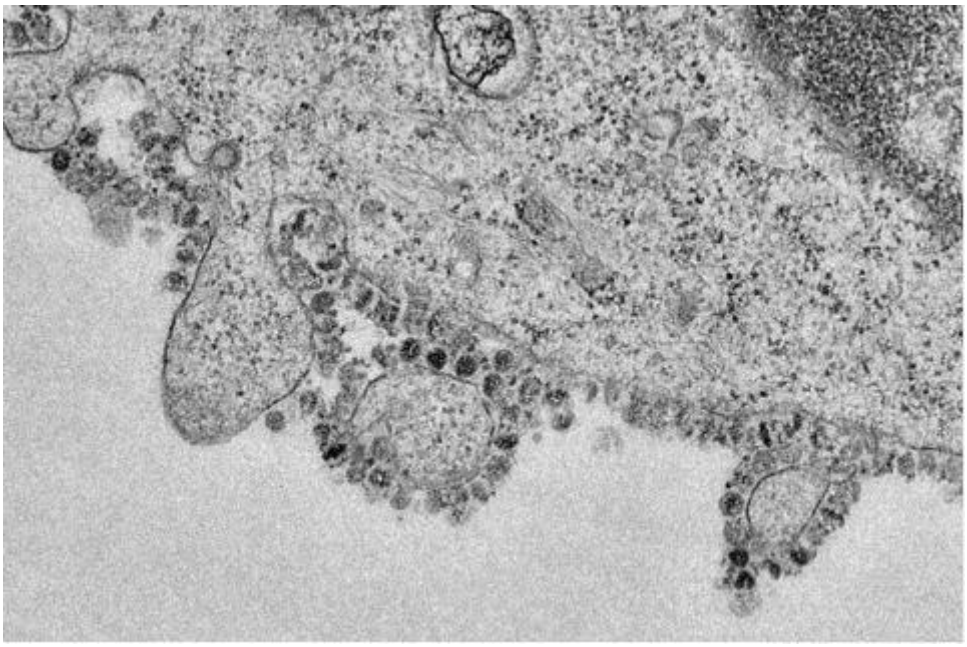


An electron microscope image showing particles of the new coronavirus being released from an infected cell. Image credit: The University of Hong Kong [**source of the image**]

Reflections on some very nasty little things (episode 3)

Author : Jérôme Vétillard, March 30, 2020

Episode 3: Physiopathology of COVID19. What are the cells infected by SARS-COV2 ? Biomarkers of serious illness ?

Disclaimer : *I am a Digital Architect at Microsoft Consulting Services who worked as an oncologist in a past life, pioneering stem cells research and biomolecular engineering (cell sorting, genetically modified cells, cell culture …) to transplant blood stem cells. The content and the opinions expressed herein are my own personal opinions and do neither officially (or unofficially) represent my employer's views in anyway nor are intended to convey the views of Microsoft Corporation.*

How does the SARS-COV2 virus infect the cell ?

