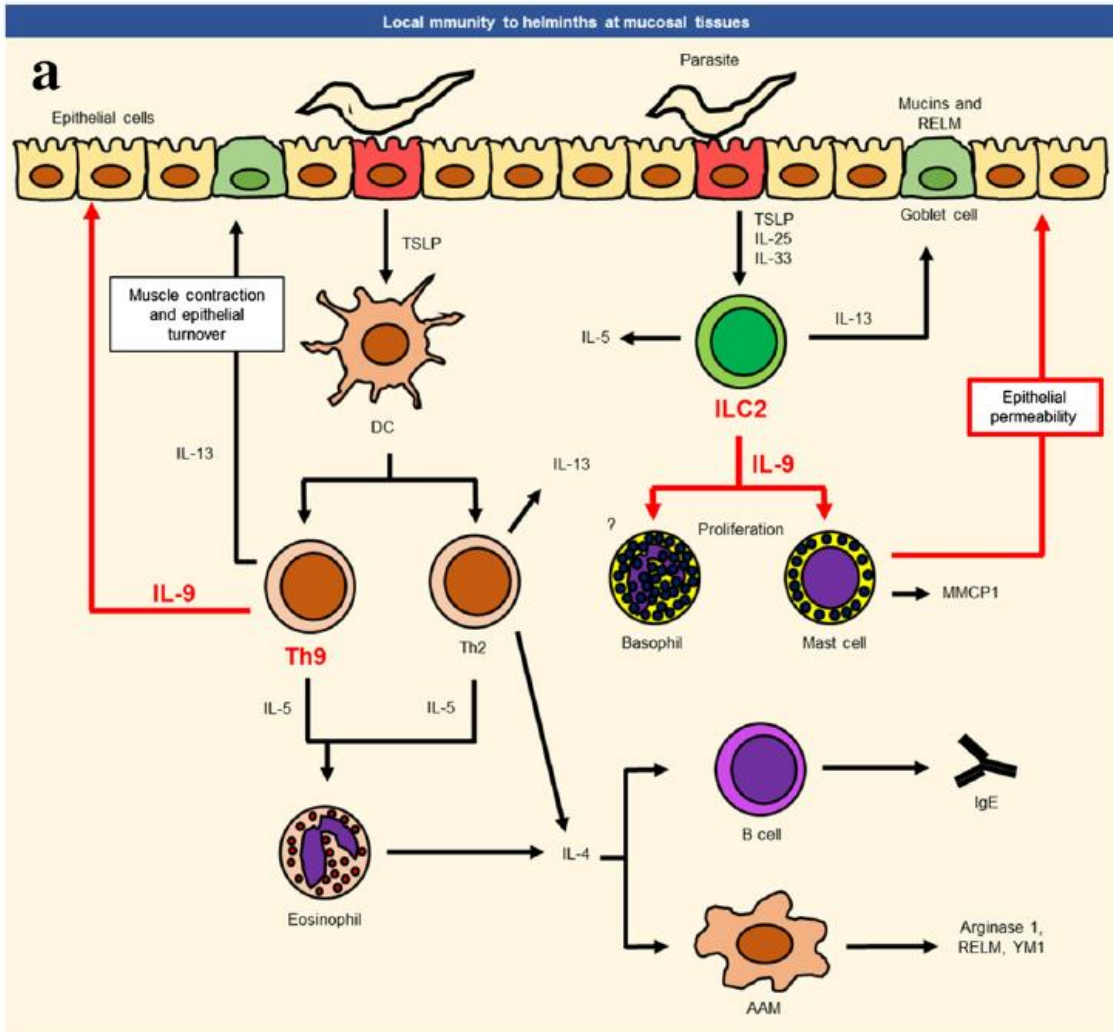
USP – 2024

Resposta imune contra agentes infecciosos

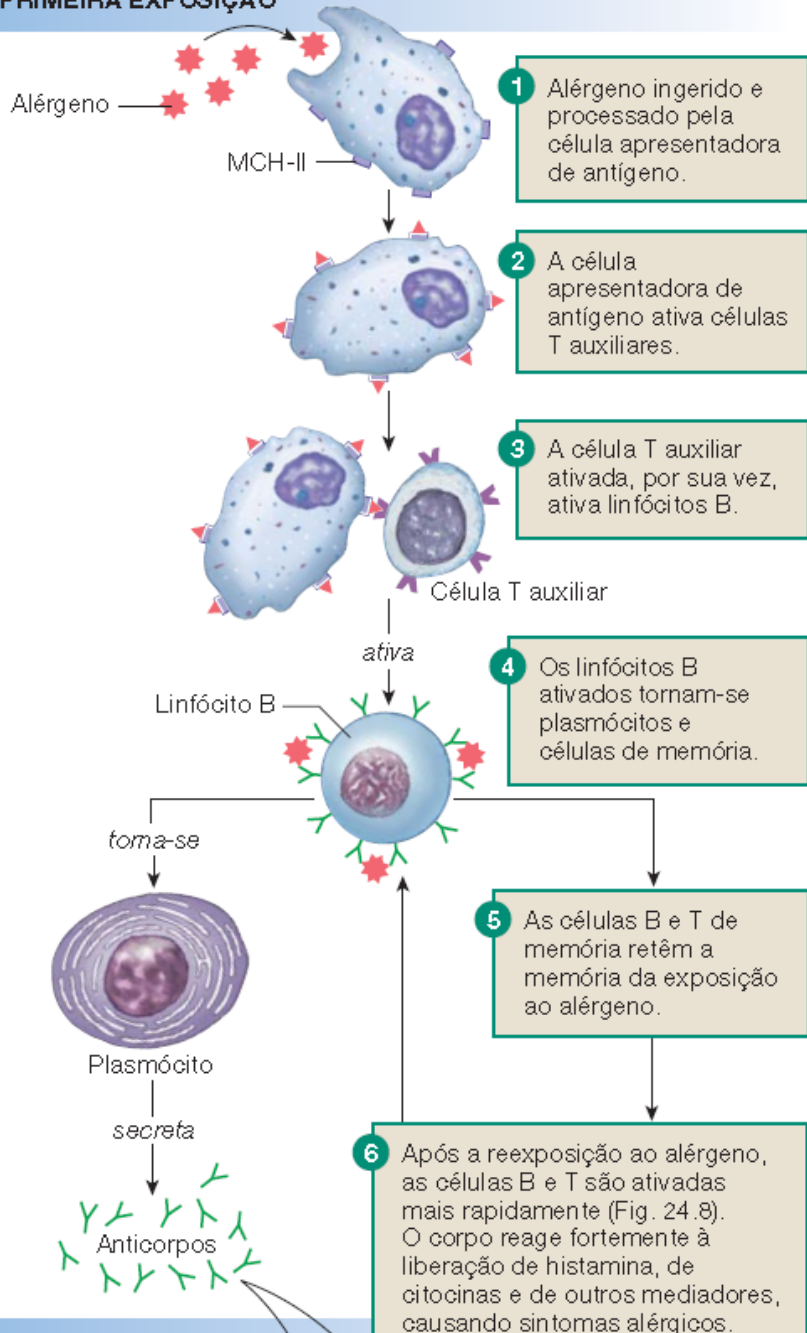
Generalidades:

- A defesa contra microrganismos é mediada pelos mecanismos efetores da **imunidade inata e adaptativa**.
- O sistema imunológico responde de maneira **especializada e distinta a diferentes** tipos de microrganismos para combater esses agentes infecciosos da forma mais eficaz possível.
- A sobrevivência e a patogenicidade dos microrganismos em um hospedeiro são criticamente influenciadas pela capacidade dos **microrganismos para evadir-se ou resistir aos mecanismos efetores da imunidade**.
- Muitos microrganismos estabelecem **infecções latentes ou persistentes**, nas quais a resposta imune controla, mas **não elimina o microrganismo** e, o microrganismo sobrevive sem propagar a infecção.
- Em muitas infecções, as **lesões teciduais** e doenças podem ser causadas pela **resposta do hospedeiro** ao microrganismo mais do que pelo próprio microrganismo.
- **Defeitos hereditários e adquiridos** na imunidade inata e adaptativa são importantes causas de susceptibilidade a infecções.

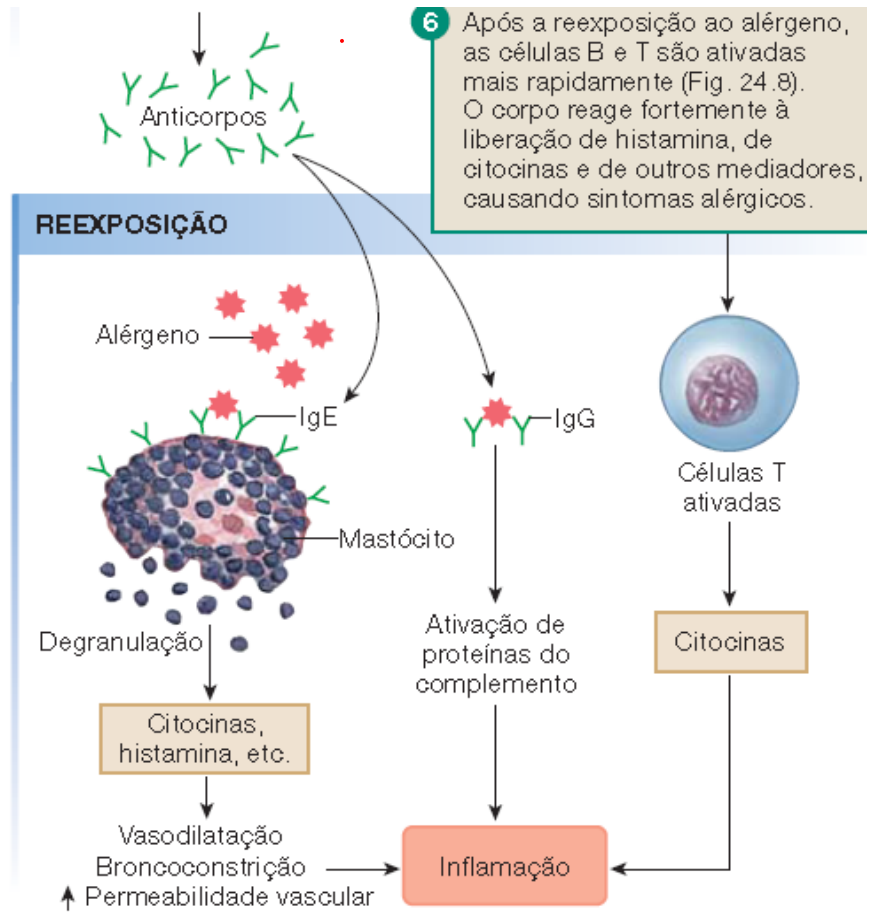
Resposta imune a Parasitas



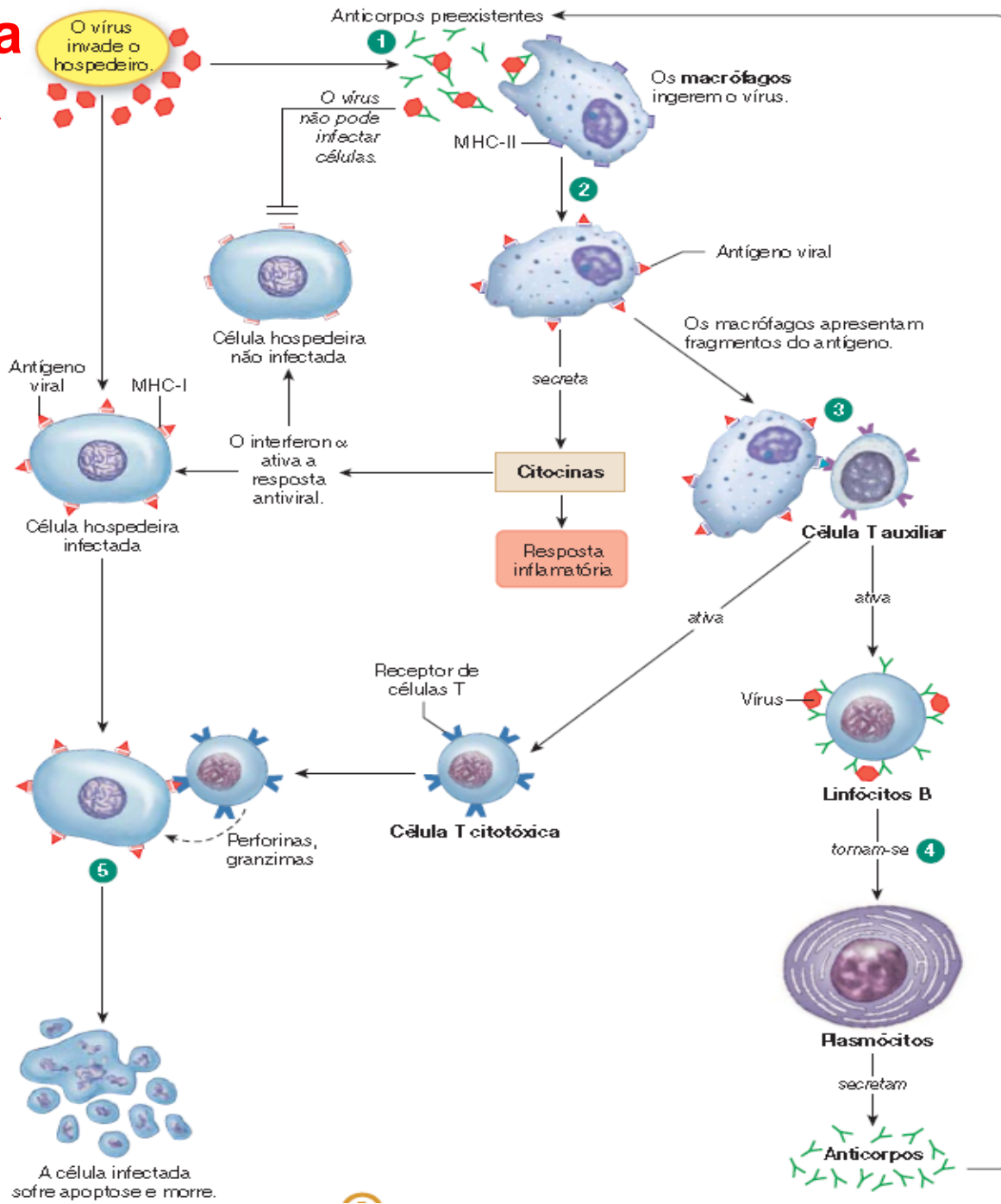
PRIMEIRA EXPOSIÇÃO



Resposta alérgica



Resposta imune a Vírus



1 Os anticorpos atuam como opsoninas, recobrando as partículas virais e tornando-as alvos melhores para os macrófagos.

2 Os macrófagos ingerem vírus e inserem antígenos virais nas moléculas de MHC-II em suas membranas. Os macrófagos ativados também secretam citocinas.

3 As células T auxiliares ligam-se a antígenos virais nos macrófagos e tornam-se ativadas. Estas células T_H ativadas estimulam linfócitos B e células citotóxicas.

4 Os linfócitos B de memória ativados tornam-se plasmócitos, o que resulta em produção adicional de anticorpos.

5 As células T citotóxicas ativadas atacam e destroem células hospedeiras infectadas.

Vírus

Morfologia, classificação e replicação Doenças causadas por vírus em humanos, animais e plantas

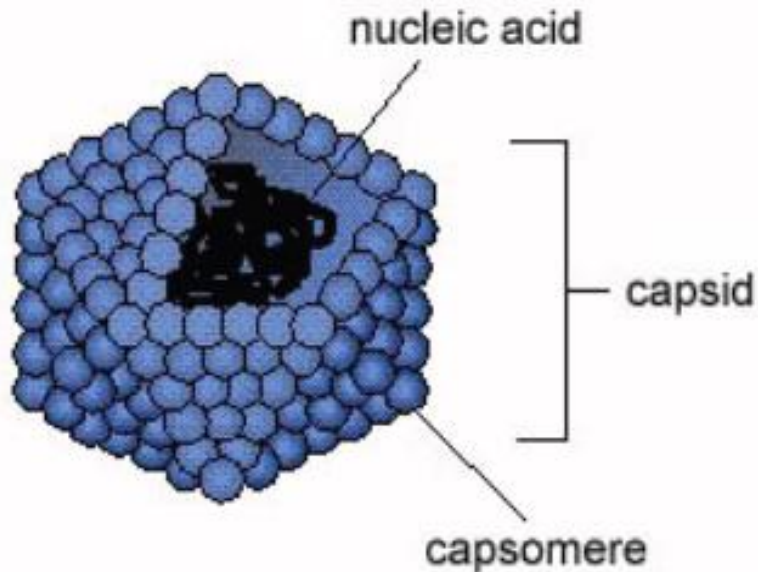


Estimado de aproximadamente 10^{31} vírus no planeta
(Breitbart and Rohwer, 2005)

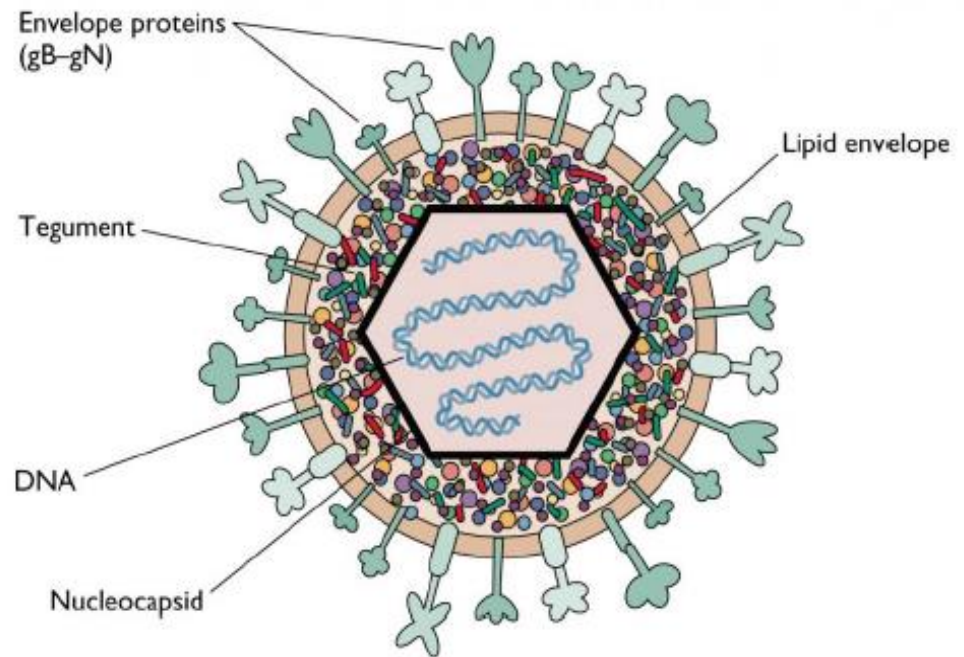
Estrutura dos vírus

Proteção do genoma pelo capsídeo viral.

Os capsídeos são constituídos por proteínas virais com propriedades de auto reconhecimento, o que propicia a formação de complexos chamados protômeros.



Não Envelopados
(Adenovírus)



Envelopados (Herpesvírus)

No caso dos vírus envelopados o capsídeo passa a ser chamado de nucleocapsídeo.