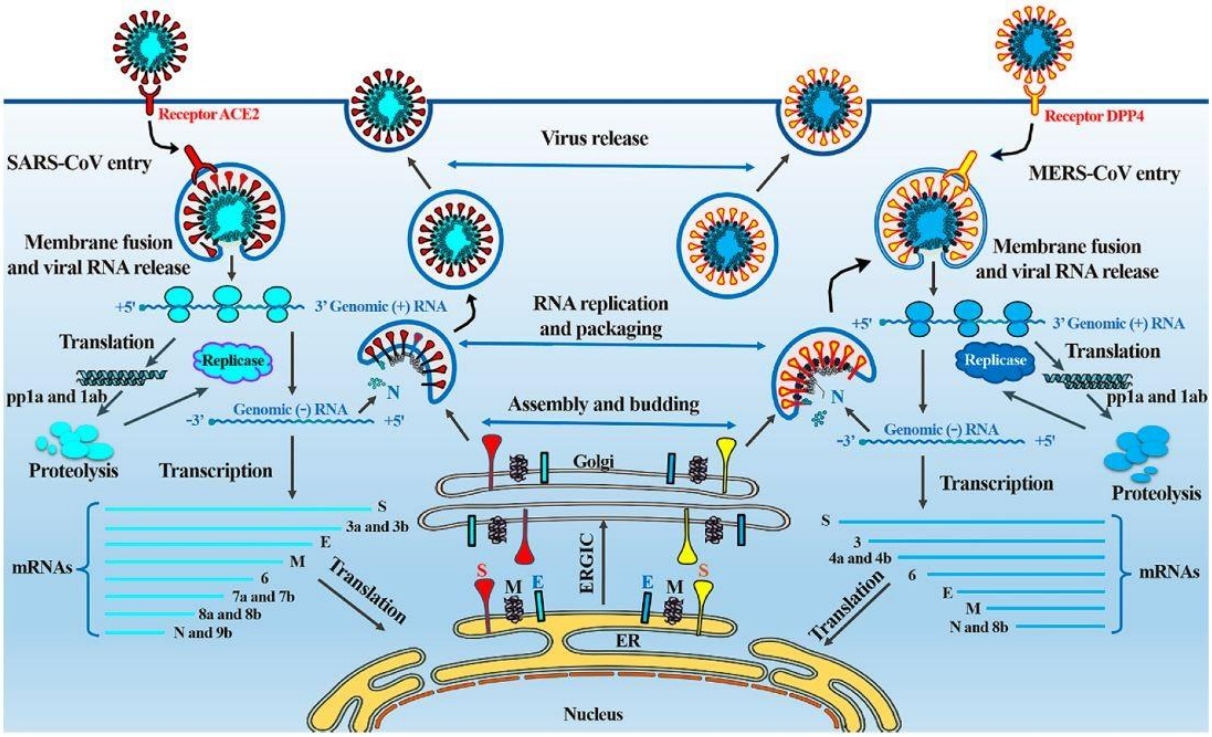
Remember in **Episode1**, we discuss about the SARS-COV2 as being a retrovirus, with a spike protein able to bind to some receptor on human cells.

It appears that the binding of SARS-COV2 is done on a specific receptor named ACE2. [**source**] This binding would also require another human cell surface protein acting as an "activator"/"catalyst" to infect the cell, *i.e.* fusion its enveloppe with the cell's membrane and free its genetic payload.

Despite what we have written in **Episode1** about coronavirus mutability, the Spike-protein of SARS-COV2, is very alike the one of SARS-COV(1) as they both target the ACE-2 receptor on human cells [source]. Differences between SARS-COV(1) Spike protein of 2003, and SARS-COV2 Spike protein of 2019 seems to be limited.

SARS-COV(1) replication cycle

Here is a detailed and didactic diagram about SARS-COV(1) and MERS-COV replication lifecycle. As written above, the SARS-COV2 replication lifecycle and entry point is very alike SARS-COV(1) replication lifecycle (with slight differences about receptors used that we shall explain below).



"[...] *Legend : S, spike; E, envelope; M, membrane; N, nucleocapsid*. [...]".

[**Source**] "From SARS to MERS, Thrusting Coronaviruses into the Spotlight",
[***Viruses* 2019**, *11*(1), 59].

Let's focus on SARS-COV entry. Once bound onto ACE2 receptor, the complex "SARS-COV+ACE2 receptor" in internalized through endocytosis. The S protein changes its 3D structure to facilitate envelope's fusion and delivery of genetic payload into the cytoplasm of the infected cell. Complex cascade of virus-enzymes activation, and interaction with the infected cell biomolecular factory enable the transcription of the virus genetic payload into something the infected cell can "read"/understand: mRNAS, *i.e.* messenger-RNA which you can envision like "plans" for virus's components. The whole collection of mRNAS is the "virus' blueprint / master plan". Then, it is quite