VÍRUS DNA



PARVOVIRIDAE



HERPESVIRIDAE



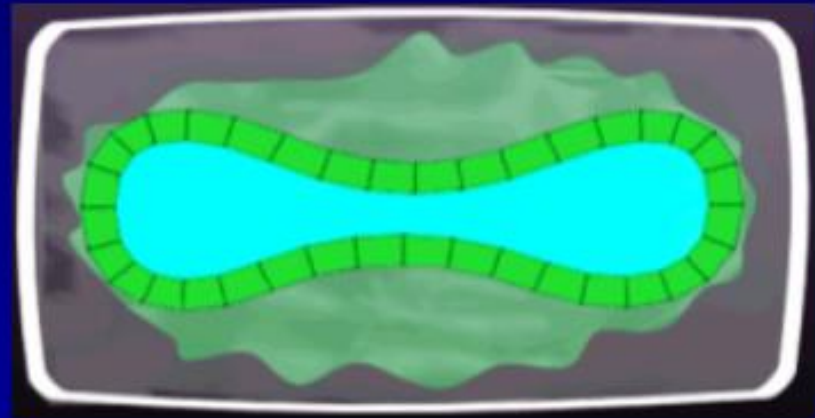
HEPADNAVIRIDAE



PAPOVAVIRIDAE



ADENOVIRIDAE



POXVIRIDAE

VIRUS RNA



REOVIRIDAE



TOGAVIRIDAE
FLAVIVIRIDAE



BUNYAVIRIDAE



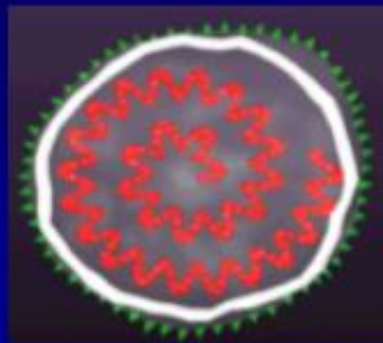
ARENAVIRIDAE



RHABDOVIRIDAE



PICORNAVIRIDAE



PARAMYXOVIRIDAE



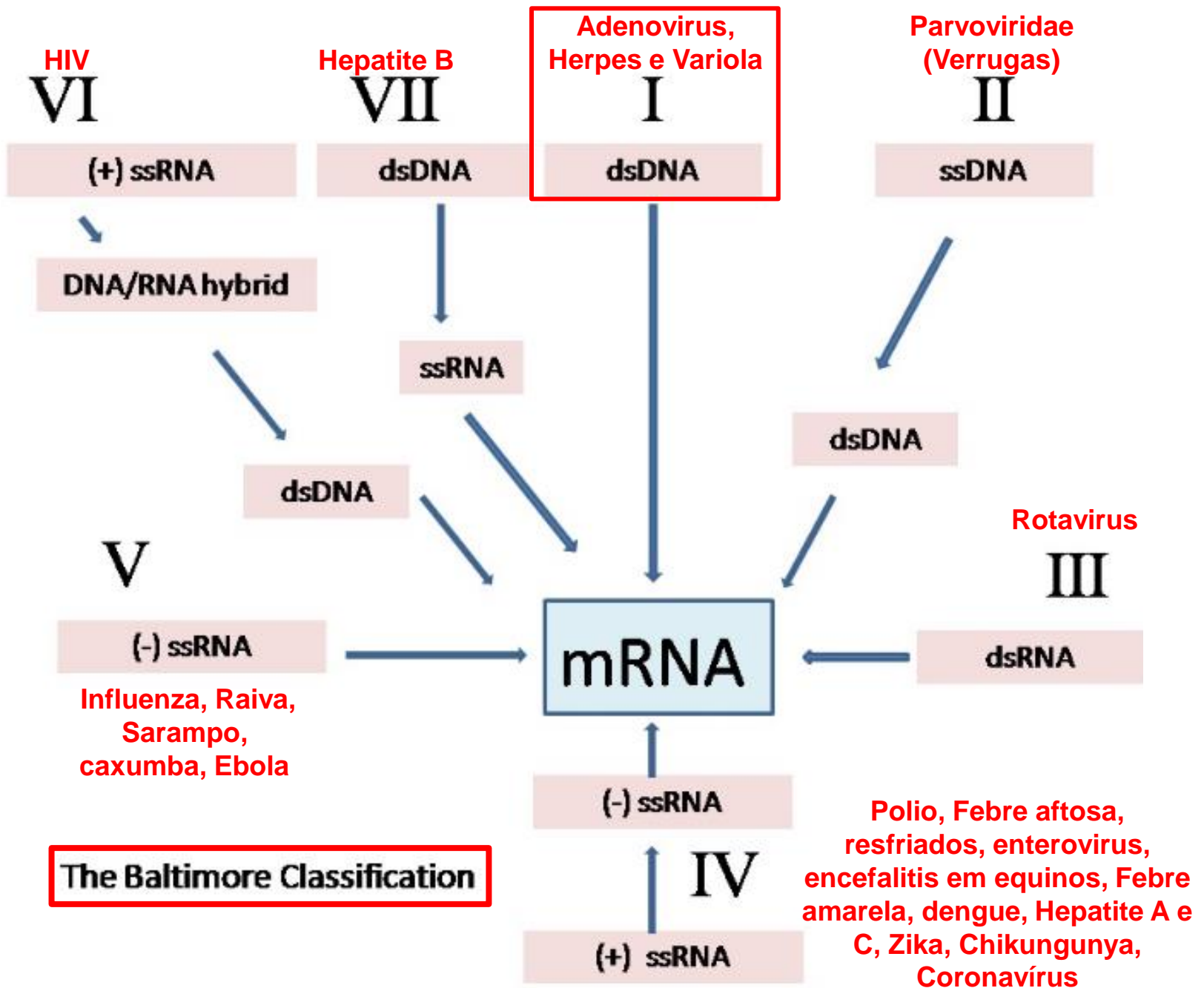
ORTHOMYXOVIRIDAE














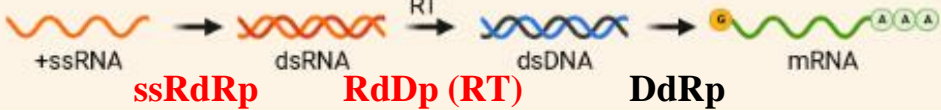


RETROVIRIDAE



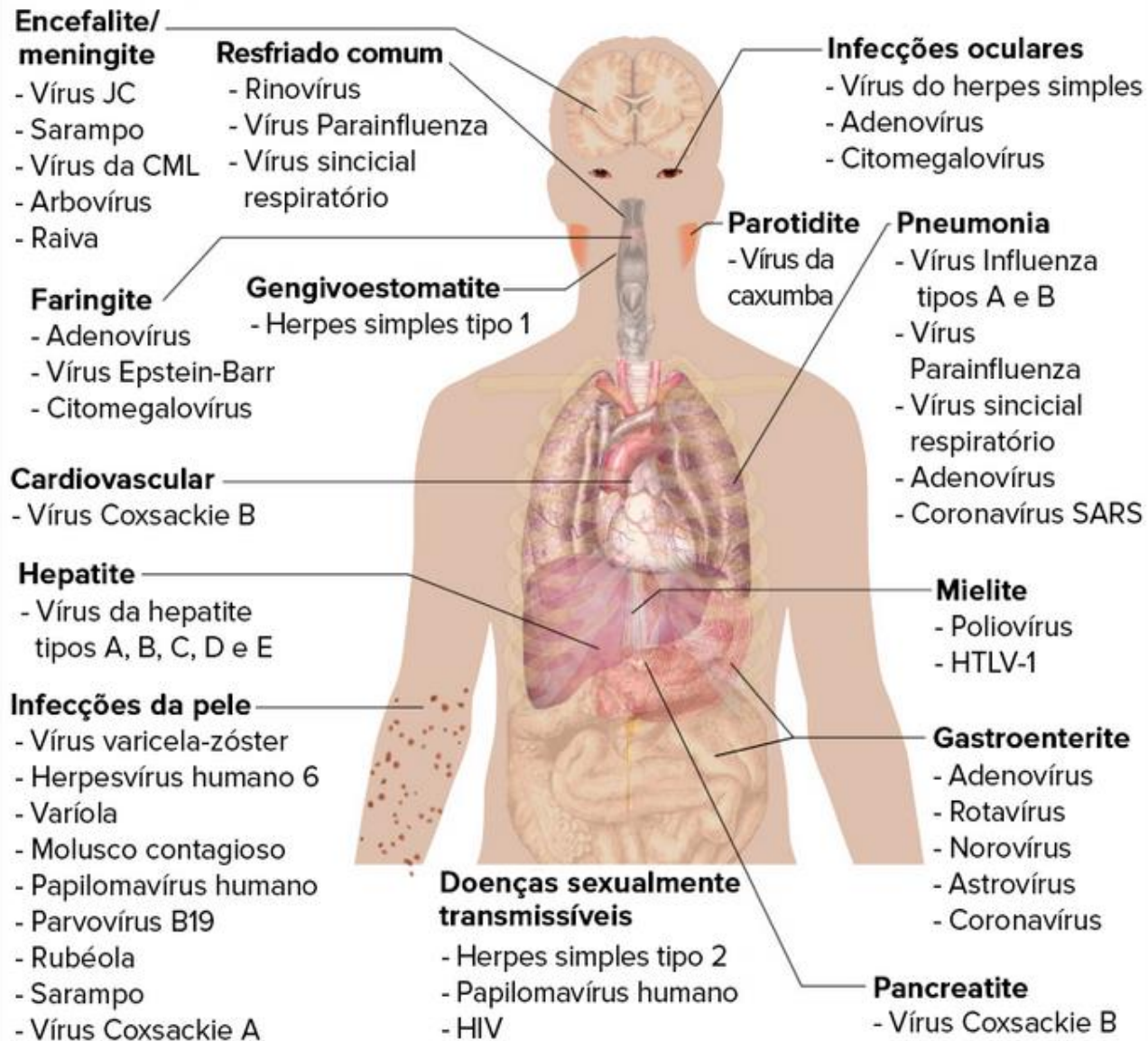
CORONAVIRIDAE



The Baltimore Classification e Transcrição Viral

Group	Example	Genetic Material Processing	
Group 1 dsDNA	 Smallpox		Adenovírus, Herpes e Varíola
Group 2 +ssDNA	 Parvovirus		Parvoviridae (Verrugas)
Group 3 dsRNA	 Rotaviruses		Rotavírus
Group 4 +ssRNA	 Coronaviruses		Polio, Febre aftosa, resfriados, enterovirus, encefalitis em equinos, Febre amarela, dengue, Hepatite A e C, Zika, Chikungunya, Coronavírus
Group 5 -ssRNA	 Measles		Influenza, Raiva, Sarampo, caxumba, Ebola
Group 6 +ssRNA-RT	 HIV		HIV
Group 7 dsDNA-RT	 Hepatitis B		

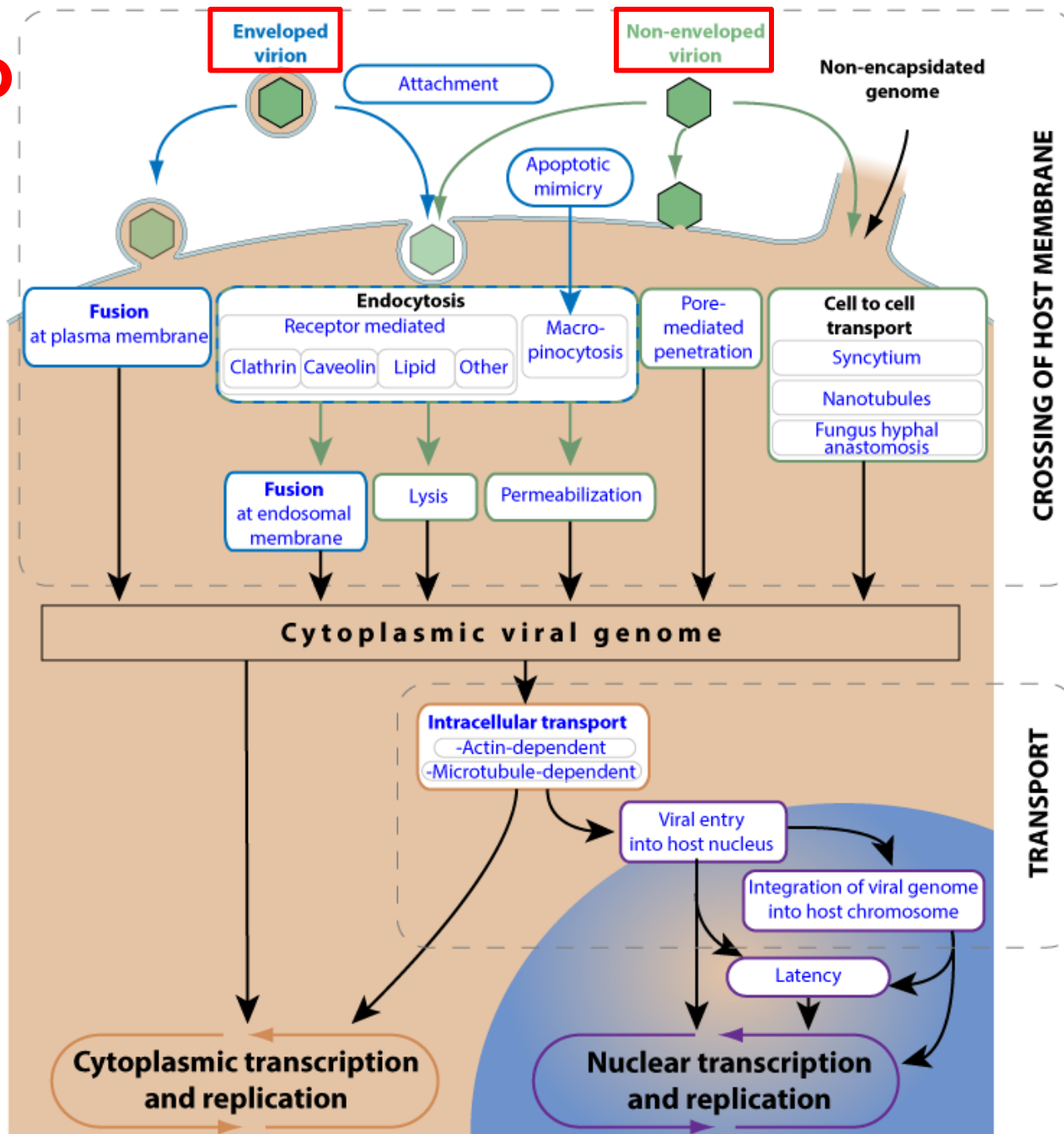
Visão geral das infecções virais



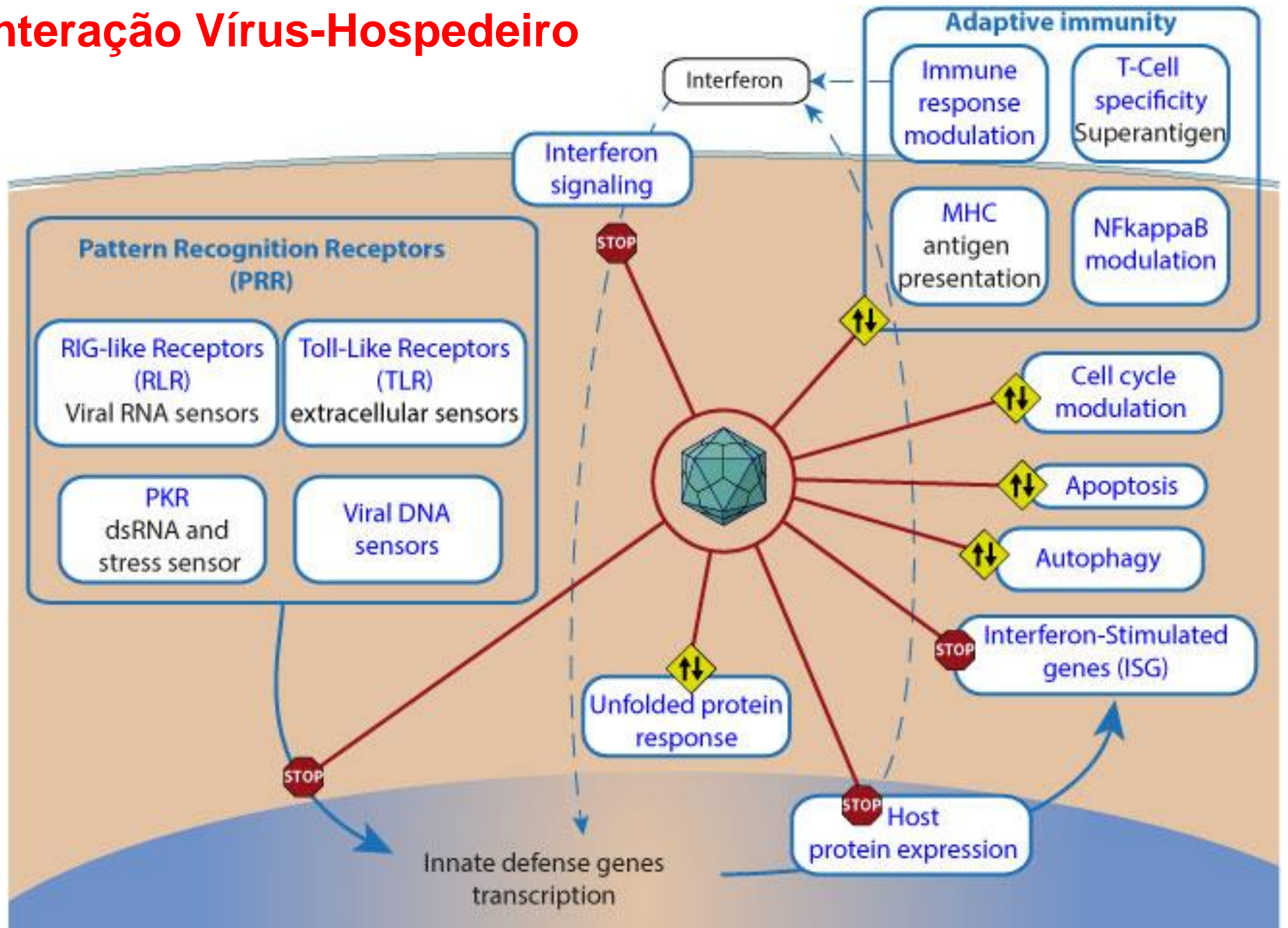
*John Cunningham (JC)

Créditos da imagem: "[Prevention and treatment of viral infections: Figure 1](#)", por OpenStax College, Biology, [CC BY 4.0](#). Modificado do trabalho original de Mikael Häggström.

Infecção Viral



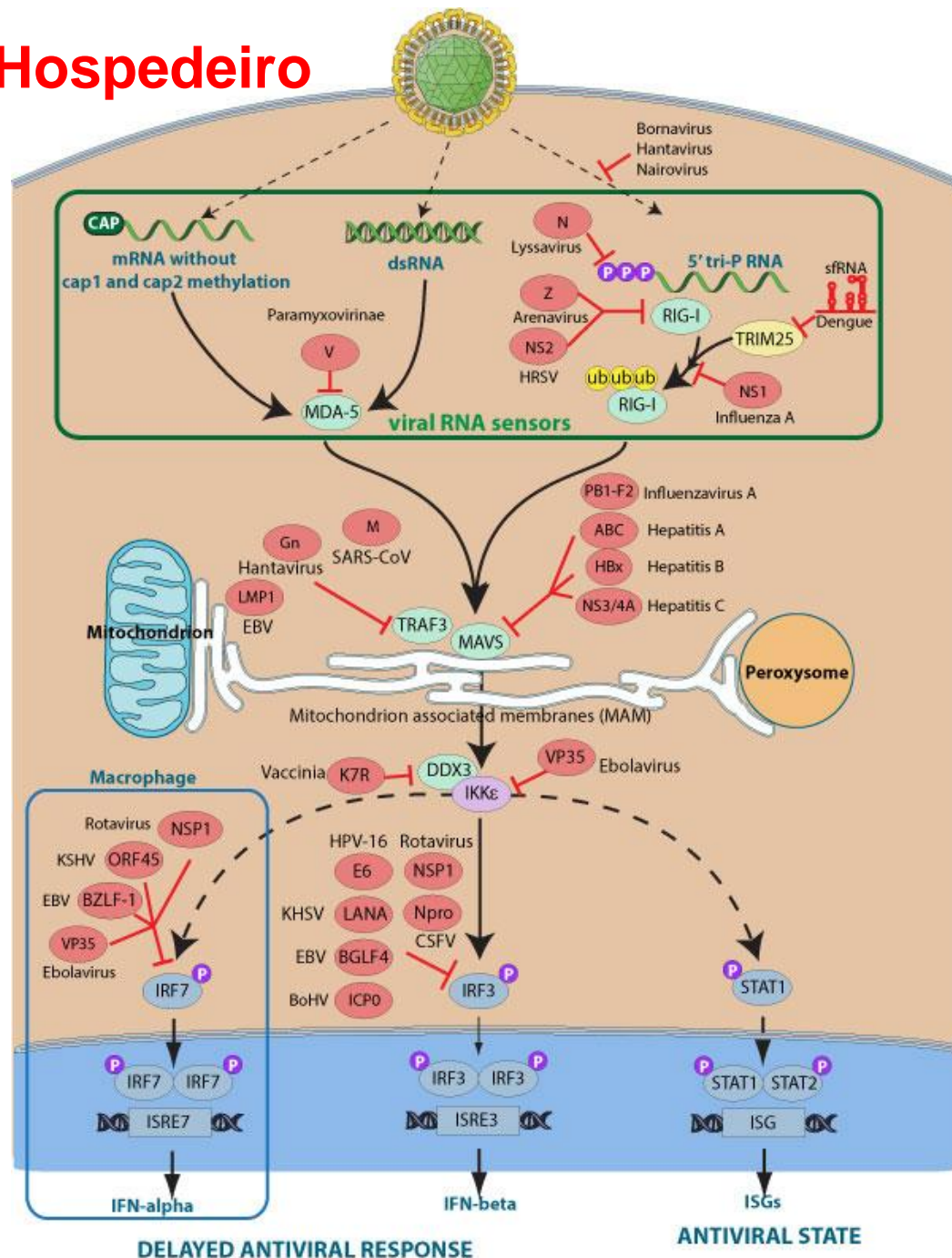
Interação Vírus-Hospedeiro



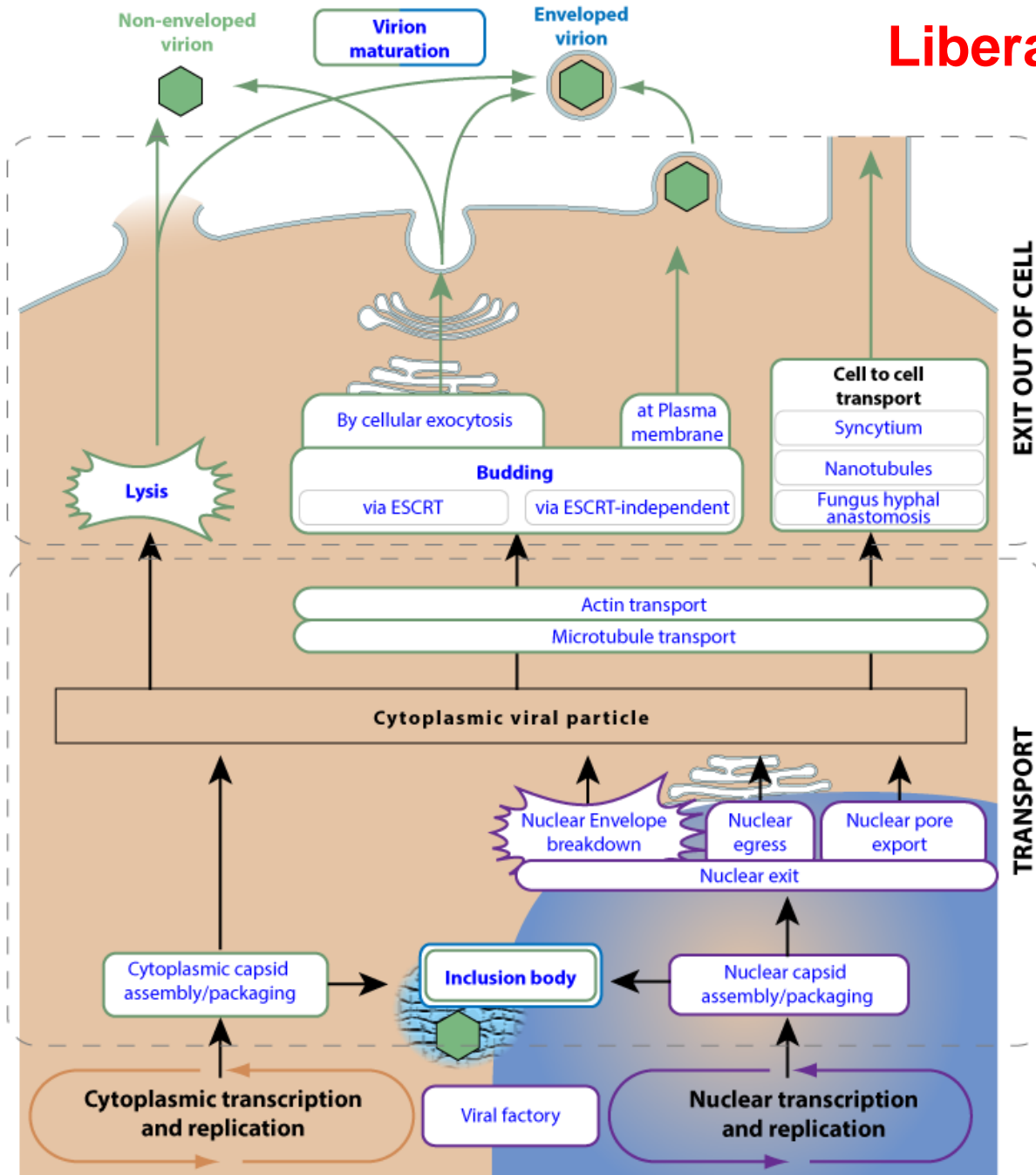
Interação Vírus-Hospedeiro

The cellular RIG-like receptors (RLR) belong to an innate sensor pathway that recognize RNA virus products and activates cellular antiviral state.