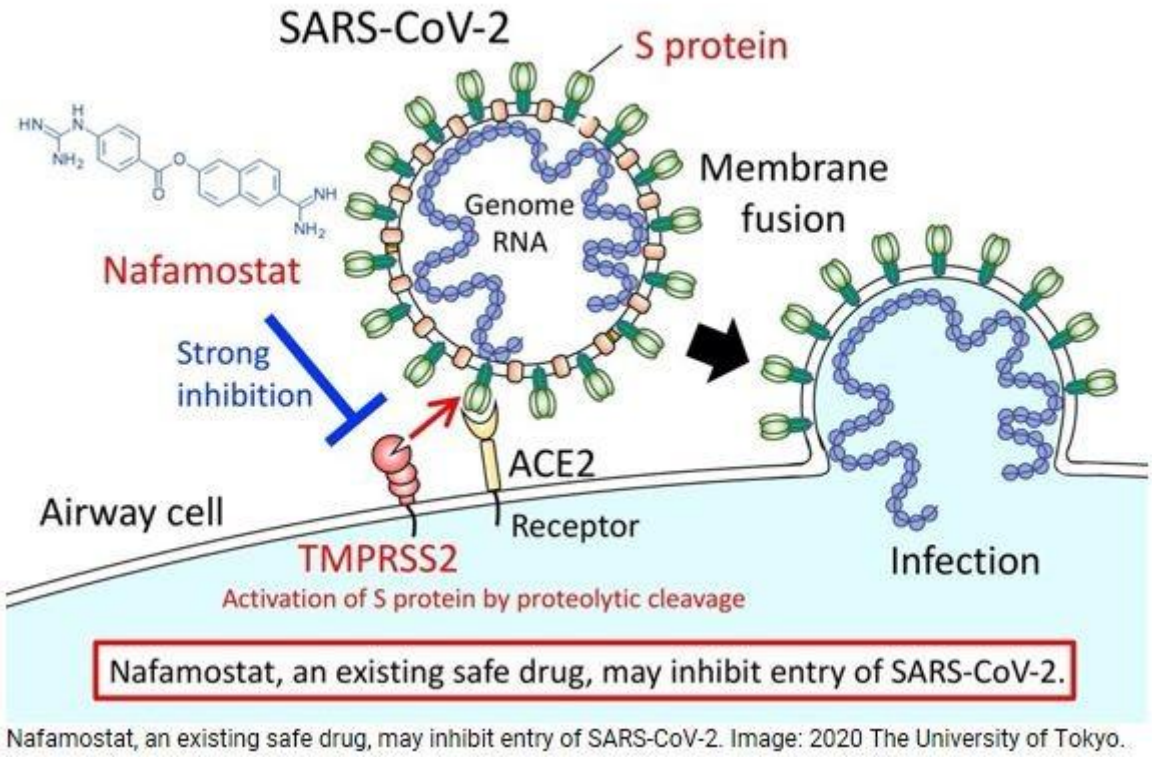
straight forward, the cell has readable plans, it produces virus' components as ordered. The beauty of this biomolecular puzzle is the "auto assembly" of new viruses from its newly produced components due to specific molecular high affinity between specific regions of newly produced viral proteins, genetic information, among which the famous newly produced NP nucleoprotein (see **Episode1**) which starts to package the newly produced RNA into a ribonucleic capside. As the virus continues to be assembled, it goes toward the cell's membrane where it is excreted through exocytosis. The cycle is done, one virus in, one out… Issue is the cell will continue to produce new virus particles. **Interestingly enough… the virus does not "kill" the infected cell (it needs it), it diverts its biomolecular factory to replicate. However it somehow boost its anabolism to produce new virus particles…** Which could eventually trigger apoptosis (programmed cellular death) and tissue necrosis.

SARS-COV2 slight differences with SARS-COV(1)

S proteins of SARS-CoV-2 share about 76% and 97% of amino acid identities with SARS-CoV and RaTG13 (a SARS-like (SL) Coronavirus discovered in a cave of Yunnan, China, in 2013). [source : **Nature**]. Meaning, while SARS-COV2 is close to SARS-COV(1), it has some slight differences in its infection pattern that could explain why it is more infectious than SARS-COV(1).

"[…] *Genomic analyses of the new coronavirus have revealed that its spike protein differs from those of close relatives, and suggest that the protein has a site on it which is activated by a host-cell enzyme called furin*.

This is significant because furin is found in lots of human tissues, including the lungs, liver and small intestines, which means that the virus has the potential to attack multiple organs. […]" [source: **Nature**].



Nafamostat, an existing safe drug, may inhibit entry of SARS-CoV-2. Image: 2020 The University of Tokyo.

We can see here that the Spike protein needs to be activated by TMPRSS2 furin before it binds to ACE2 receptor. We can see also that "Nafamostat" an existing drug for pancreatis, can block this activation, and could be a drug candidate. No, Hydroxychloroquine is not the only one drug that could save your lungs. [**source**]

TMPRSS2 is the "famous" furin that would "activate" Spike-protein, by slightly modifying its 3D structure so it can bind tightly to ACE2 receptor. It has been initially identified in MERS-COV infection pattern (Yamamoto *Et Al. 2016* - [**Source**]).

"[...] *MERS-CoV is an enveloped virus, and its envelope protein (S protein) mediates membrane fusion at the plasma membrane or endosomal membrane. Multiple proteolysis by host proteases, such as furin, transmembrane protease serine 2 (TMPRSS2), and cathepsins, causes the S protein to become fusion competent. TMPRSS2, which is localized to the plasma membrane, is a serine protease responsible for the proteolysis of S in the post-receptor-binding stage*. [...]". [**Source**]

We can see that the infection pattern of this sub-family of Coronavirus is quite simular and stable in different "variants". It is explained by darwinism: it is stable, because it is needed and efficient.

Not all scientists are aligned with the assumption that the furin-activation of the SARS-COV2 spike protein is the root cause for it being very infectious. They underlined that the deadliest flu (1918) did not have this furin-based activation mechanism. But, it appears that the SARS-COV2's spike protein has at least a 10-time stronger affinity to ACE2 receptor when compared to SARS-COV(1)'s S-protein.

Deep understanding of these molecular mechanisms are very important to find antivirus drug patterns that could prevent the SARS-COV2 from infecting human cells.

What kind of cells SARS-COV2 could infect ?

It could infect cells that exhibit ACE2 Receptor, as we've written above that the TMPRSS2 furin is found in lots of human tissues: it is an ubiquitous protein (lungs, liver and small intestines at least).

Let us investigate on which cells (ACE2 is a membrane-surface protein) ACE2 is expressed/present. ACE2 mRNA (remember messenger-RNA) is known to be present in virtually all organs, but its protein expression is largely unknown.

ACE2 acronym means angiotensin-converting enzyme 2. Its "normal" job is to catalyze the cleavage of "Angiotensin 2" a vasoconstrictor peptide into "Angiotensin1" a vasodilator. Hence ACE2 does lower the blood pressure.

High blood pressure comorbidity explained

People with high blood pressure might use a drug family named **Angiotensin-converting-enzyme inhibitors** (**ACE inhibitors**). These drugs inhibit ACE(1) enzyme, which does the opposite job to ACE2. ACE1 transforms Angiotensin1 into Angiotensin2 which is a powerful vasoconstrictor peptide. As SARS-COV2 will "consume" ACE2 receptor through endocytosis, it will not be available for its "normal job", *i.e.* reduce blood pressure. Furthermore, it seems that patients using ACE1 inhibitors, have an overexpression of ACE2 receptors, *i.e.* more entry gates for SARS-COV2, which could explain why high blood pressure might be a comorbidity in favor of serious COVID19 [**Source**].

ACE2 & TMPRSS2 are ubiquitous membrane proteins

So, now we know the "normal function" of ACE2, to produce a vasodilator peptide to lower blood pressure, it is quite normal to find ACE2 receptor in every endothelial vascular cells (in the inner membrane of blood vessels). Major organs are "exchange surface" (lung air/blood, liver blood/blood, kidney blood/urine, intestine intestine-track/blood), or require high oxygenation like the brain... hence they are hyper-vascularized, and the endothelial vascular cells of the millions of capillaries are expressing ACE2 receptor which is also ubiquitous. "[...] *ACE2 was present in arterial and venous endothelial cells and arterial smooth muscle cells in all organs studied. In conclusion, ACE2 is abundantly present in humans in the epithelia of the lung and small intestine* [...]" [**Source**].

SARS-COV2 uses ACE2+TMPRSS2 to infect human cells. These membrane proteins are ubiquitous, and specifically are present in the lung and small intestine epithelia at least (liver and brain are other possibilities). This explains that COVID19 can have

clinical presentations like, viral pneumonia with possibly Acure Distress Respiratory Syndrome, diarrhea, anosmia, ageusia...

Since identifying the possible route of infection has major implications for understanding infection patterns of SARS-COV2 and identify potential therapeutical targets.

Clinical findings of COVID19

Phase 0: Incubation period

The virus might pass through the mucous membranes, especially nasal and larynx mucosa, then enters the lungs through the respiratory tract. The median incubation period was estimated to be 5.1 days (95% CI, 4.5 to 5.8 days), and 97.5% of those who develop symptoms will do so within 11.5 days (CI, 8.2 to 15.6 days) of infection. These estimates imply that, under conservative assumptions, 101 out of every 10 000 cases (99th percentile, 482) will develop symptoms after 14 days of active monitoring or quarantine [**source**]. During this phase, the virus will infect lung cells as a primary target, and will replicate until the first symptoms exhibition. During this phase, patients are asymptomatic. By analogy with the influenza virus, asymptomatic patients are supposed to be contaminant 24-hours prior to first symptoms' exhibition, but this assumption do not rely on statistically validated clinical data.