- Apoptosis
- Produção de IL
- Síntese de Interferon



Liberação/Brotamento



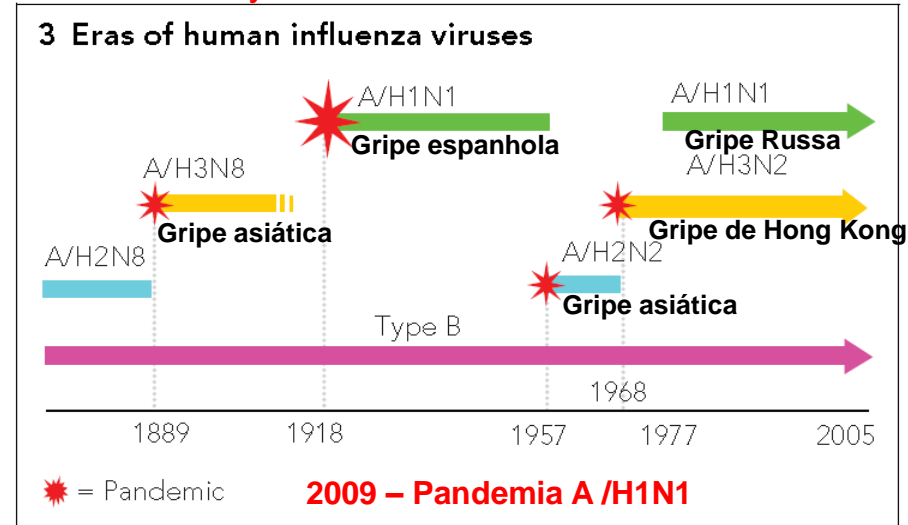
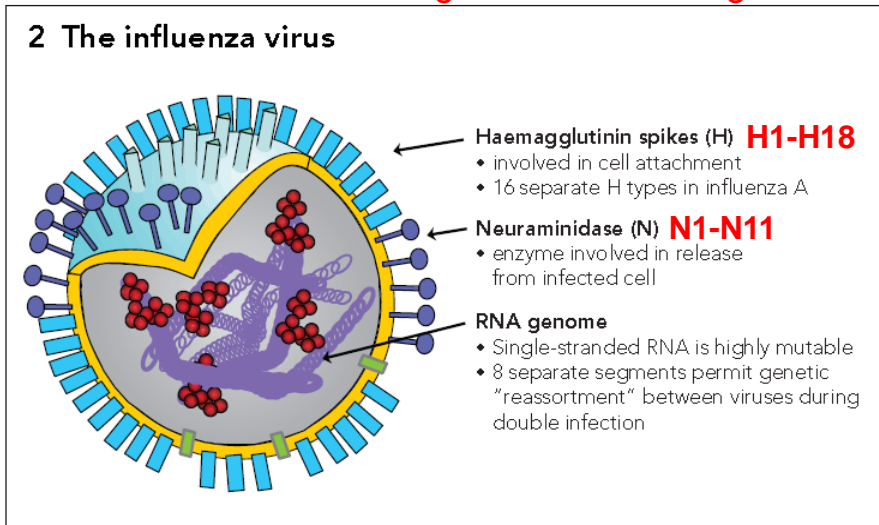
Pandemia

De acordo com a OMS, uma pandemia pode começar quando se reúnem três condições:

- O aparecimento de uma **nova doença** na população;
- O agente **infecta humanos**, causando uma doença séria;
- O agente **espalha-se fácil** e sustentavelmente entre humanos.

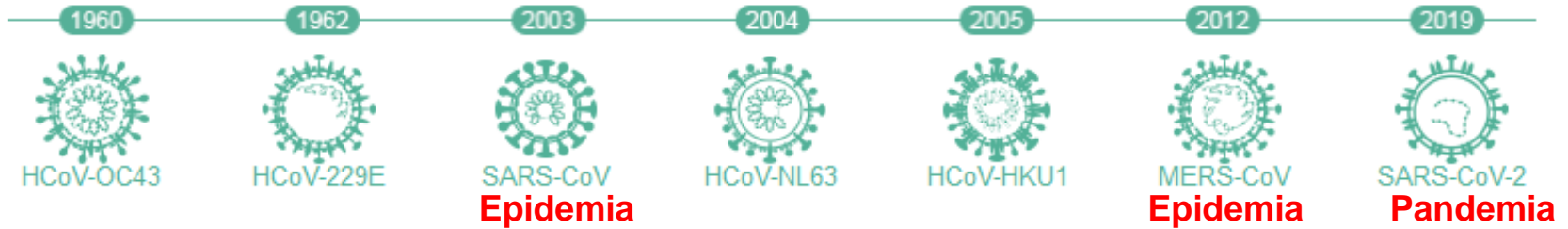
PREPARING FOR AN INFLUENZA PANDEMIC

Pandemic influenza occurs at irregular and unpredictable intervals, and is the result of a major antigenic change known as “antigenic shift”, which occurs only in influenza A.



Myxovirus influenzae, também denominado vírus influenza. Vírus envelopados de RNA de fita simples segmentadas e subdividem-se nos tipos A, B e C, sendo que apenas os do tipo A e B têm relevância clínica em humanos.








Coronavírus

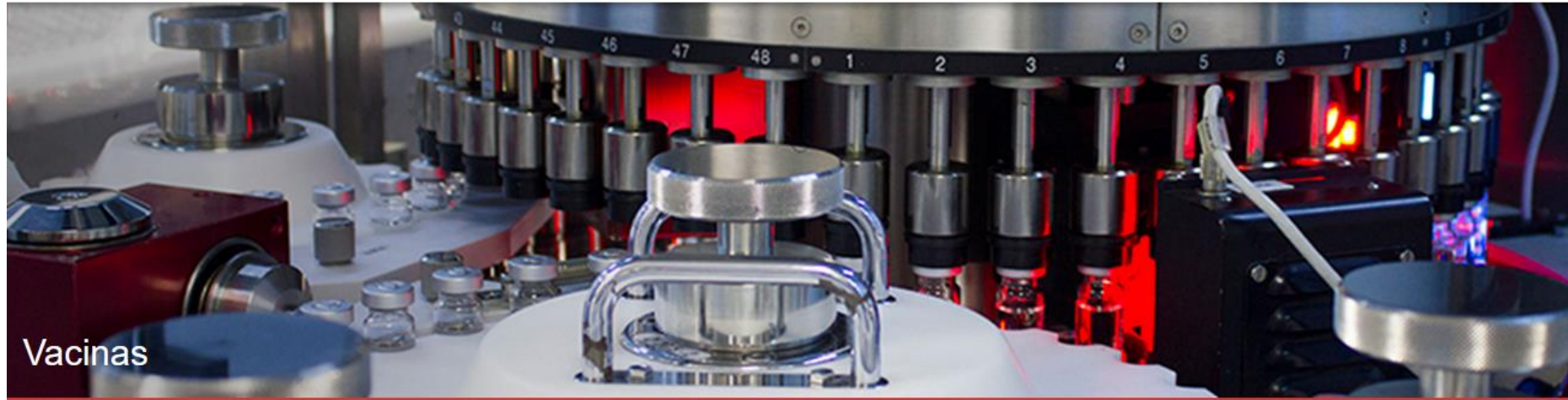


Types

Genera

Disease

	SARS-CoV-2 (2019-nCoV)	Betacoronavirus	Coronavirus disease 2019 (COVID-19). As of 20th Mar, >240,000 infected, >9,800 death
	SARS-CoV	Betacoronavirus	Severe acute respiratory syndrome(SARS), mortality rate 9%
	MERS-CoV	Betacoronavirus	Middle East respiratory syndrome(MERS), mortality rate >30%
	HCoV-HKU1	Betacoronavirus	Upper and lower respiratory tract disease
	HCoV-NL63	Alphacoronavirus	Common cold
	HCoV-OC43	Betacoronavirus	Common cold
	HCoV-229E	Alphacoronavirus	Common cold



Vacinas

Vacinas em desenvolvimento

Um longo caminho tem sido percorrido pelo Butantan no desenvolvimento de vacinas e adjuvantes, desde a pesquisa básica e a aplicação em etapas piloto, até o escalonamento em nível industrial, envolvendo equipes multidisciplinares em colaborações nacionais e internacionais.

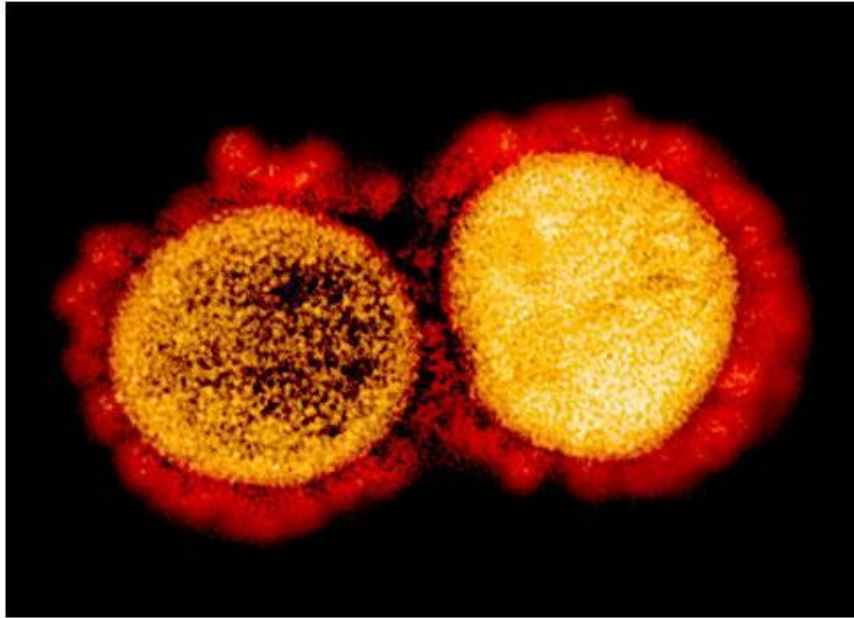
A aplicação de ferramentas biotecnológicas tem contribuído para avanços no desenvolvimento de novos antígenos e adjuvantes, o que aumenta a segurança sem comprometer a eficácia dos novos produtos.

Vacina da Dengue 1, 2, 3, 4 (atenuada)

Vacina Influenza H7N9

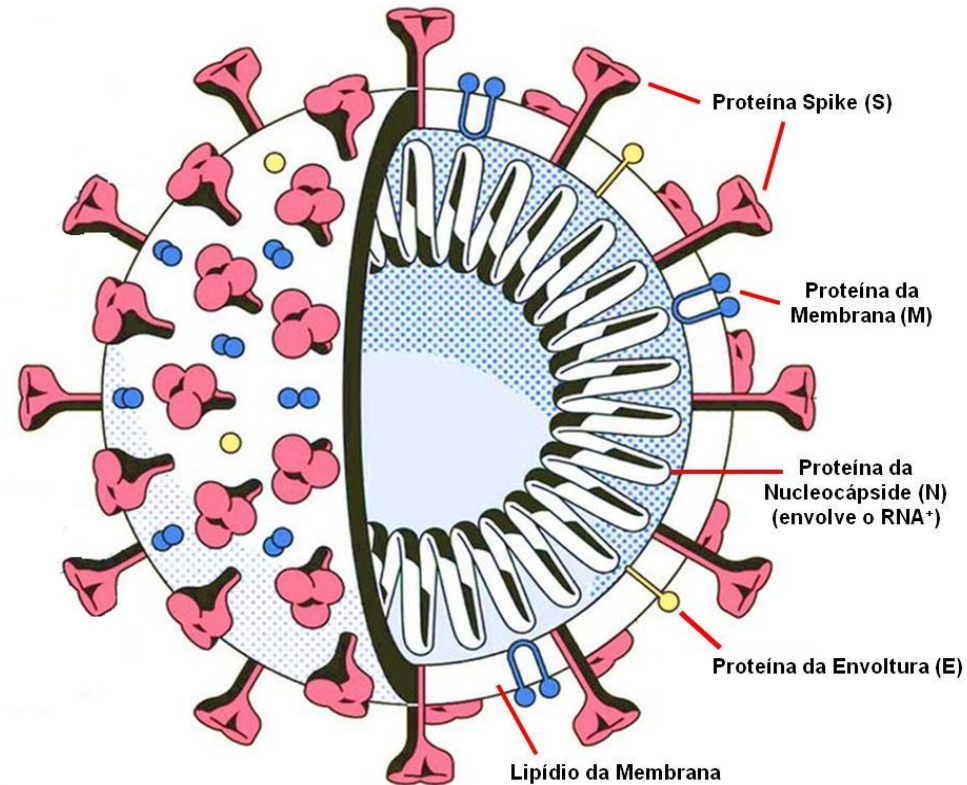
Uma estratégia da Organização Mundial da Saúde (OMS) definiu o desenvolvimento e a produção de uma vacina contra a gripe causada pelo vírus Influenza aviário A (H7N9). O Butantan foi escolhido para ser um dos laboratórios mundiais a produzir e fornecer essa vacina, na iminência de uma pandemia pelo vírus H7N9. A vacina encontra-se em ensaio clínico fase II para a comprovação da segurança e eficácia.

SARS-CoV-2



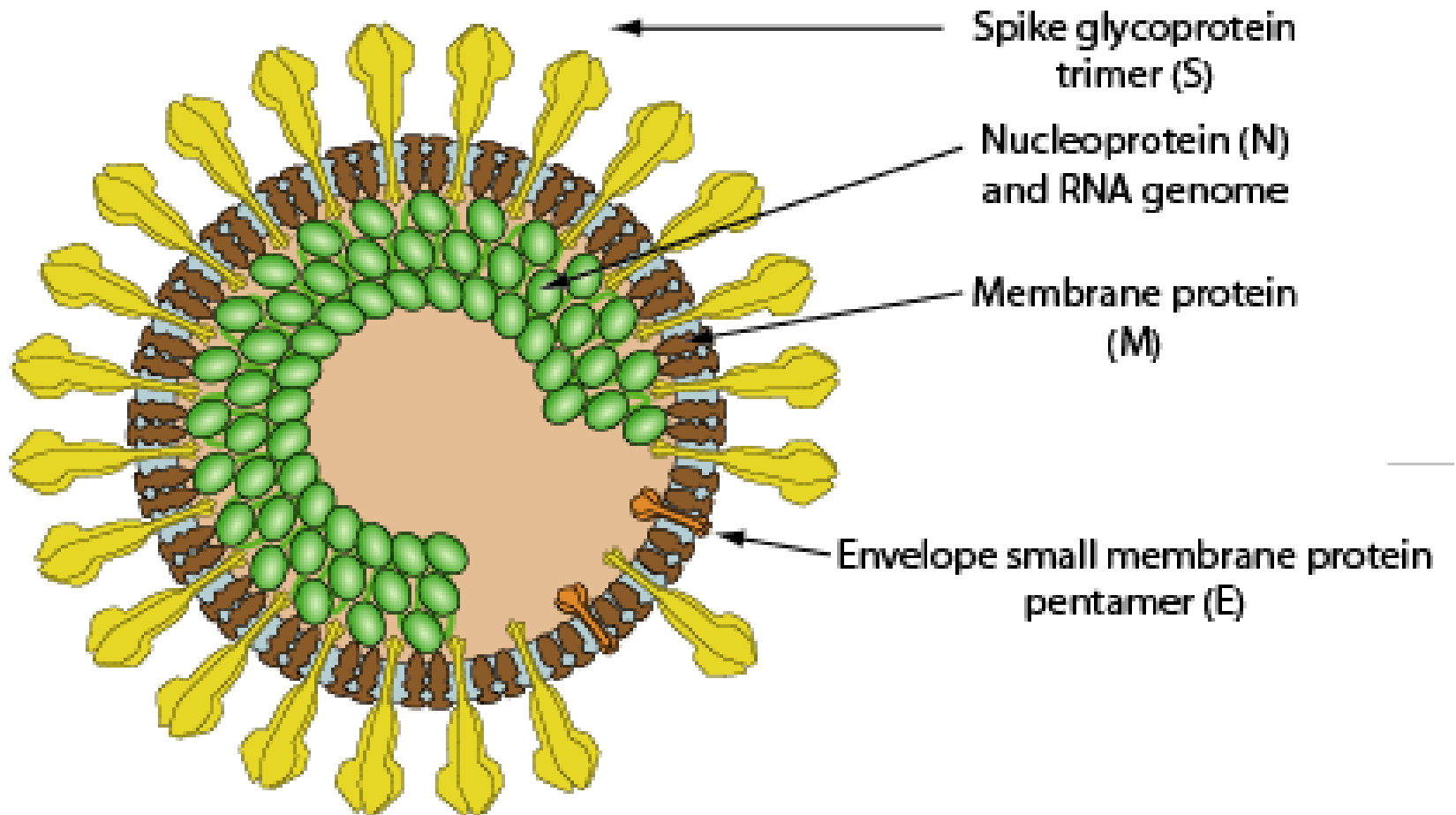
Novel Coronavirus SARS-CoV-2

Transmission electron micrograph of SARS-CoV-2 virus particles, isolated from a patient. Image captured and color-enhanced at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID



É um novo vírus do gênero β coronavírus, família de vírus RNA (~30 kb) de cadeia simples e positivo, com envoltório, de morfologia esférica ou pleomórfica com um diâmetro de 80-120 nm, que podem infectar, principalmente o trato respiratório e intestinal, de diferentes espécies, causando uma ampla gama de sintomas em animais e pessoas

Um novo coronavírus (SARS-CoV-2) é uma nova cepa que não foi previamente identificada em humanos e causa a doença que foi denominada "doença de coronavírus 2019" (abreviada como "COVID-19")



In the last two decades, coronavirus has caused two large-scale pandemics, SARS in 2002 and the Middle East respiratory syndrome (MERS) in 2012. In December 2019, a novel coronavirus (SARS-CoV-2) induced an outbreak of pneumonia in Wuhan, China, restated the risk of coronaviruses posed to public health. The infection routes and pathogenesis of SARS-CoV-2 are not fully understood, and the study of SARS-CoV-2 host cell receptor ACE2 could be valuable for the prevention and treatment of the COVID-19.

Origem de SARS-CoV-2

“O salto de Espécie”

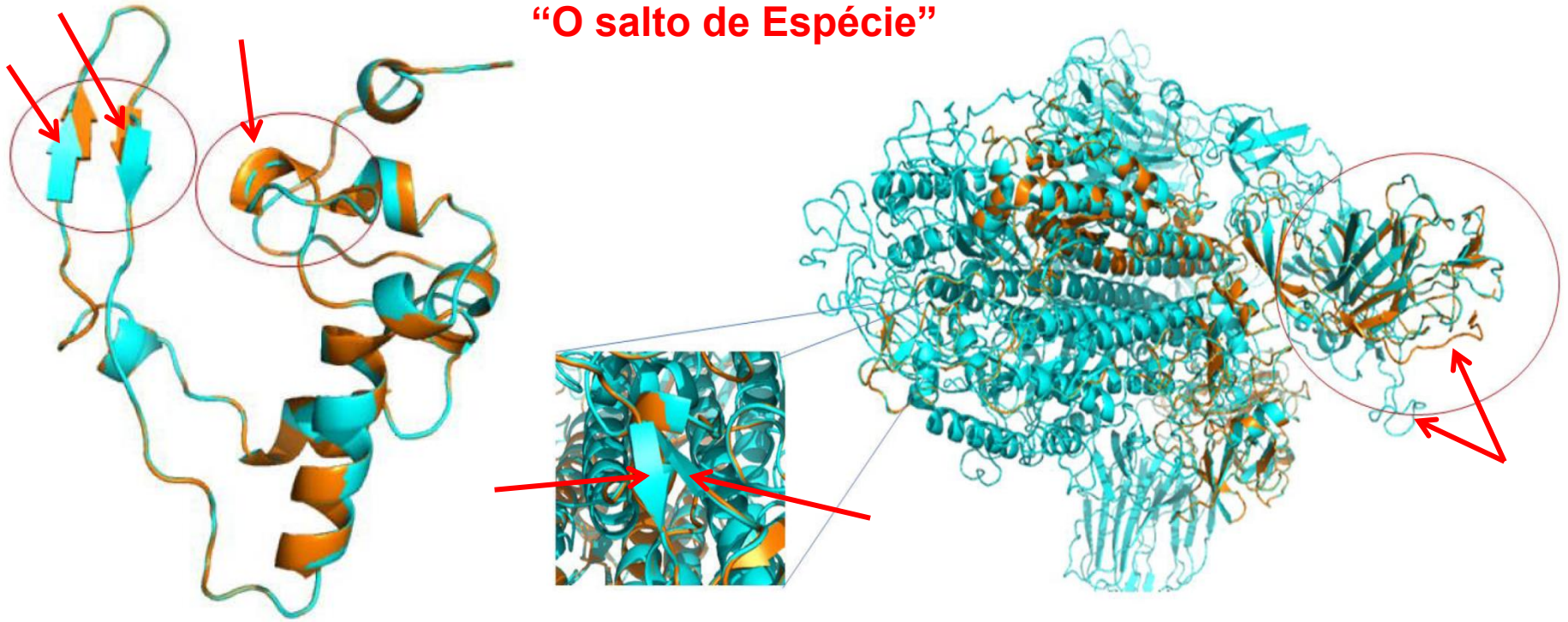
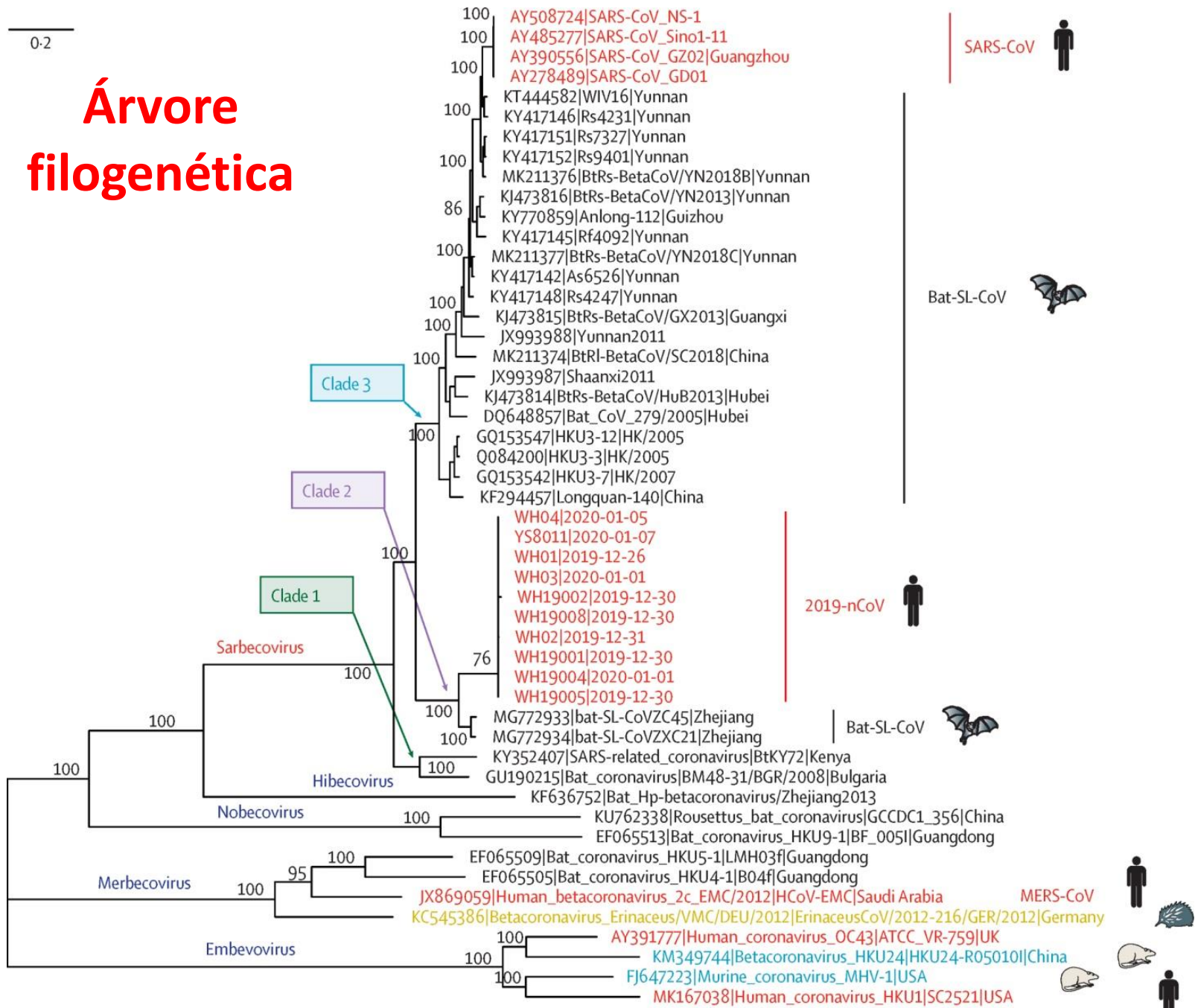


Figure 2. Cartoon model of the structural superposition between the homology model of the 2019-nCoV in blue and the **Nucleocapsid protein** of SARS Coronavirus (PDB code 2jw8.1) in orange. the presence of an alpha-helix on the SARS-CoV and not present on the 2019 n-CoV structure and e positional difference of the beta-sheets.

Figure 3. Cartoon model of the structural superposition between the homology model of the 2019-nCoV in blue and the **spike glycoprotein** of SARS Coronavirus (PDB code 6acc.1) in orange. the red circle highlights the presence of a variable region on the 2019 n-CoV at the beginning of the protein while the blue square highlights the presence of 2 beta-sheets on the 2019 n-CoV (401:KYR and 440:LND) that are not present on the SARS-CoV structure.

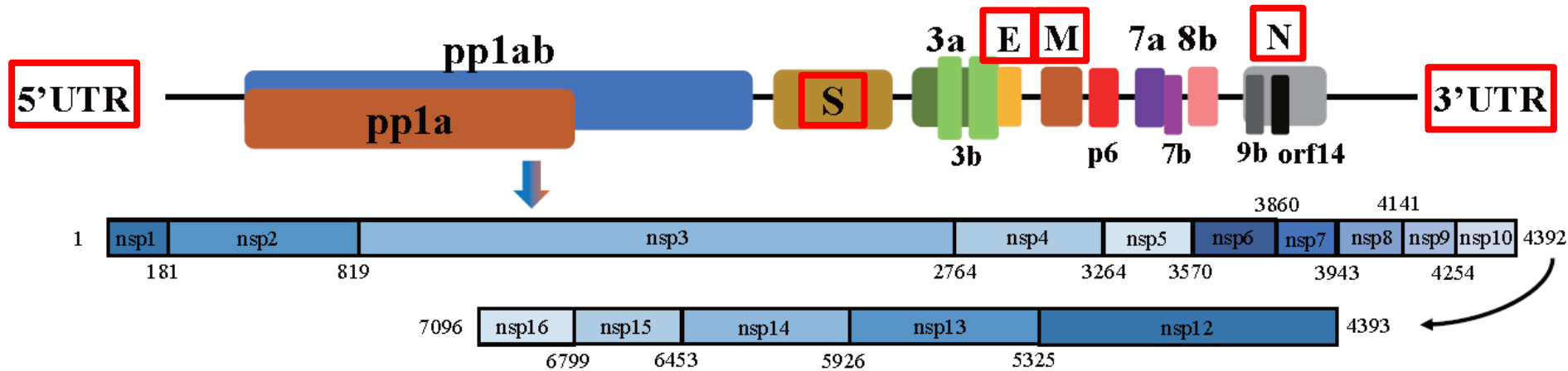
Árvore filogenética



SARS-CoV-2 Genoma

A

IVDC-HB-01/2019 (~29.8kb)



O genoma **de ~30 kb**, codifica para **~14 ORFs**, com a seguinte ordem:

5' - UTR 265 nucleotídeos (nt)-ORF1ab (codifica 16 proteínas não estruturais, nsp) - proteína Spike (**S**) - ORF3a-ORF3b-proteína do envoltório (**E**); proteína da membrana (**M**)-ORF6-ORF7a--ORF7b- ORF8-proteína do nucleocapsídeo (**N**)---ORF9b-ORF9c-ORF10(14)-229 nt UTR **-3'**

Os genes S, E, M e N codificam proteínas estruturais, sendo a proteína S a responsável pela ligação ao receptor Enzima Conversora da Angiotensina 2 (ECA 2) em humanos.

SARS-CoV-2 - Transdução

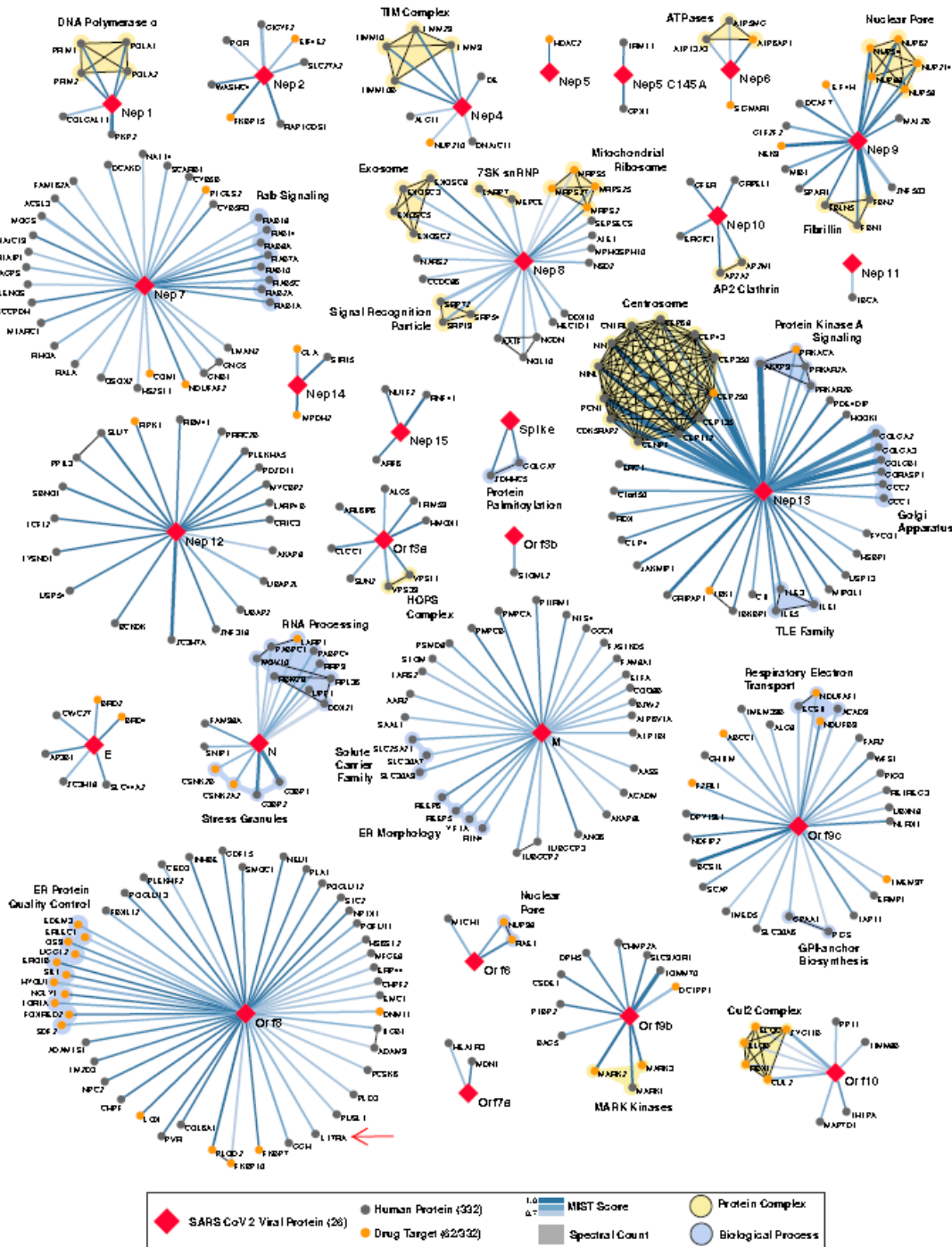
Protein	Mol. weight (kDa)	Seq. similarity with SARS-CoV	Description
Nsp1	19.8	91.1%	Suppresses host antiviral response
Nsp2	70.5	82.9%	
Nsp3	217.3	86.5%	Nsp3-Nsp4-Nsp6 complex involved in viral replication
Nsp4	56.2	90.8%	Nsp3-Nsp4-Nsp6 complex involved in viral replication
Nsp5	33.8	98.7%	Main protease (3C-like)
Nsp6	33.0	94.8%	Nsp3-Nsp4-Nsp6 complex involved in viral replication
Nsp7	9.2	100.0%	Nsp7-Nsp8 complex is part of RNA polymerase
Nsp8	21.9	99.0%	Nsp7-Nsp8 complex is part of RNA polymerase
Nsp9	12.4	98.2%	ssRNA binding
Nsp10	14.8	99.3%	Essential for Nsp16 methyltransferase activity
Nsp11	1.3	92.3%	Short peptide
Nsp12	106.7	98.3%	RNA polymerase
Nsp13	66.9	100.0%	Helicase/triphosphatase
Nsp14	59.8	98.7%	3'-5' exonuclease
Nsp15	38.8	95.7%	Uridine-specific endoribonuclease
Nsp16	33.3	98.0%	RNA-cap methyltransferase
S	141.2	87.0%	Spike protein, mediates binding to ACE2
Orf3a	31.1	85.1%	Activates the NLRP3 inflammasome
Orf3b	6.5	9.5%	
E	8.4	96.1%	Envelope protein, involved in virus morphogenesis and assembly
M	25.1	96.4%	Membrane glycoprotein, predominant component of the envelope
Orf6	7.3	85.7%	Type I IFN antagonist
Orf7a	13.7	90.2%	Virus-induced apoptosis
Orf7b	5.2	84.1%	
Orf8	13.8	45.3%	
N	45.6	94.3%	Nucleocapsid phosphoprotein, binds to RNA genome
Orf9b	10.8	84.7%	Type I IFN antagonist
Orf9c	8.0	78.1%	
Orf10	4.4	-	

Alvo para Inibidor
Alvo para Vacina

Alvo para Inibidor
Alvo para Vacina

Alvo para Inibidor
Alvo para Vacina

Alvo para Inibidor
Alvo para Vacina

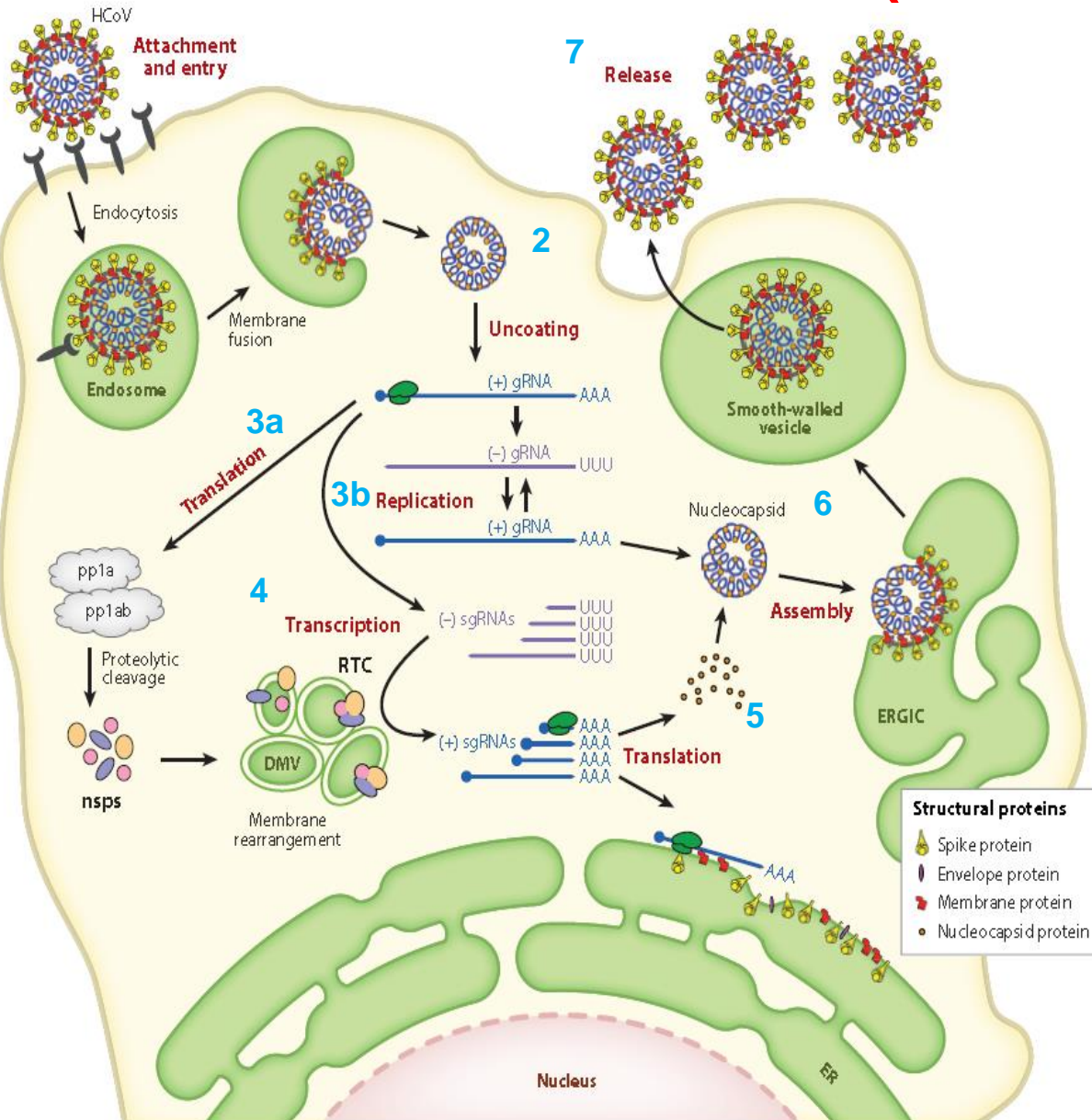


Mapa de Interação Proteínas SARS-CoV-2 - Humano

Figure 3: SARS-CoV-2 Protein-Protein Interaction Network. In total, **332** high confidence interactions are represented between **26 SARS-CoV-2** proteins and their human interactors. Red diamonds represent a SARSCoV-2 viral protein, interacting human host proteins are represented with circles, with drug targets in orange. Edge color is proportional to MiST score and edge thickness proportional to spectral counts. Physical interactions among host proteins are noted as thin black lines, protein complexes are highlighted in yellow, and proteins sharing the same biological process are highlighted in blue.