Phase 1: virus replication and flu-like clinical presentation

It is important to recall here that some patients are asymptomatic during the whole COVID19 episode. A study conducted on the "confined-by-design" population of the Diamond Princess Cruise Ship, says that there are 18% of patients that were asymptomatic. [**source**]. This is consistent with the figure estimated for SARS-COV(1) outbreak in 2003: 13% [**source**]. Interestingly enough, among Health Care Professionals in Singapore fighting the SARS outbreak in 2003, asymptomatic SARS was associated with lower SARS antibody titers and higher use of masks when compared to pneumonic SARS (non-asymptomatic SARS). This reflects the importance of reducing the exposure to virus particles to avoid massive infection and replication from the very start. After all, the virus load at the time of infection, and the virus load during the infection (viremia) might have an impact on the severity of the COVID19. In the absence of masks (Episode 4?), physical distance with possibly infecting people is key, along with the adoption of other barrier gesture and reinforced disinfection of your close environment.

Then, for those who will exhibit a "noisy" clinical presentation:

From a cohort of 62 patients admitted to hospital with laboratory confirmed SARS-Cov-2 infection. Data were collected from 10 January 2020 to 26 January 2020. [**Source**].

Among 56 patients who could provide the exact date of close contact with someone with confirmed or suspected SARS-Cov-2 infection, the median incubation period from

exposure to symptoms was 4 days (interquartile range 3-5 days). The median time from onset of symptoms to first hospital admission was 2.0 (1.0-4.3) days.

The most common symptoms at illness onset were fever (48, 77%), cough (50, 81%), expectoration (35, 56%), headache (21, 34%), myalgia or fatigue (32, 52%), diarrhea (3, 8%), and hemoptysis (2, 3%). Only two (3%) patients developed shortness of breath

The clinical presentation of the 1st phase of COVID19 looks like a flu and is very similar to SARS (2003) as SARS-CoV2 virus shares highly homological sequence with SARS-CoV, and causes acute, highly lethal pneumonia coronavirus disease 2019 (COVID-19) [**source**].

Additional testimonies say that the fatigue might be very important and the cough quite spectacular. Recently, some additional symptoms like anosmia and ageusia which suggests that SARS-COV2 could also present a neurotropism.

"[...] *The infection of SARS-CoV has been reported in the brains from both patients and experimental animals, where* ***the brainstem was heavily infected****. Furthermore, some coronaviruses have been demonstrated able to spread via a synapse-connected route to the medullary cardiorespiratory center from the mechanoreceptors and chemoreceptors in the lung and lower respiratory airways. Considering the high similarity between SARS-CoV and SARS-CoV2, it remains to make clear whether the potential invasion of SARS-CoV2 is partially responsible for the acute respiratory failure of patients with COVID-19* [...]" [**source**].