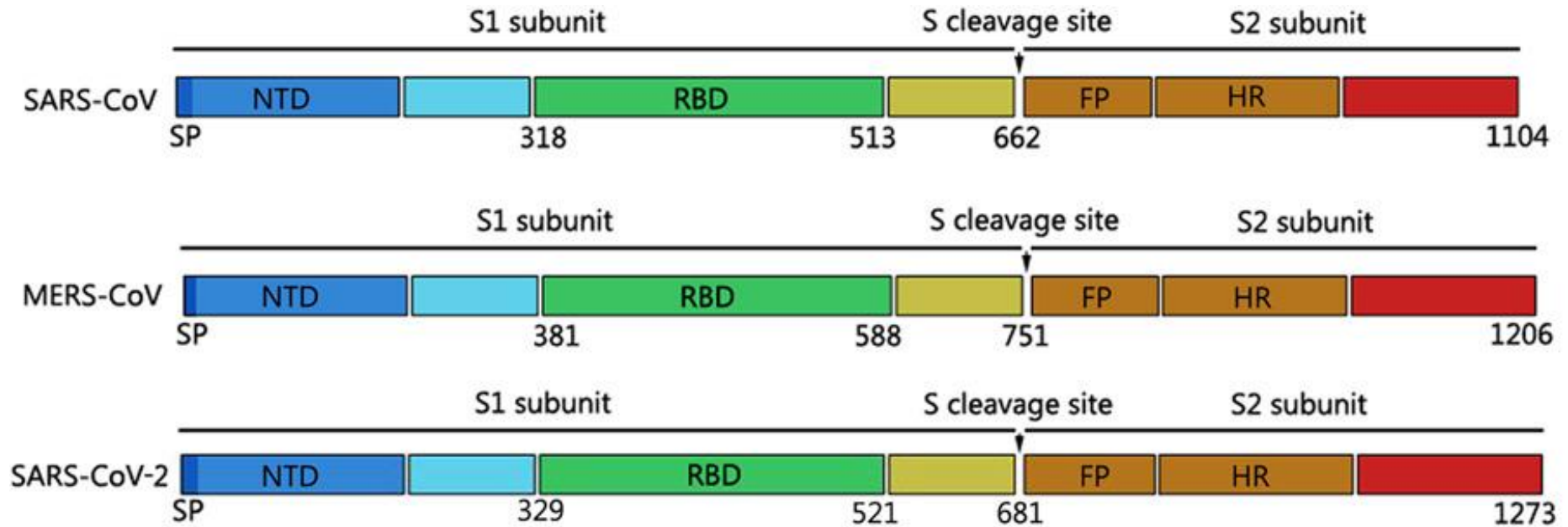
Ciclo geral de replicação de coronavírus humanos (HCoVs)

1



1. Adesão e ingresso do vírus à célula hospedeiro;
2. Remoção da envoltura e liberação do gRNA;
- 3a. Síntese de proteínas virais;
- 3b. Síntese de novas moléculas de gRNA;
4. Síntese de pequenas e novas moléculas de gRNA;
5. Síntese de proteínas virais;
6. Montagem de novas partículas virais;
7. Liberação ao meio extracelular

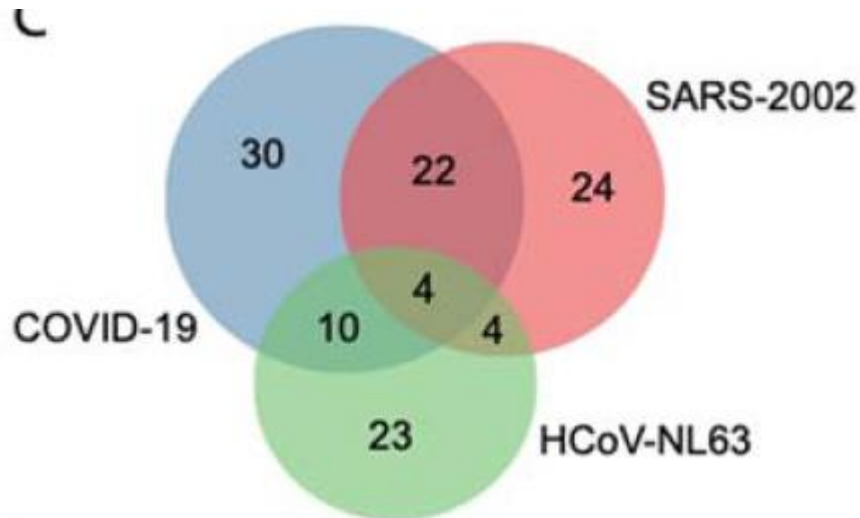
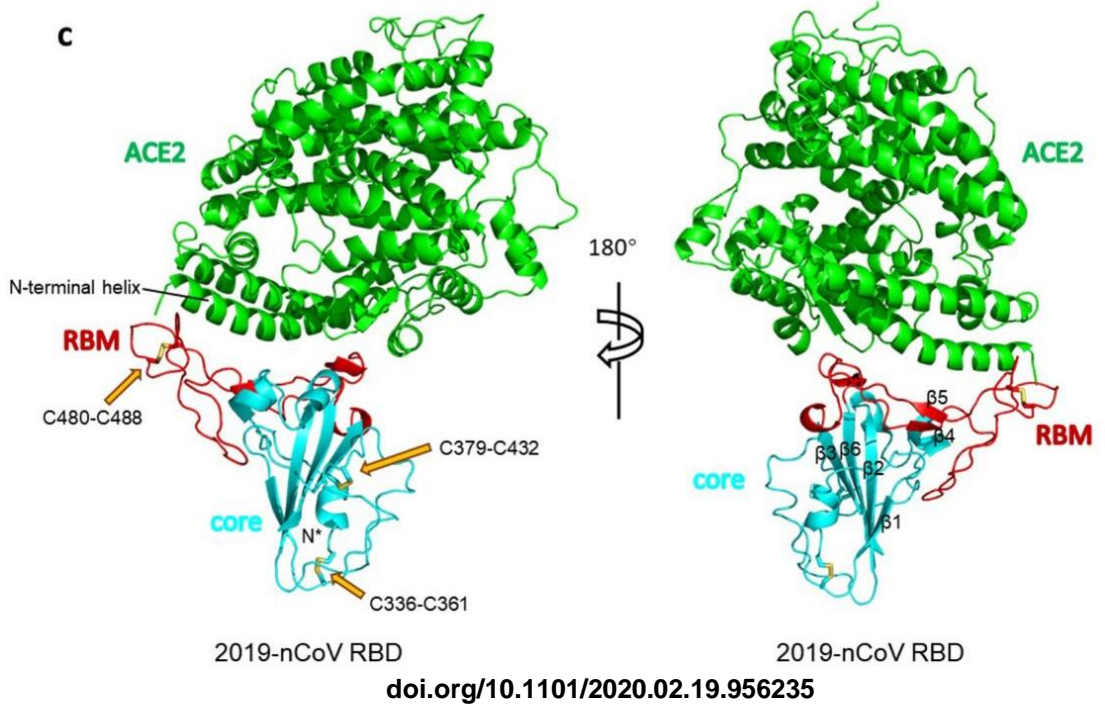
Proteína S



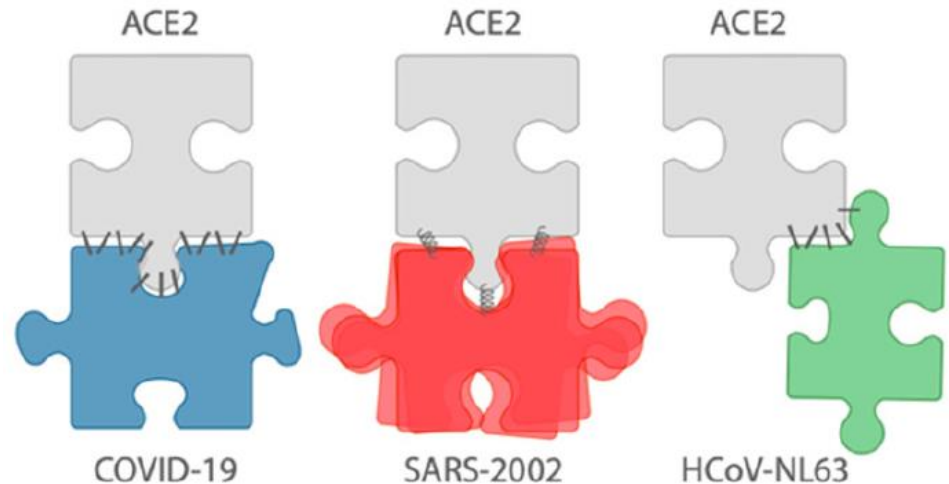
Proteína S e ECA2

1273 aa e 141.2 kD.
 SARS-CoV-2 utiliza an extensivamente glicosylated spike (S) protein that protrudes from the viral surface to bind to angiotensin-converting enzyme 2 (ACE2), the host cell receptor, to mediate cell entry. The S protein is a trimeric class I fusion protein that is composed of two functional subunits responsible for receptor binding (S1 subunit) and membrane fusion (S2 subunit), with each trimer displaying 66 N-linked glycosylation sites

doi.org/10.1101/2020.03.26.010322



Interação de resíduos ECA2

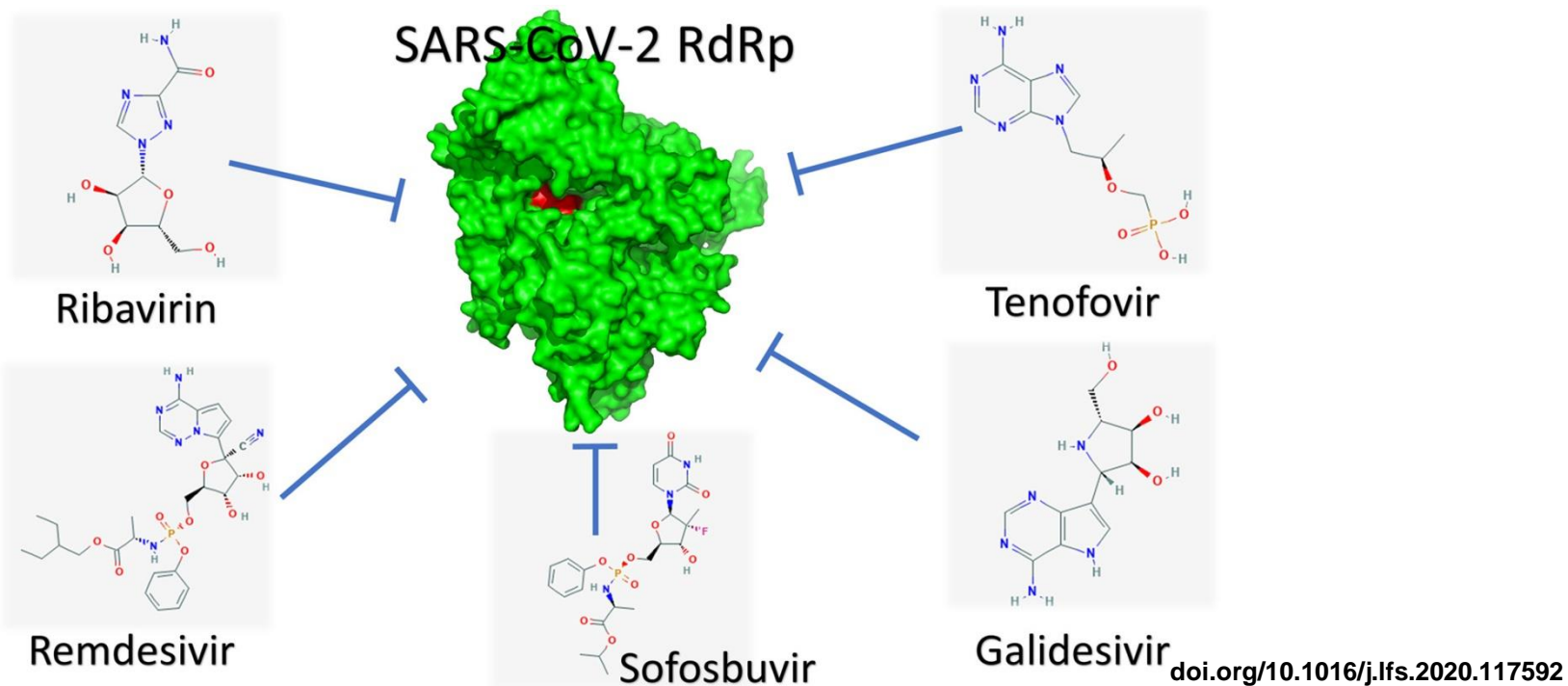


Estratégia de ligação

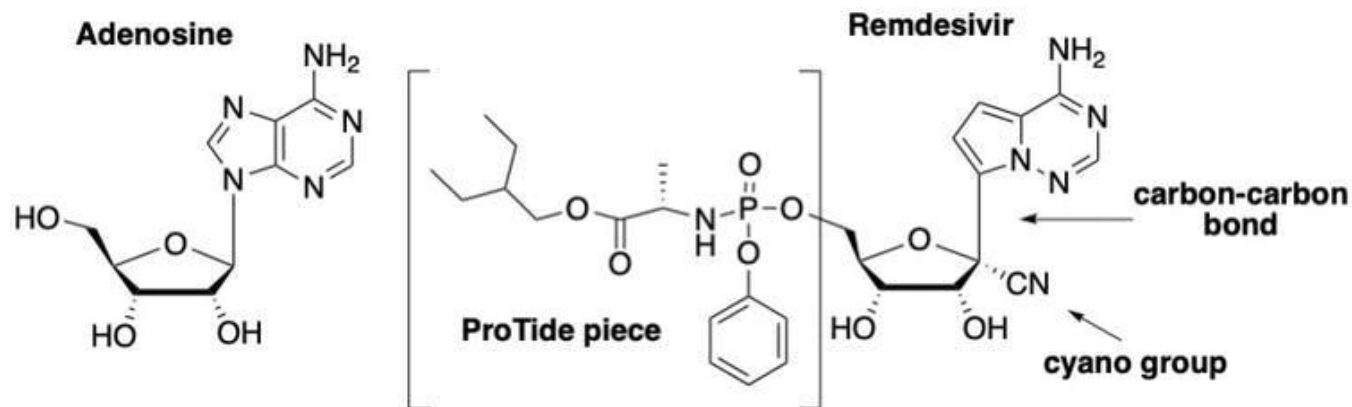
[doi:10.3390/v12050497](https://doi.org/10.3390/v12050497)

nsp12 RNA-dependent RNA polymerase (RdRp)

The SARS-CoV nsp12 is 932 a.a. in length and 106,7 kD; Complex of nsp8 and the nsp7-nsp8 complex within the viral RNA synthesis complex.



O **remdesivir** é incorporado na cadeia crescente de RNA, a presença do grupo CN faz com que o formato do açúcar fique deformado, o que, por sua vez, distorce o formato da cadeia de RNA, de forma que apenas mais três nucleotídeos possam ser adicionados. Isso termina a síntese da cadeia de RNA e a replicação do vírus.

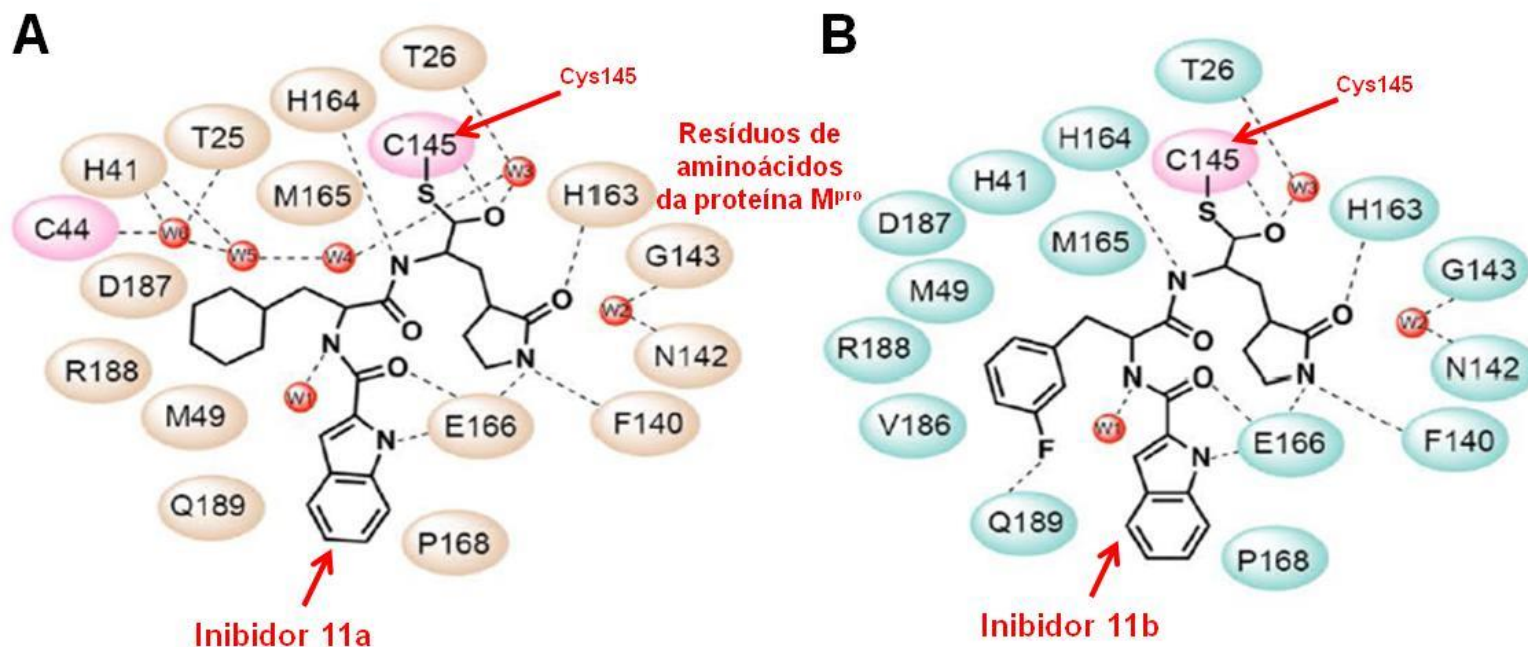


Nsp5 – Protease (3C-like main protease, M^{pro}, 3CL^{pro})

ORF1ab encodes replicase polyprotein 1 ab. After cleaved by two proteases (3CL^{pro} and PL^{pro}), replicase proteins showed multifunction involved in transcription and replication of viral RNAs. PL^{pro} cleaves three sites at the N-terminus and 3CL^{pro} cuts at the other 11 sites at the C-terminus, and forming 15 non-structural proteins. Among them, Nsp3 is proteolytic enzyme PL^{pro}; Nsp5 is 3CL^{pro}, Nsp12 is an RdRp, and Nsp13 is helicase. One crystal structure of 3CL^{pro} has been deposited in PDB (pdb code: 6LU7).

Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease

Cite as: W. Dai *et al.*, *Science* 10.1126/science.abb4489 (2020).



Ligação dos Inibidores 11a (A) e 11b (B) na Protease M^{pro} de SARS-Cov-2. Mostra que o grupo aldeído de 11a e 11b ligam na Cys145 de M^{pro}, apresentando boa atividade inibitória e contra infecção por SARS-CoV-2 *in vitro*.

(Adaptado de: DOI: 10.1126/science.abb4489)

COVID-19

HOW DOES IT AFFECT YOU?

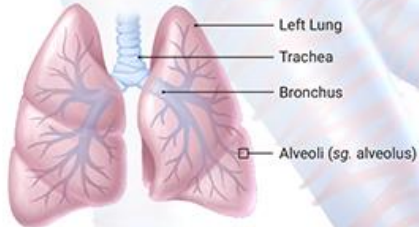
Designed by Avesta Rastan

www.azuravesta.com

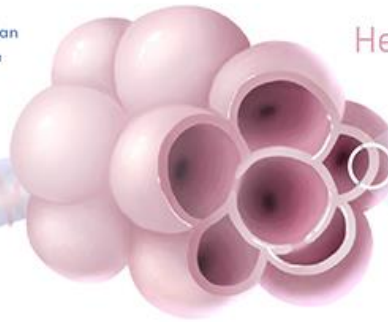
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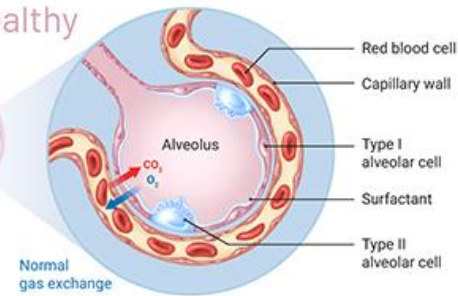
Coronavirus Disease 2019 (COVID-19) is a pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2, also called SARS-CoV-2. Despite the widespread awareness regarding COVID-19, many are still unaware about how it affects the human body.



SARS-CoV-2 starts its journey in the nose, mouth, or eyes and travels down to the alveoli in the lungs. Alveoli are tiny sacs of air where gas exchange occurs.



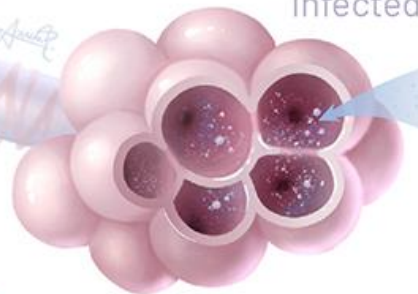
Healthy



Normal gas exchange

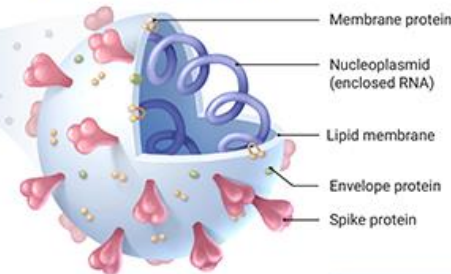
Gas Exchange

Each sac of air, or alveolus, is wrapped with capillaries where red blood cells release **carbon dioxide** (CO₂) and pick up **oxygen** (O₂). Two alveolar cells facilitate gas exchange; **Type I** cells are thin enough that the oxygen passes right through, and **Type II** cells secrete **surfactant** – a substance that lines the alveolus and prevents it from collapsing.



Infected

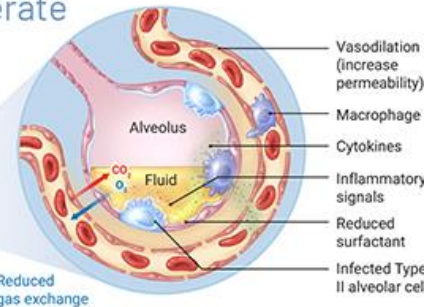
SARS-CoV-2 Structure



Viral Infection

The spike proteins covering the coronavirus bind ACE2 receptors primarily on type II alveolar cells, allowing the virus to inject its RNA. The RNA "hijacks" the cell, telling it to assemble many more copies of the virus and release them into the alveolus. The host cell is destroyed in this process and the new coronaviruses infect neighbouring cells.

Moderate



Reduced gas exchange

Stay Home

Symptoms may start to show (e.g. dry cough, fever, etc.)

Pneumonia develops

Shortness of breath

Hospitalization

Dangerous for high-risk individuals; secondary infections may occur

Intensive Care (ICU)

Patients may require ventilators and life-support

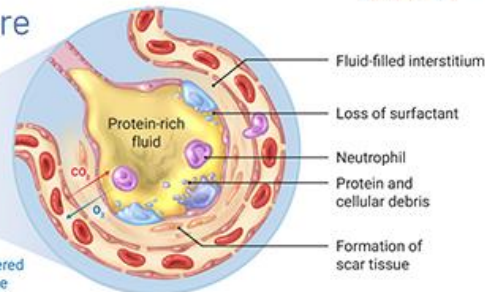
Complications unrelated to COVID-19 may occur

With proper care, patients may recover at any point during this process

Immune Response

- 1 After infection, Type II cells release **inflammatory signals** that recruit **macrophages** (immune cells).
- 2 Macrophages release **cytokines** that cause vasodilation, which allows more immune cells to come to the site of injury and exit the capillary.
- 3 Fluid accumulates inside the alveolus.
- 4 The fluid dilutes the surfactant which triggers the onset of alveolar collapse, decreasing gas exchange and increasing the work of breathing.
- 5 **Neutrophils** are recruited to the site of infection and release Reactive Oxygen Species (ROS) to destroy infected cells.
- 6 Type I and II cells are destroyed, leading to the collapse of the alveolus and causing **Acute Respiratory Distress Syndrome (ARDS)**.
- 7 If inflammation becomes severe, the protein-rich fluid can enter the bloodstream and travel elsewhere in the body, causing **Systemic Inflammatory Response Syndrome (SIRS)**.
- 8 SIRS may lead to **septic shock** and **multi-organ failure**, which can have fatal consequences.

Severe



Greatly hindered gas exchange

Impaired Gas Exchange

When the immune system attacks the area of infection it also kills healthy alveolar cells. This results in three things that hinder gas exchange:

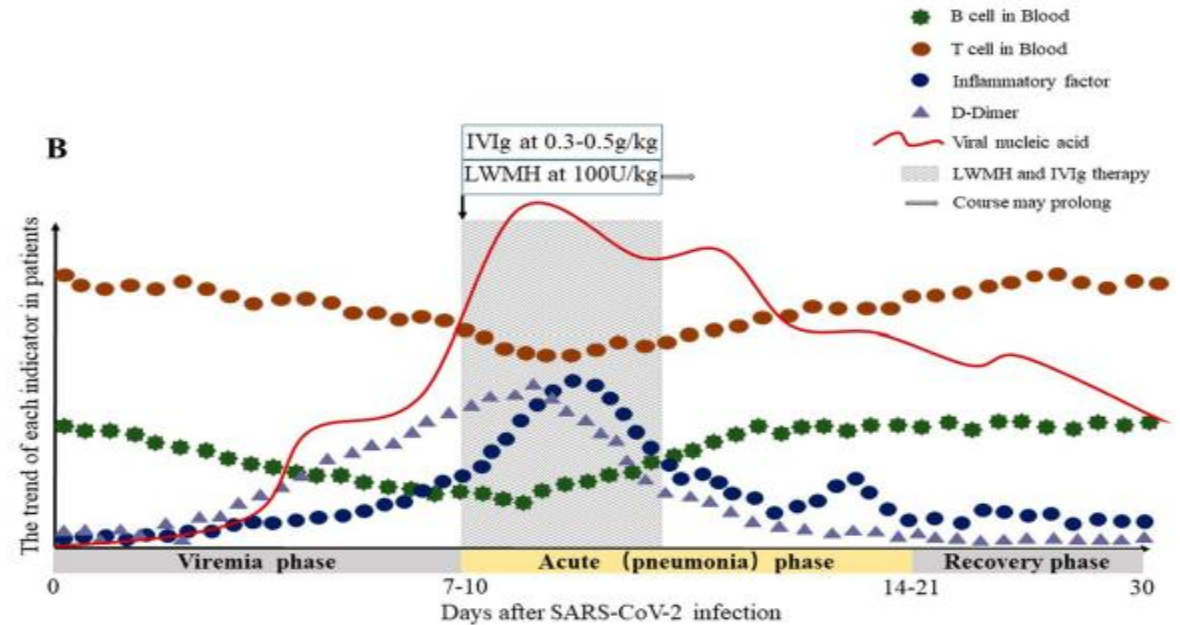
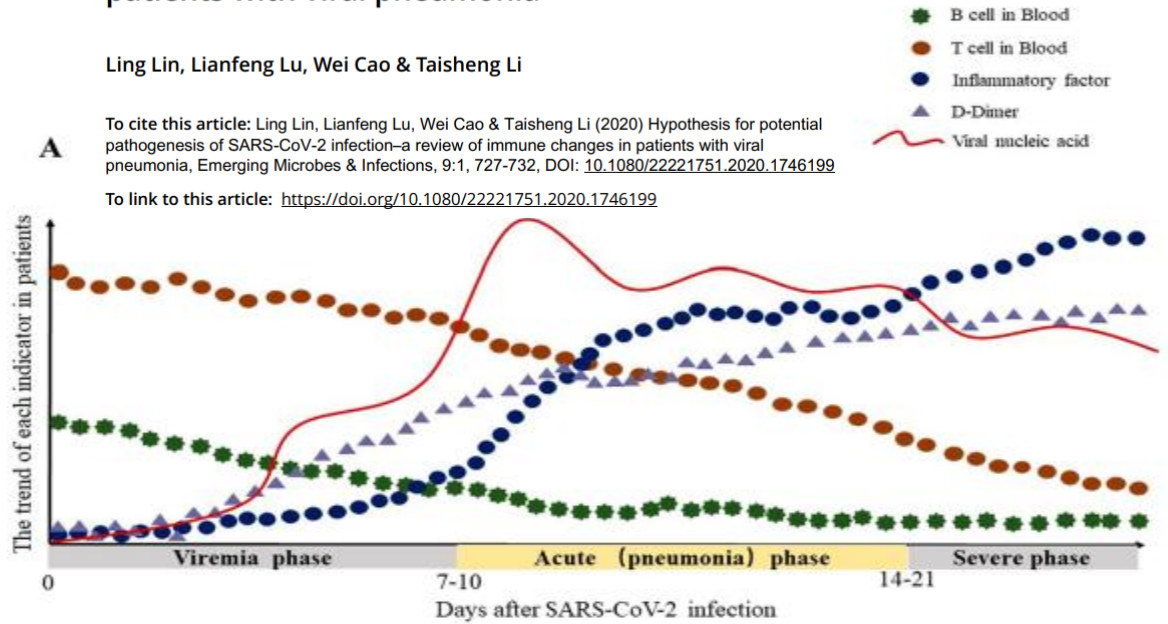
- 1) Alveolar collapse due to loss of surfactant from Type II cells
- 2) Less oxygen enters the bloodstream due to lack of Type I cells
- 3) More fluid enters the alveolus

Hypothesis for potential pathogenesis of SARS-CoV-2 infection—a review of immune changes in patients with viral pneumonia

Ling Lin, Lianfeng Lu, Wei Cao & Taisheng Li

To cite this article: Ling Lin, Lianfeng Lu, Wei Cao & Taisheng Li (2020) Hypothesis for potential pathogenesis of SARS-CoV-2 infection—a review of immune changes in patients with viral pneumonia, *Emerging Microbes & Infections*, 9:1, 727-732, DOI: [10.1080/22221751.2020.1746199](https://doi.org/10.1080/22221751.2020.1746199)

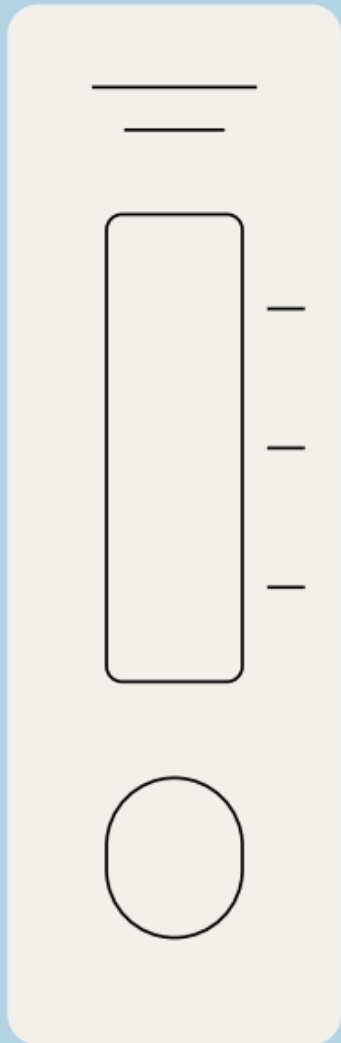
To link to this article: <https://doi.org/10.1080/22221751.2020.1746199>



Potential therapeutics for severe acute respiratory syndrome (SARS) and MERS.

Treatment	Stage of Development	
	SARS (Notes)	MERS (Notes)
Host protease inhibitors	Effective in mouse models [138]	In vitro inhibition [138]
Viral protease inhibitors	In vitro inhibition [139]	In vitro inhibition [140]
Monoclonal and polyclonal antibodies	Effective in mouse, ferrets, golden Syrian hamster [124,141,142] and non-human primate models [143,144]	Effective in mouse, rabbit, and non-human primate models [10,145]
Convalescent plasma	Off-label use in patients [146,147]	Effective in a mouse model; clinical trial approved [10]
Interferons	Off-label use in patients (often in combination with immunoglobulins or thymosins) [146,147]	Effective in non-human primate models; off-label use in patients (often in combination with a broad-spectrum antibiotic and oxygen) [10]
Ribavirin	Off-label use in patients (often in combination with corticosteroids) [146,147]	Effective in a non-human primate model; off-label use in patients (often in combination with a broad-spectrum antibiotic and oxygen) [10]
Lopinavir and ritonavir	Off-label use in patients (improved the outcome in combination with ribavirin) [146,147]	Effective in a non-human primate model; off-label use in patients [10,148]
Common Feature	None of these therapeutic agents are approved for commercial use in humans	

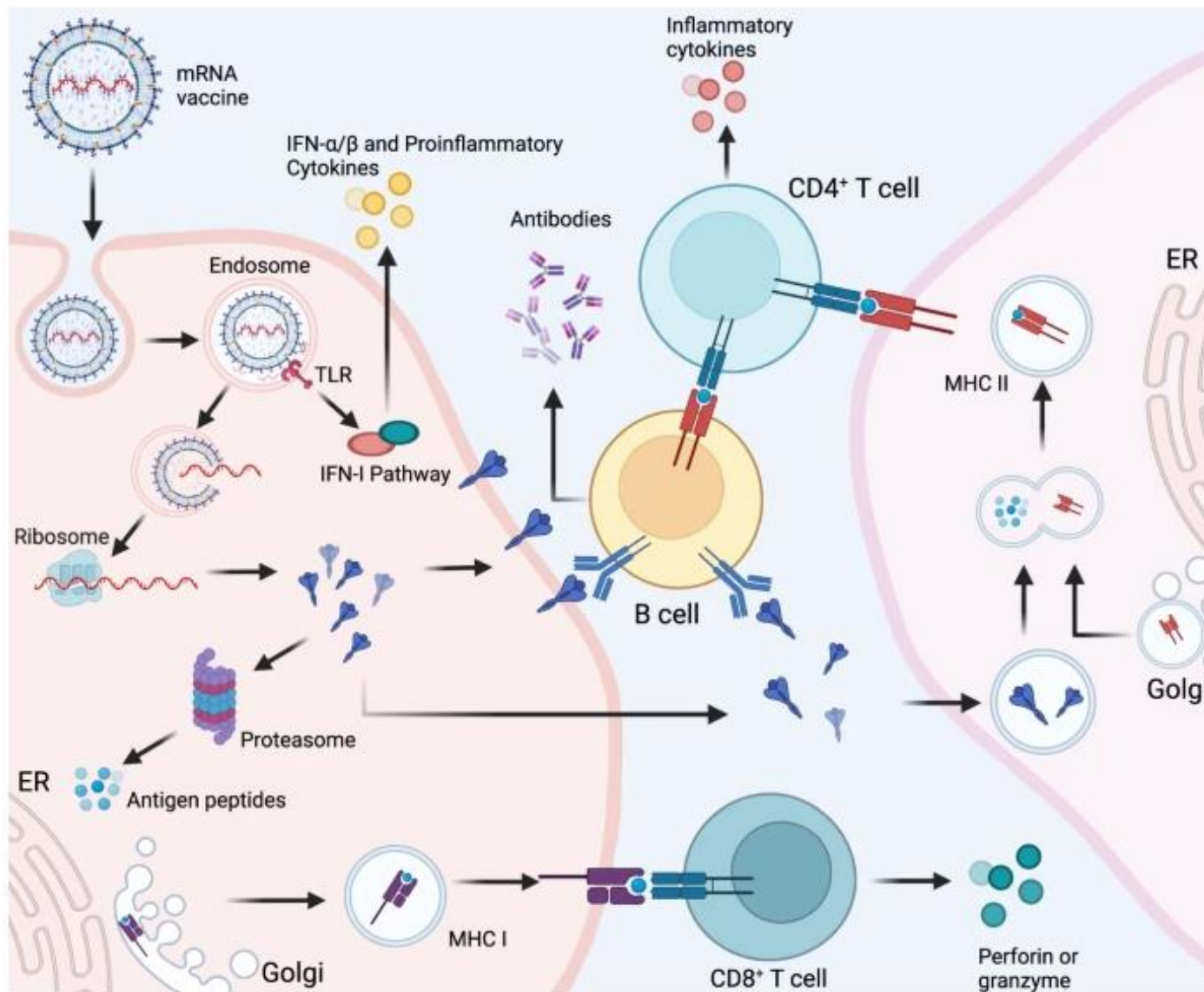
Vacina Pfizer



Componentes:

- 100 ug mRNA
- Lípidios [(4-hidroxitil)azanedil)bis(hexano-6,1-dil)bis(2-hexildecanoato)]
- 2 (polietilenogicol)-2000-N,N-ditetradecilacetamida
- 1,2-distearoil-sn-glicero-3-fosfocolina e colesterol)]
- Cloreto de potássio
- Fosfato de potássio monobásico
- Cloreto de sódio
- Fosfato de sódio dibásico di-hidratado e sacarose.

Fig. 1: Cellular and humoral immune responses induced by messenger RNA (mRNA) vaccine.



Antivirals

Entry Inhibitors
 fostemsavir
 combinectin
 (GSK3732394)

NRTIs/NtRTIs (nukes)
 EFdA (MK-8591)
 GS-9131

NNRTIs (non-nukes)
 doravirine
 elvitegravir
 rilpivirine LA

INIs (or INSTIs)
 cabotegravir
 cabotegravir LA

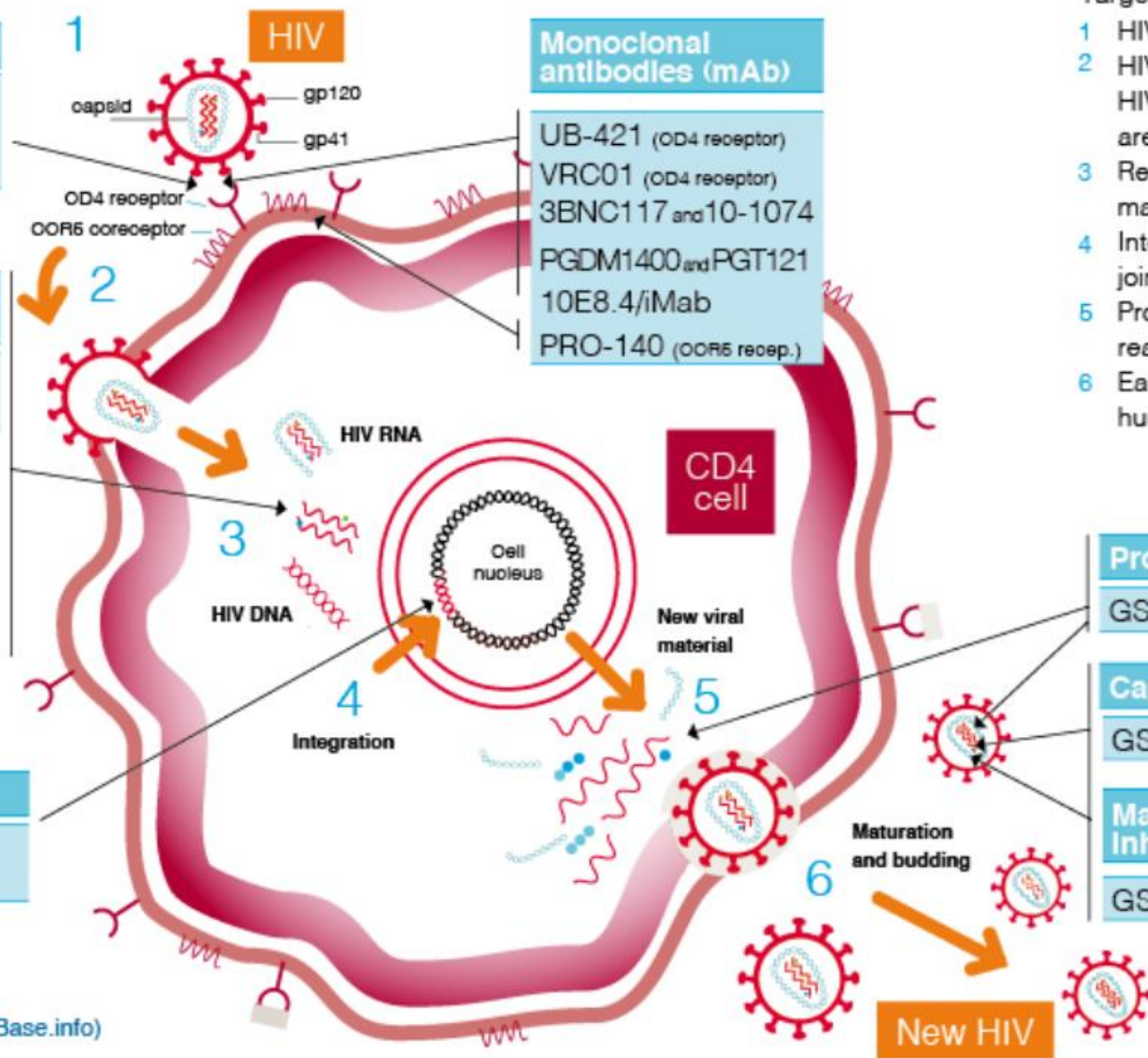
Monoclonal antibodies (mAb)
 UB-421 (CD4 receptor)
 VRC01 (CD4 receptor)
 3BNC117 and 10-1074
 PGDM1400 and PGT121
 10E8.4/iMab
 PRO-140 (CCR5 recep.)

- Targets in the HIV lifecycle
- 1 HIV attaches to a CD4 cell.
 - 2 HIV enters a CD4 cell and HIV proteins and enzymes are released into the cell.
 - 3 Reverse transcriptase (RT) makes double strand HIV.
 - 4 Integrase enables HIV to join the cell DNA.
 - 5 Protease cuts and reassembles new HIV.
 - 6 Each cell produces hundreds of new virions.

Protease Inhibitor
 GS-PS1

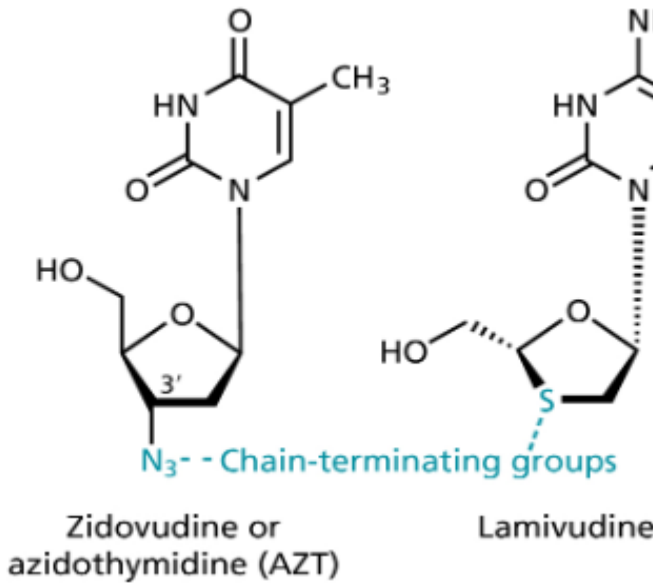
Capsid Inhibitor
 GS-CA1

Maturation Inhibitor
 GSK3640254

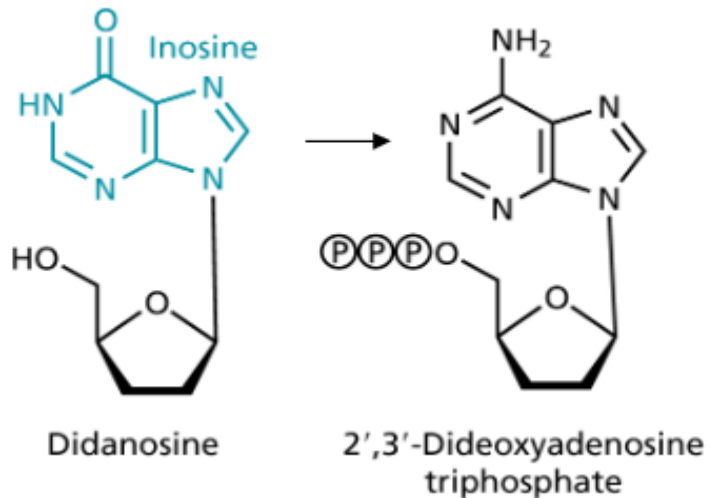


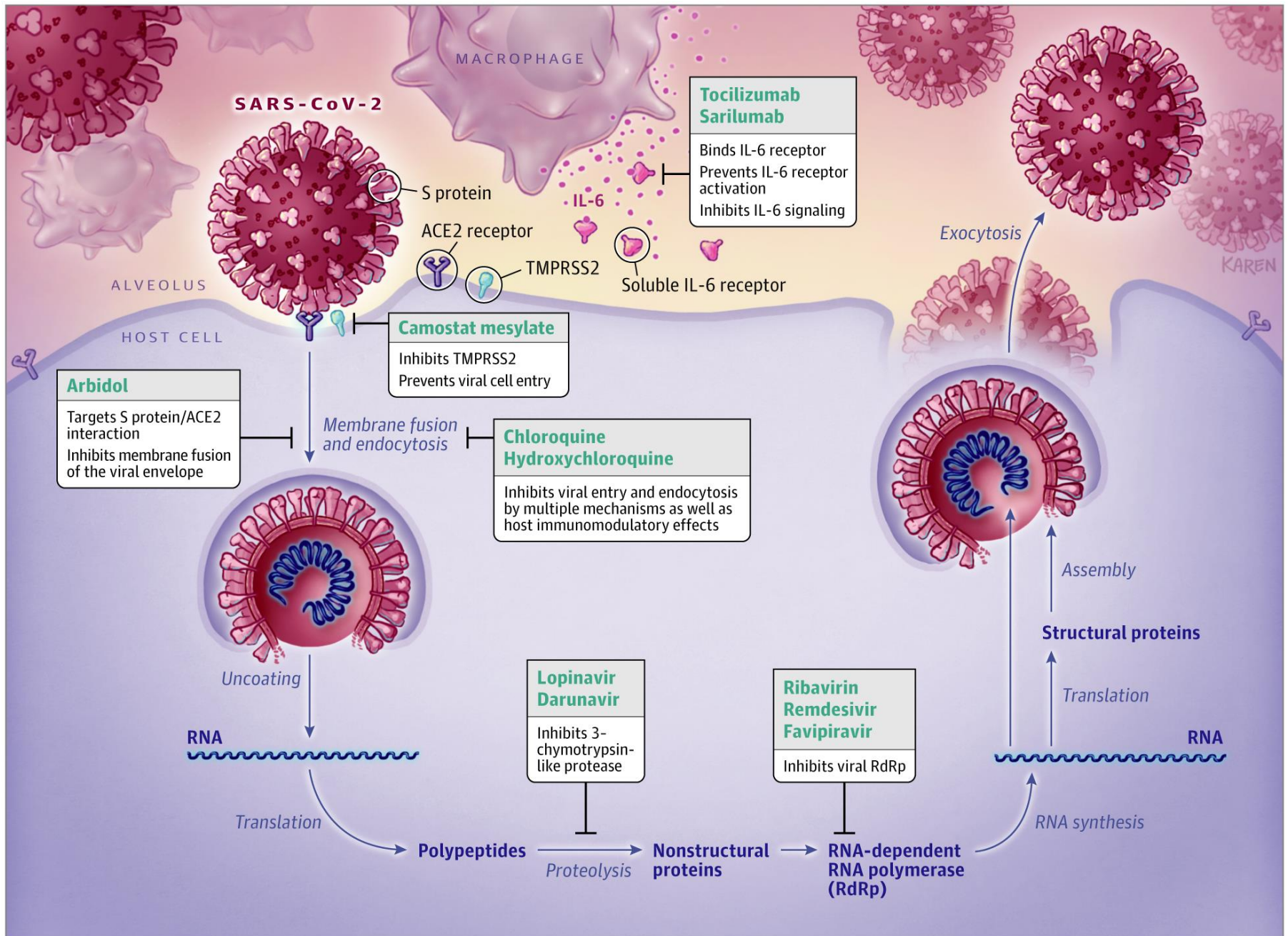
HIV i-Base (www.i-Base.info)

Nucleosídeos Inibidores de Transcriptase reversa (NRTIs)



→ Também utilizada para tratamento da hepatite B





Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α -ketoamide inhibitors

Linlin Zhang^{1,2}, Daizong Lin^{1,3}, Xinyuanyuan Sun^{1,2}, Ute Curth⁴, Christian Drosten⁵, Lucie Sauerhering^{6,7}, Stephan Becker^{6,7},...