Phase 2: Acute Distress Respiratory Syndrome

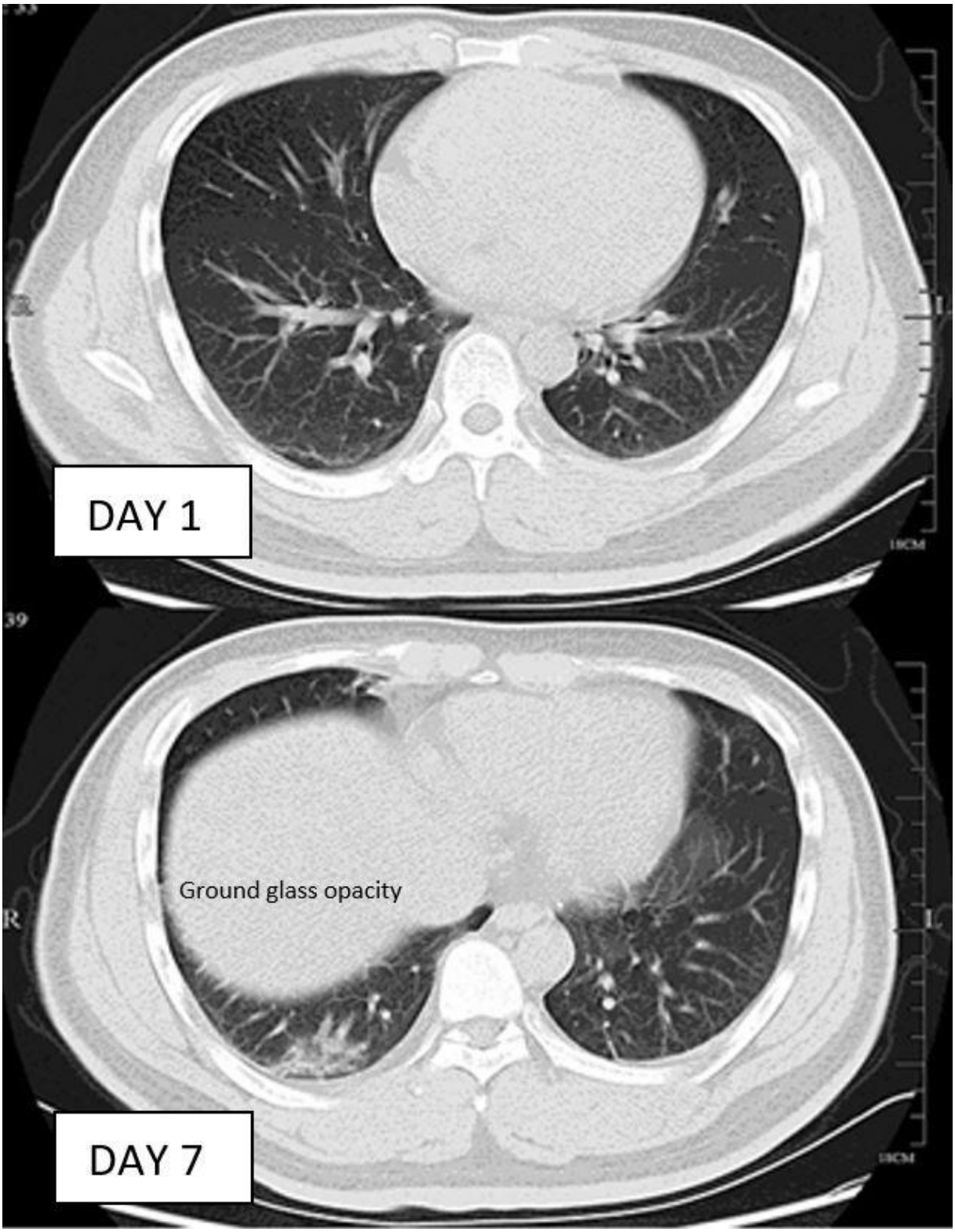
While in the vast majority of case (~80%), the COVID19 will not go beyond this phase-1 stage that lasts around a week, and patients will fully recover.

For some (~20%), their clinical conditions will worsen around days 7 to 10 and they will develop signs of Acute Distress Respiratory Syndrome (ADRS) which will require their admission at the hospital in an intensive care unit for breath support.

- Pa02: Partial Oxygen Pressure assesses how oxygen is able to travel from the lungs to the blood. It therefore reflects the amount of oxygen carried by the blood and delivered to the organs. Normal value : above 80mm Hg.
- Sa02: Oxygen Saturation (of Hemoglobin). 97% of oxygen is transported bound to Hemoglobin which can transport up to 4 molecules of O2.

Patients with ADRS exhibits lower values for SaO2 (below 70%) and then artificial ventilation with 100% O2 tries to increase SaO2 up to 94%. They exhibit lower values for PaO2 too, around 60 mm Hg [**source**].

Their lungs exhibits ground glass opacity:



[Source] : Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series / BMJ 2020; 368.

Phase 2: Immune exacerbation

In the acute phase of SARS-CoV infection (in 2003), rapid reduction of lymphocytes in peripheral blood [Source], mainly T lymphocytes, was observed, and both CD4+ and CD8+ T lymphocytes were decreased. The loss of lymphocytes precedes even the abnormal changes on the chest X-ray.

A retrospective study for MERS-CoV (2016) comparing 45 patients in South Korea has suggested that a decreasing number of peripheral lymphocyte (lymphopenia as an absolute lymphocyte count lower than 1,000 cells/mm3), thrombocytopenia (platelet count lower than 150000 cells/mm3) and high CRP level could predict pneumonia development and progression to respiratory failure at the early course of the disease. [Source]. This article suggests that **biological analysis might predict the progression or SARS-CoV X diseases to pneumonia and respiratory failure at the early course of the disease. This suggest another approach to the pandemic management: biological surveillance of blood counts to anticipate future hospitalization.** Instead of developing Artificial Intelligence algorithms to "diagnose" COVID19-pneumonia on lung imaging which have already been done, it might be more efficient to focus on algorithm and data that can predict the future worsening of clinical presentation, because this is the clinical pattern that puts high pressure on our Health System, while other people could stay home with symptoms ranging from asymptomatic presentation up to a severe flu.

What happens is the exacerbation of the immune response due to a "cytokine storm". Cytokine are specific proteins that are widely use to coordinate, moderate, manage the immune response. Some of you might have heard about "Interleukines" (Interleukine-1 IL-1, to Interleukine-17, IL-17). Among those, you have what is called "Pro Inflammatory" cytokines, which are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. **IL-1β**, **IL-6**, and **TNF-α** are the typical proinflammatory cytokines. **IL-10** is an anti-proinflammatory cytokine (remember the balance).

Some of you might have heard about "Interferon", among which type I Interferons (type I IFNs) which induce the innate immune response and provide the first critical line of immune defense, which then activate a cascade of intercellular messages mediated through Interleukines that could amplify the immune response facing a new antigen (a piece of new pathogen). Like often with biological systems and molecular architecture, you have a balance between retroactive loops to slow down the immune response, and retroactive loops to boost the immune response. Most of the time, this balance is very well managed… sometimes it derails (auto immune diseases… COVID19 cytokine storm).

To (over)simplify things, the infected cell produces type I interferon… saying to the other cells around "beware, there's a new vilain in town". It is also a kind of "suicide", as it also says "I'm infected, kill me" to which CD8+ lymphocytes (CD8 is a surface protein, CD8

Lymphocytes are also named cytotoxic lymphocytes) will respond by killing the infected cell using perforin-mediated-cytolysis. Perforin is a glycoprotein responsible for pore formation in cell membranes of target cells. Perforin is able to polymerize and form a channel in target cell membrane. Many research groups focus on the role of perforin in various diseases, immune response to bacterial and viral infections, immune surveillance and immunopathology. In addition, perforin is involved in the pathogenesis of autoimmune diseases and allogeneic transplant rejection. Natural killer (NK) cells and CD8-positive T-cells are the main source of perforin. [**Source**].

CD4+ lymphocytes are also named "helper lymphocytes" will also participate in modulating the immune response. They will produce local inflammatory to attract macrophages to "clean the mess on the crime scene". Activated macrophages will in return call for some support by releasing pro-inflammatory cytokines such as **IL-1β**, **IL-6**, and **TNF-α.** New comers (immuno-competent cells) will in turn "talk a lot on the 911 radio communication network". To avoid the storm, we need strong helpers CD4+ lymphocytes to slow down the talking of immuno-competent cells. **But remember the early biological sign of COVID19?** "[...] *rapid reduction of lymphocytes in peripheral blood [**Source**], mainly T lymphocytes, was observed, and both CD4+ and CD8+ T lymphocytes were decreased* [...]". Mo.. Fu... !!!

To summarize the cytokine storm : (1) Viruses infect lung epithelial cells and alveolar macrophages to produce progeny viruses and release cytokines/chemokines (mainly contains interferons). (2) Cytokine/chemokine-activated macrophages and virally infected dendritic cells lead to a more extensive immune response and the initiation of cytokine storm mediated by IL-1, IL-6 and TNF-α. (3) Released chemokines attract more inflammatory cells to migrate from blood vessels into the site of inflammation, and these cells release additional chemokines/cytokines to amplify cytokine storm. This results in general inflammation (sepsis), could lead to multi-visceral organ failure, and septic shock with disseminated intravascular coagulation and terminal heart failure and death.