+ See all authors and affiliations

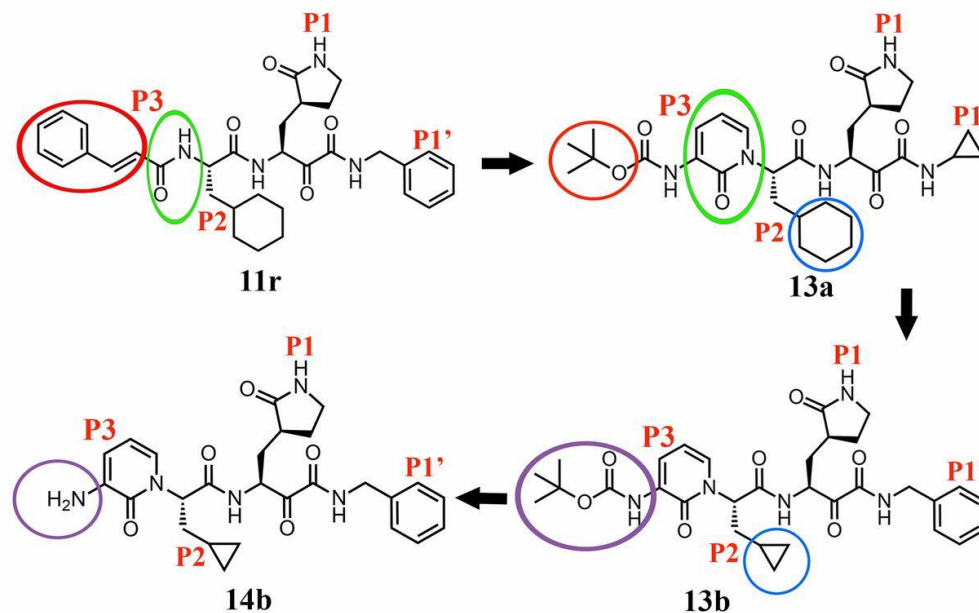
Science 20 Mar 2020:

eabb3405








DOI: 10.1126/science.abb3405

Abstract

An attractive drug target among coronaviruses is the main protease (M^{pro} , 3CL^{pro}), due to its essential role in processing the polyproteins that are translated from the viral RNA. We report the X-ray structures of the unliganded SARS-CoV-2 M^{pro} and its complex with an α -ketoamide inhibitor. This was derived from a previously designed inhibitor but with the P3-P2 amide bond incorporated into a pyridone ring to enhance the half-life of the compound in plasma. Based on the structure, we developed the lead compound into a potent inhibitor of the SARS-CoV-2 M^{pro} . The pharmacokinetic characterization of the optimized inhibitor reveals a pronounced lung tropism and suitability for administration by the inhalative route.

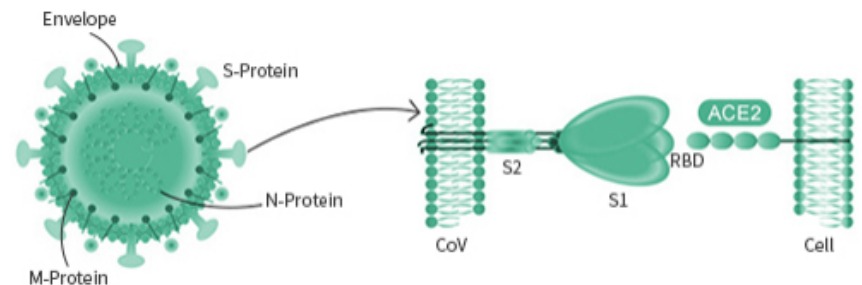


Human Coronavirus Antigens

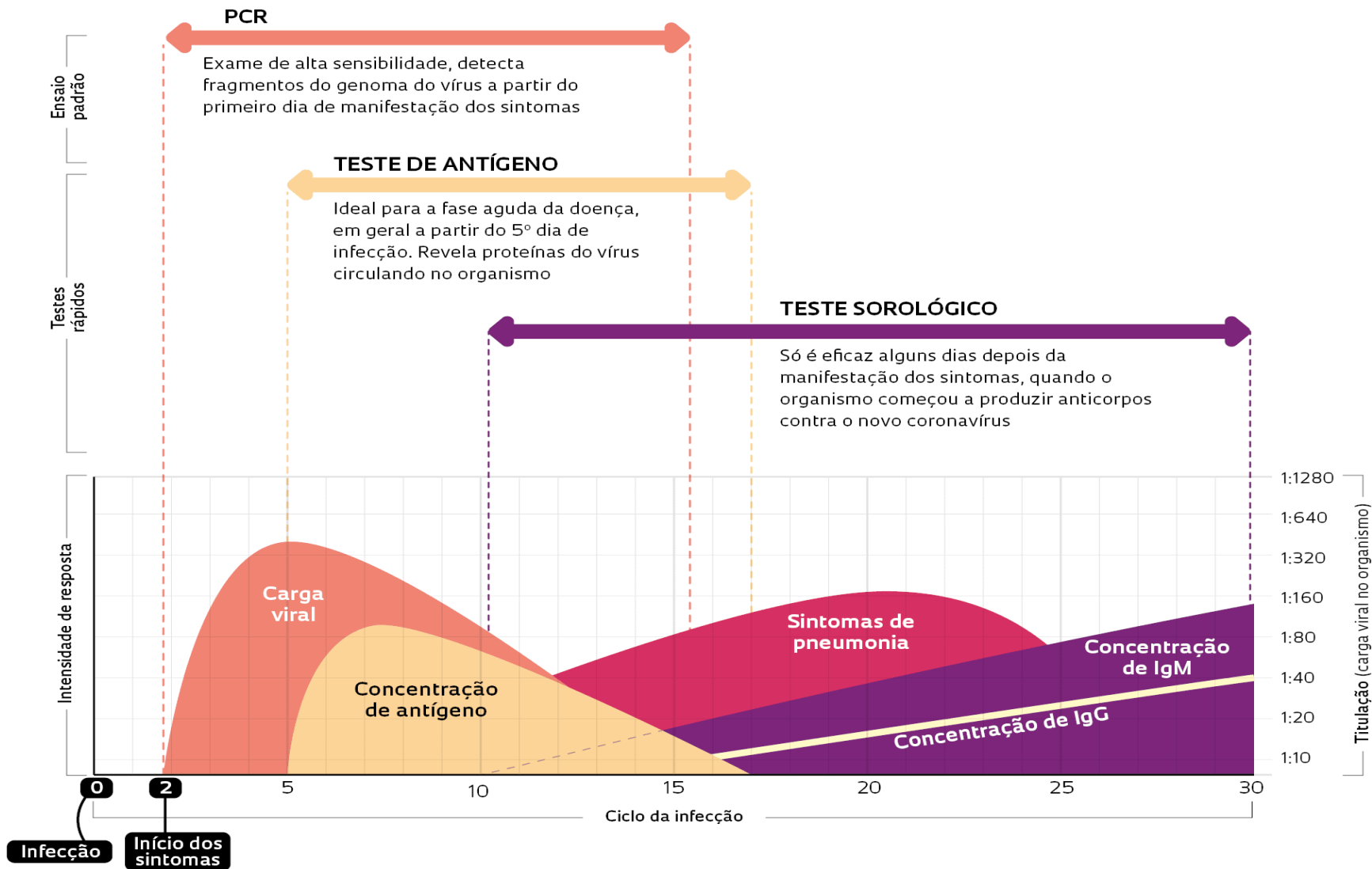
CoV Antigens	Description
 Spike	Receptor binding and membrane fusion Target for antiviral treatment and vaccines
 Nucleocapsid	Genome replication and cell signaling regulation A diagnostic marker
 HE	Receptor interaction
 PIpro	Viral polyprotein cleavage and host innate immune response blockage; Target for drugs development
 3CLPro	Polypeptides cleavage and IFN signaling inhibition Target for drugs development
 E	Assembly and release of the virus Vaccine candidates; Target for drugs development
 M	Membrane and virion structure

Host Receptor of Human Coronavirus

HCoV Types	Host receptors
HCoV-229E	APN (aminopeptidase N, CD13)
HCoV-NL63	ACE2 (angiotensin-converting enzyme 2)
HCoV-HKU1	O-ac Sia
HCoV-OC43	O-ac Sia
MERS-CoV	DPP4 (dipeptidyl peptidase 4)
SARS-CoV	ACE2 (angiotensin-converting enzyme 2)
SARS-CoV-2	ACE2 (angiotensin-converting enzyme 2)



Diagnóstico








FONTE ECO DIAGNÓSTICA, UTILIZANDO COMO REFERÊNCIA O ARTIGO CELLULAR IMMUNE RESPONSES TO SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS (SARS-COV) INFECTION IN SENESCENT BALB/C MICE: CD4+ T CELLS ARE IMPORTANT IN CONTROL OF SARS-COV INFECTION

Diagnóstico

ENSAIO RT-PCR

EXAME SOROLÓGICO

TESTE DE ANTÍGENO

 O que é	Sigla para transcrição reversa seguida de reação em cadeia de polimerase. Esse teste molecular é o exame padrão para detecção da doença no país. Revela a presença de fragmento do genoma do vírus na amostra coletada	Teste rápido que verifica a resposta imunológica do organismo ao vírus, detectando a presença dos anticorpos IgM e IgG	Exame rápido que demonstra a presença de proteínas próprias do vírus na amostra
 Amostra	Secreções do fundo do nariz (nasofaringe) e da garganta (orofaringe)	Sangue, soro ou plasma sanguíneo	Secreções do fundo do nariz (nasofaringe) e da garganta (orofaringe)
 Maior eficácia	A partir do primeiro dia de manifestação dos sintomas	A partir de alguns dias de manifestação dos sintomas, período em que o organismo já está produzindo anticorpos	Na fase aguda da doença, quando os sintomas começam a se manifestar
 Onde é feito	Em laboratório, com uso de equipamento específico e mão de obra capacitada	Em hospitais, postos de saúde e unidades volantes, com aparelho portátil fácil de manusear	Em hospitais, postos de saúde e unidades volantes, com aparelho portátil fácil de manusear
 Resultado	Cerca de 24 horas	Em até 30 minutos	Em até 30 minutos

FONTES PESQUISADORES E EMPRESAS CONSULTADOS PELA REPORTAGEM

Diagnóstico: RT-PCR

Protocolos já estabelecidos e reconhecidos (Protocolo de Berlim e Pasteur)

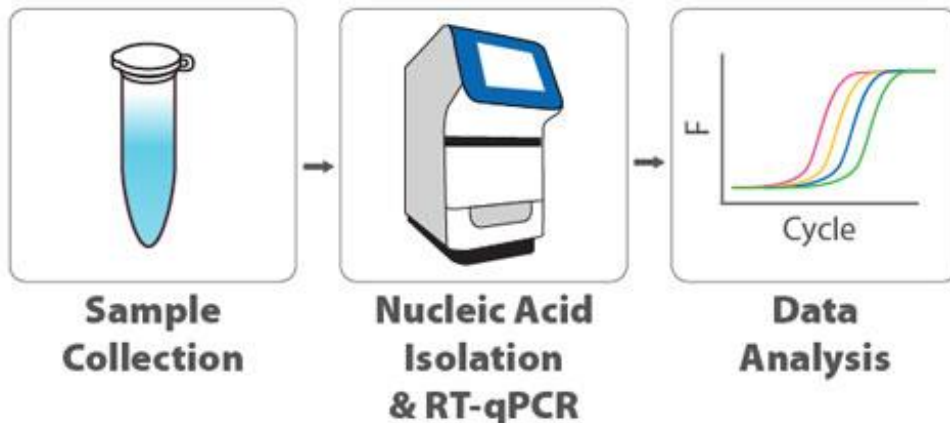
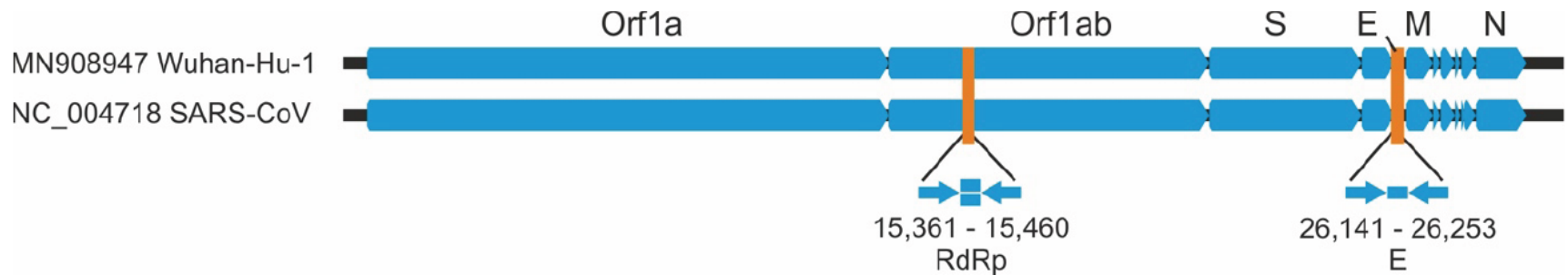
- **Extração do RNA** do material proveniente do Swab pelo método do Brazol

- RNA submetido a análise por PCR quantitativo (**qPCR** ou **PCR em Tempo Real**), usando um conjunto de três primers, em reações individuais, em duplicatas:

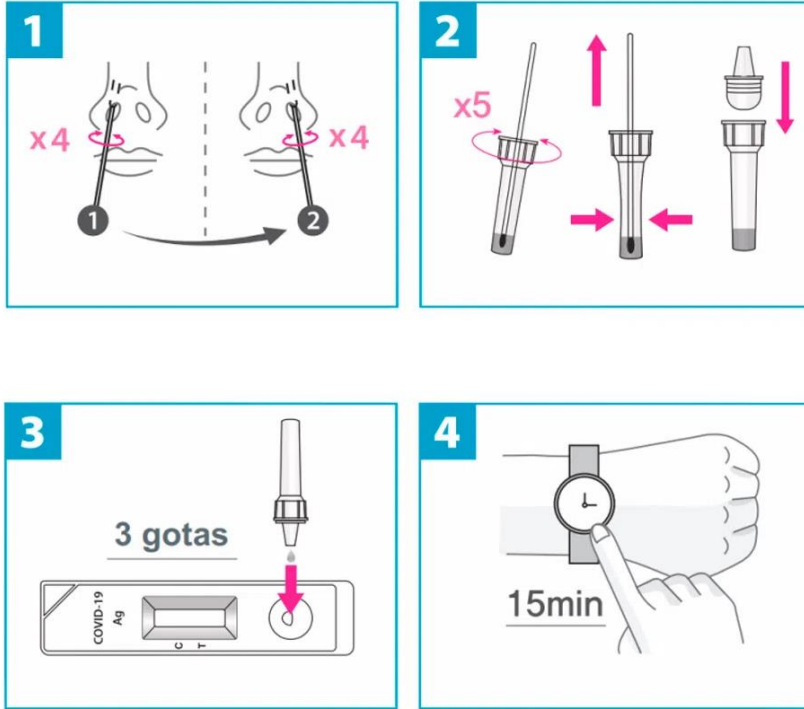
1) Primer para avaliação da presença de RNA humano (para a **RNaseP**), o qual indica a presença de células humanas nas amostras e o sucesso tanto da coleta quanto da **extração do RNA**. Deve ser positivo para todas as amostras.

2) Primer para o gene **E** do vírus. Deve ser **positivo** para amostras contendo RNA viral e para o controle positivo (RNA sintético do vírus).

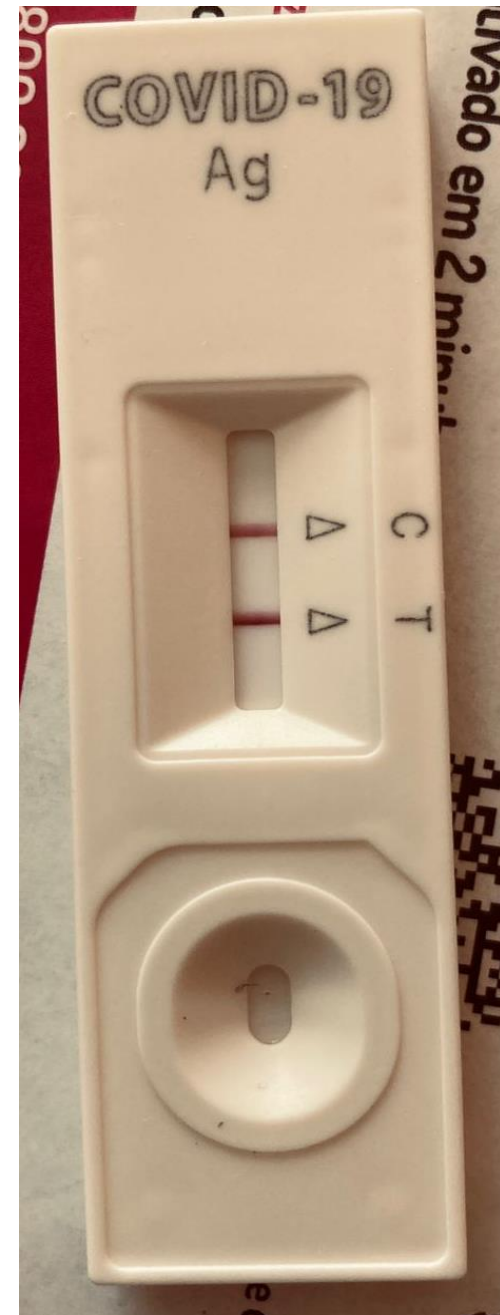
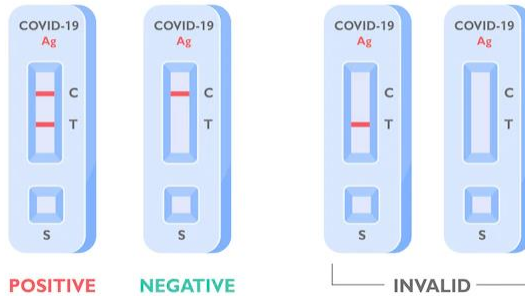
3) Primer para o gene **RdRp** do vírus. Deve ser positivo para amostras contendo RNA viral e para o controle positivo, correspondendo a um teste **confirmatório** que pode discriminar entre outros coronavírus.



Autoteste Antígeno

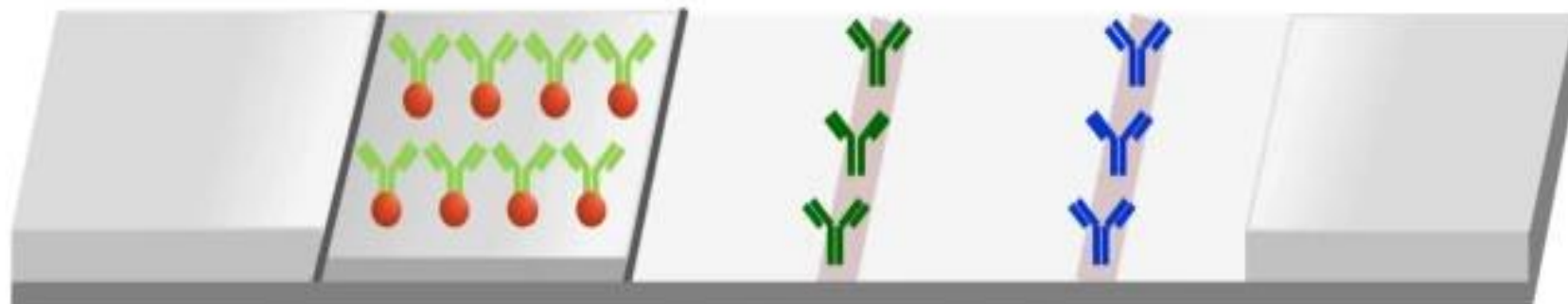


COVID 19 ANTIGEN TEST RESULT



Aplicação – Kit de Deteção

Antigen test



Sample pad

Conjugation pad

Test line

Control line

Absorbent pad



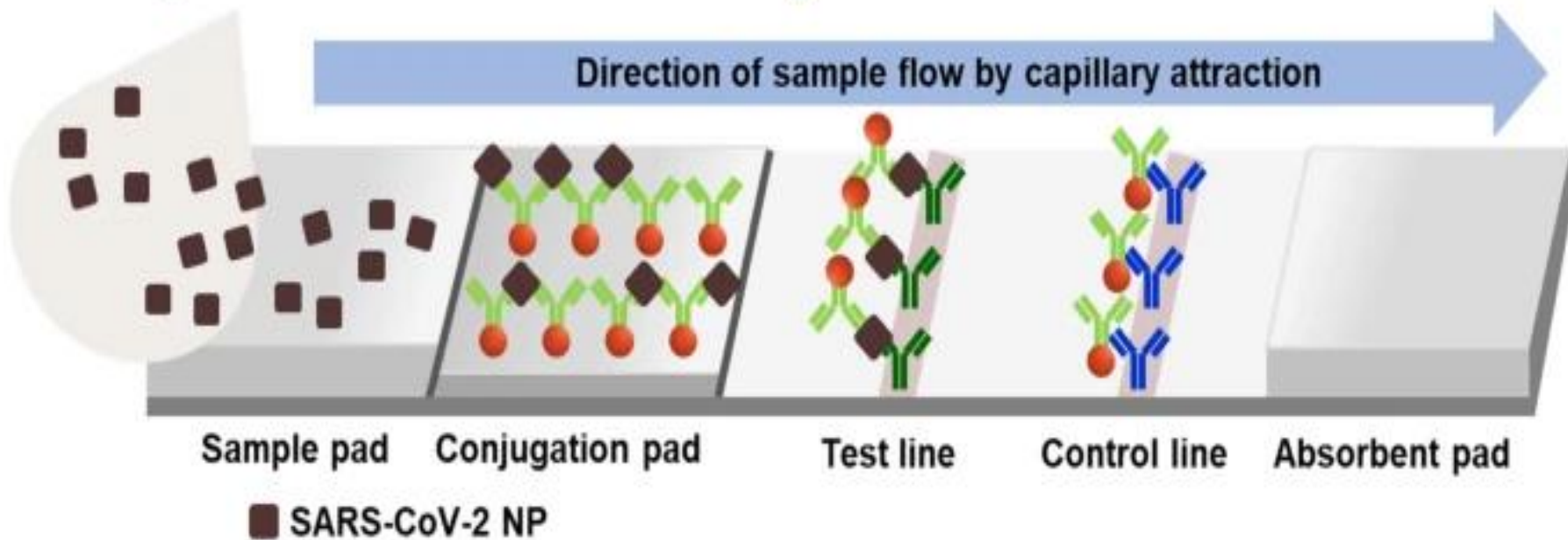
Nanoparticle conjugated anti-SARS-CoV-2 NP mAb



anti-SARS-CoV-2 NP mAb



anti-mouse IgG



Sample pad

Conjugation pad

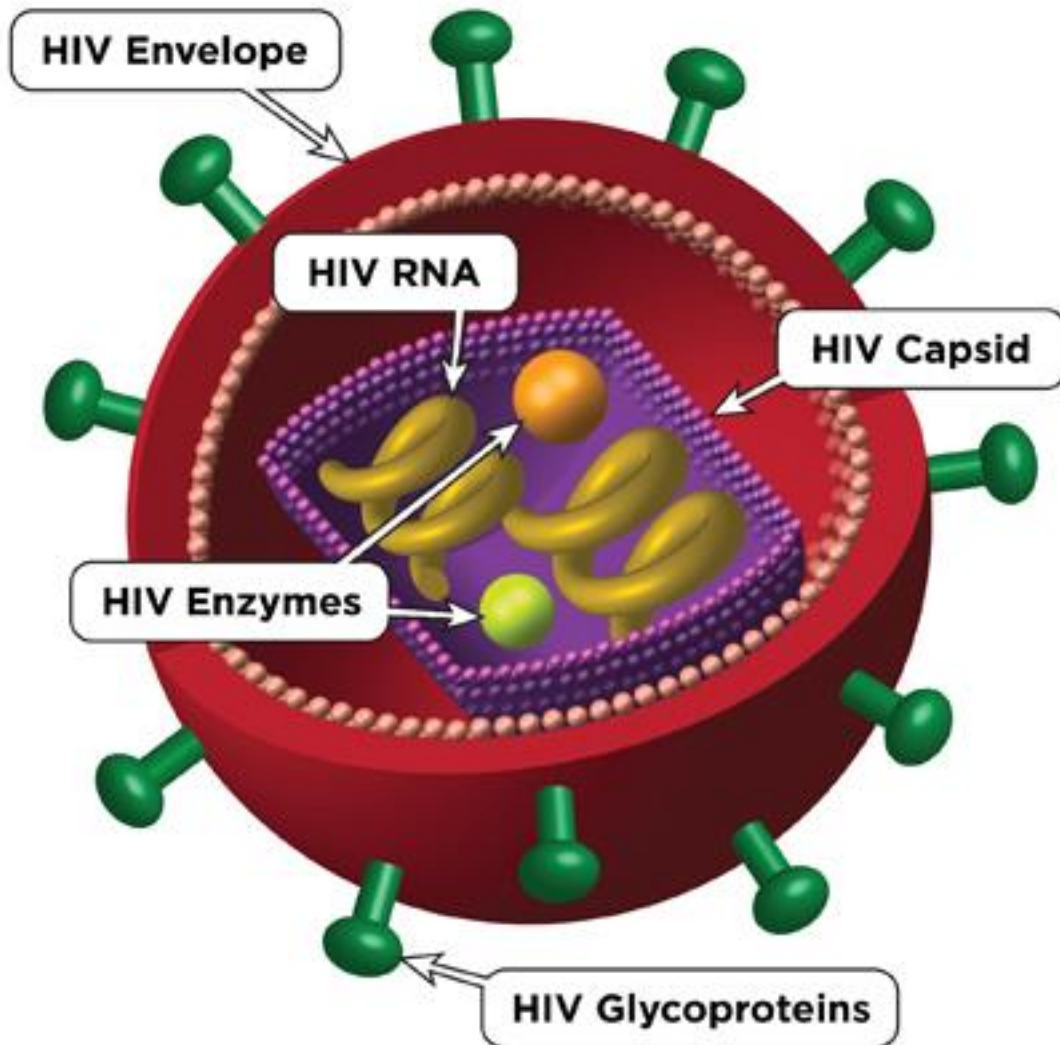
Test line

Control line

Absorbent pad

■ SARS-CoV-2 NP

HIV Genome



Key to Terms

HIV capsid: HIV's bullet-shaped core that contains HIV RNA

HIV envelope: Outer surface of HIV

HIV enzymes: Proteins that carry out steps in the HIV life cycle

HIV glycoproteins: Protein "spikes" embedded in the HIV envelope

HIV RNA: HIV's genetic material

Vírus e o Meio Ambiente

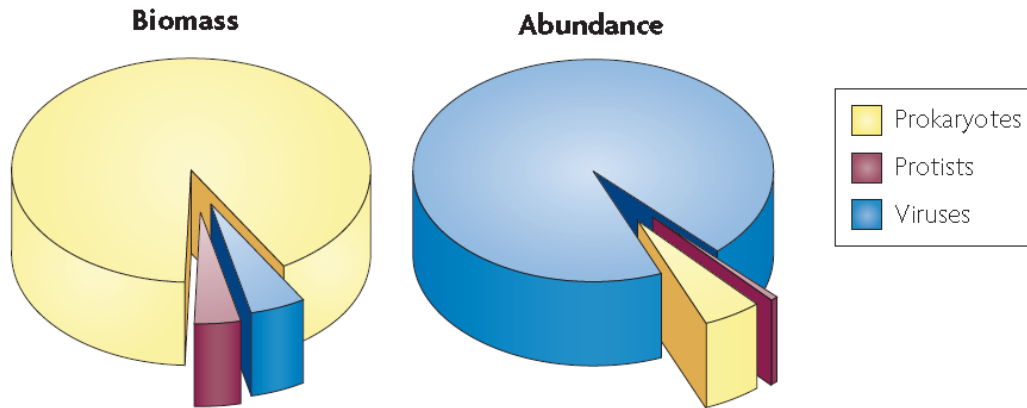


Figure 1 | **Relative biomass and abundances of prokaryotes, protists and viruses.** Viruses are by far the most abundant biological entities in the oceans, comprising approximately 94% of the nucleic-acid-containing particles. However, because of their

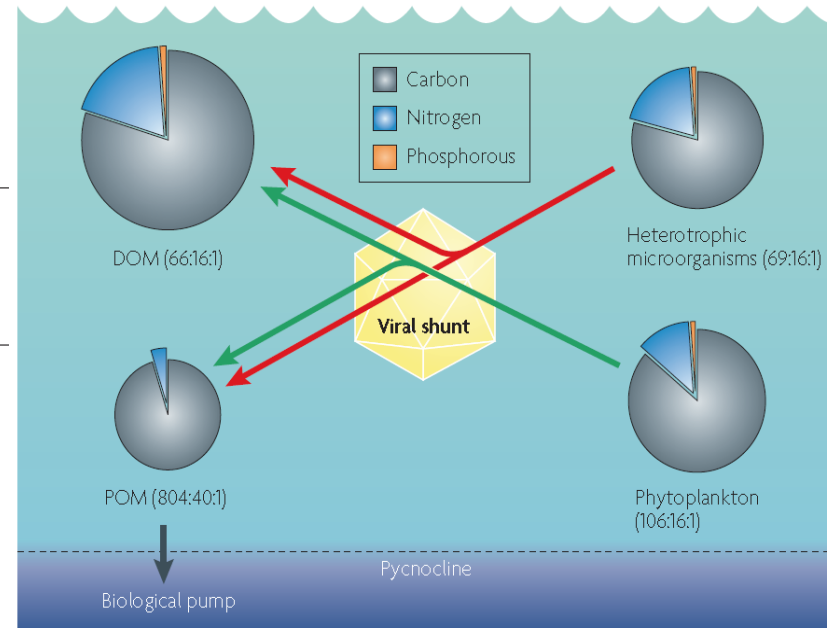
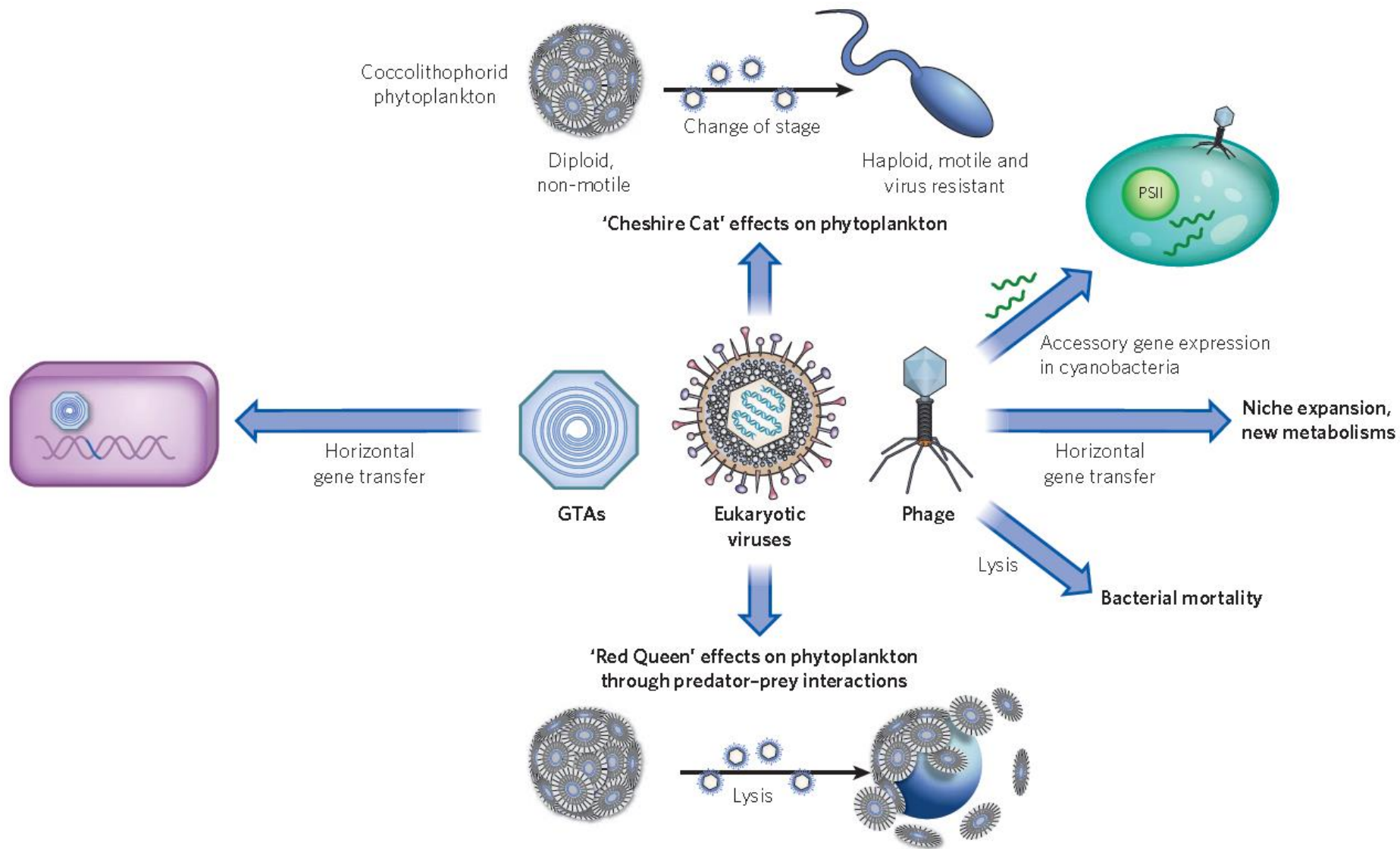
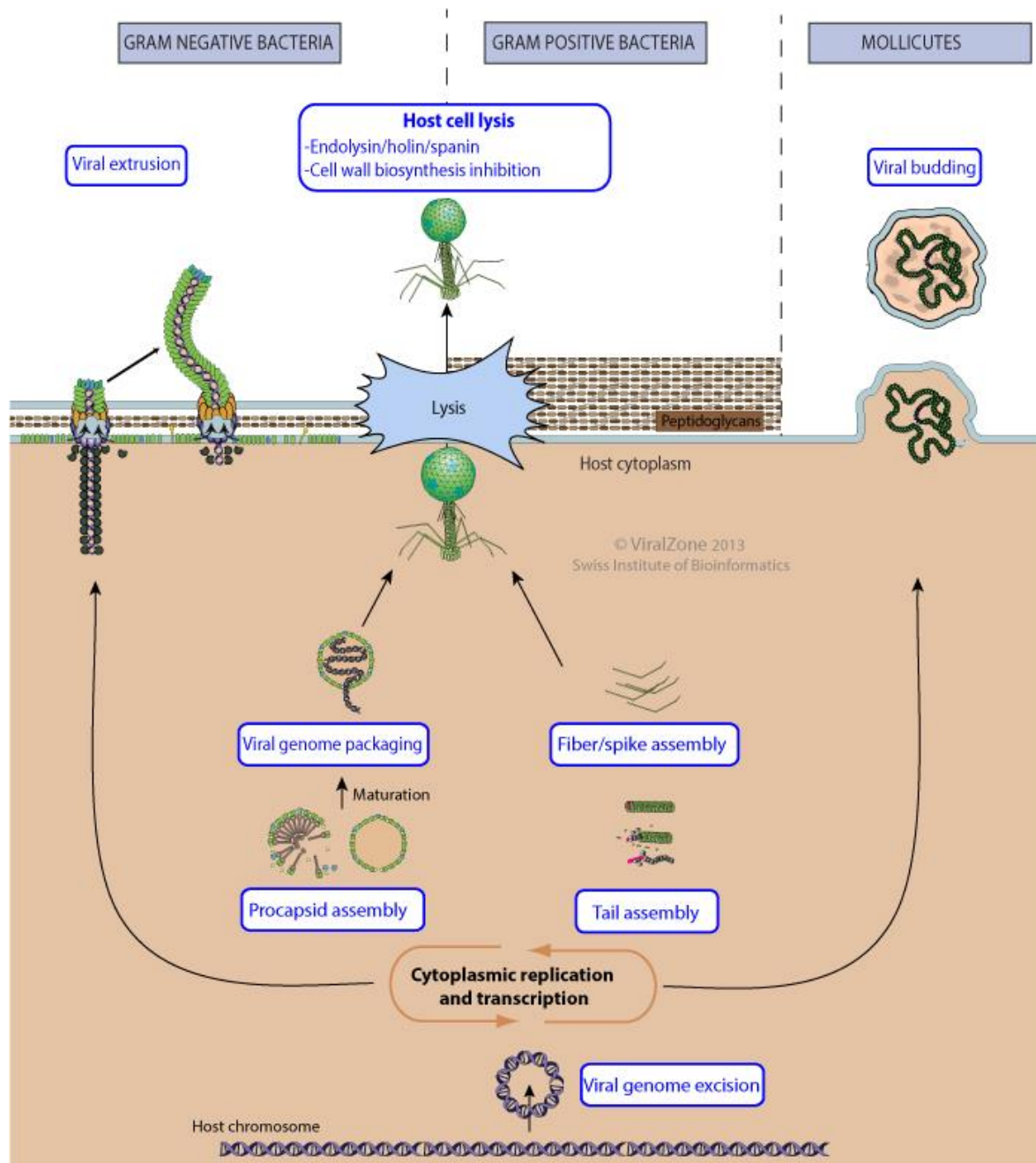
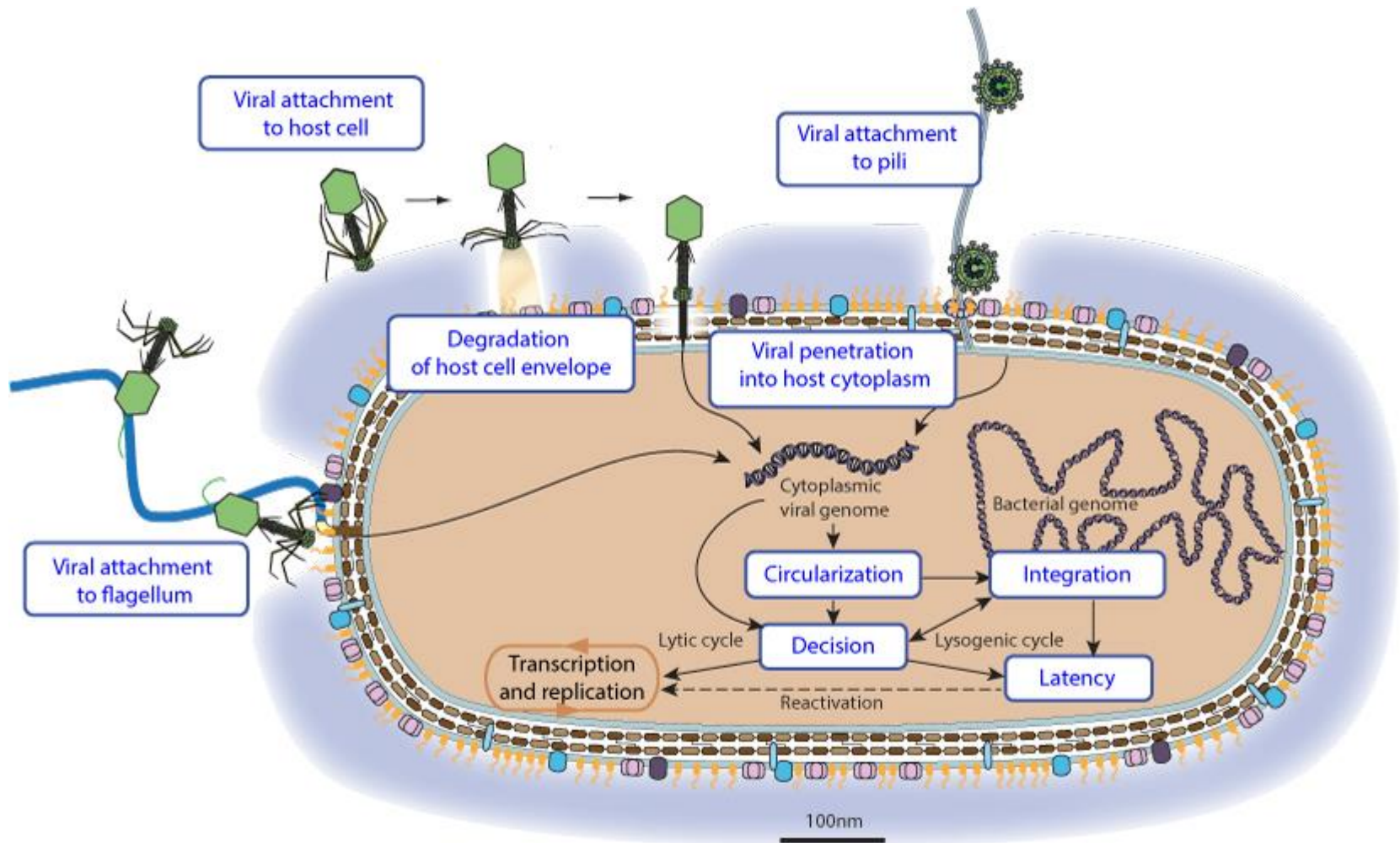


Figure 2 | **Shunt and pump.** The viral shunt moves material from heterotrophs and photoautotrophs (represented by red and green arrows, respectively) into particulate organic matter (POM) and dissolved organic matter (DOM). In this process there is a



Saída Bactérias







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2024