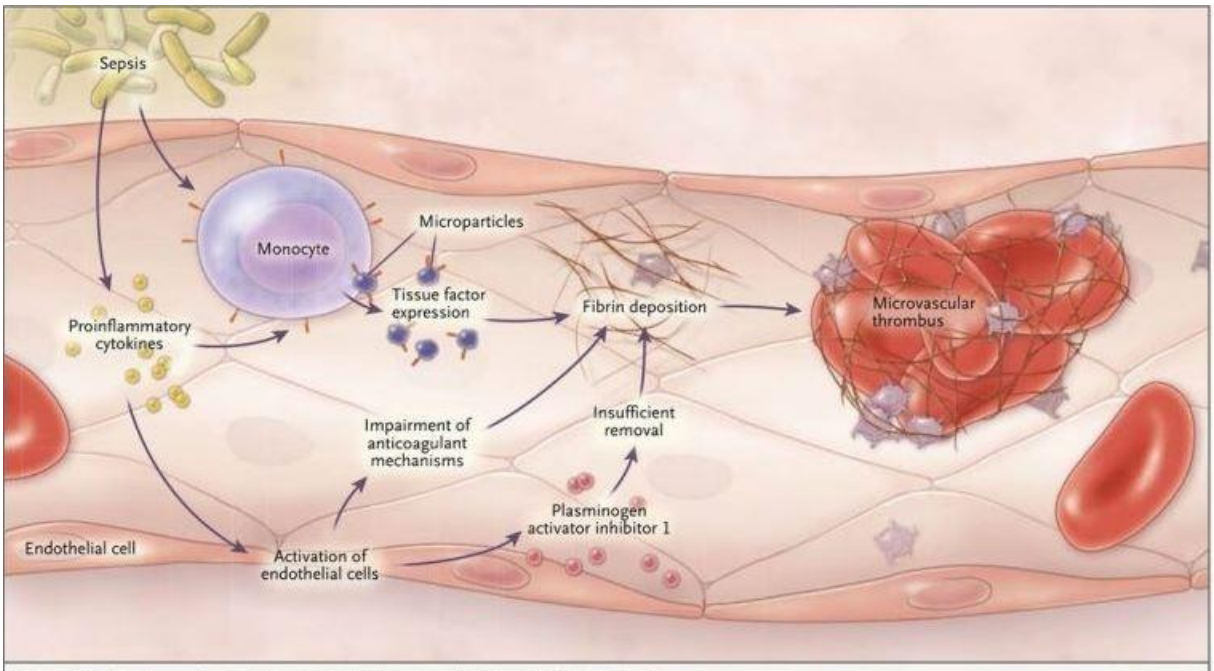


Figure 2. **Pathogenesis of Disseminated Intravascular Coagulation in Sepsis.**
Through the generation of proinflammatory cytokines and the activation of monocytes, bacteria cause the up-regulation of tissue factor as well as the release of microparticles expressing tissue factor, thus leading to the activation of coagulation. Proinflammatory cytokines also cause the activation of endothelial cells, a process that impairs anticoagulant mechanisms and down-regulates fibrinolysis by generating increased amounts of plasminogen activator inhibitor.

[image source]

Meanwhile, the reduction in CD4+ and CD8+ lymphocytes counts reduce the ability to manage the cytokine storm and slow down the immune response. In 2003, similar situation has been identified: Depletion of CD4+ T cells resulted in an enhanced immune-mediated interstitial pneumonitis and delayed clearance of SARS-CoV from the lungs [Source].

"[…] *We speculate that lymphocytes in patients with COVID-19 might gradually decrease as the disease progress. But the mechanism of significant lymphocyte reduction in severe type patients remains unclear. Besides, the inflammatory factors associated with diseases mainly containing IL-6 were significantly increased, which also contributed to the aggravation of the disease around 7 to 14 days after onset.* […]" [Source1] [Source2]

The result is this general inflammation (sepsis) which is a critical clinical condition. As the lung parenchyme is inflammated, edematized, and unable to perform its function, *i.e.* transfer the oxygen in the air to the red blood cells, and transfer the CO2 from the blood to the exhaled air. Bad oxygenation of organs can lead to a multi-visceral organ failure. SARS-COV2 replication results in the production of inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1), and interleukin-6 (IL-6) leading to macrophages activationin initiation of the coagulation process by producing thrombin, which eventually results in a fibrin clot. Activation of macrophages can result in vasculature damage. Fibrinolysis (the destruction of a fibrin clot) is also

impaired in sepsis because of the release of plasminogen-activator inhibitor (PAI-1) by both thrombin and inflammatory cytokines.

This is confirmed by studies on deceased patient at the molecular level. Deceased patients more often had increased concentrations of interleukin 2 receptor, interleukin 6, interleukin 8, interleukin 10, and tumor necrosis factor α than did recovered patients. [**Source**].

Once again, early blood cell counts of CD4+ and CD8+ lymphocytes might be an early indicator of future serious clinical presentation.

Another biomarker could be the SARS-COV 2 RNAemia (counts in circulating blood). Result showed that cases with RNAaemia were exclusively confirmed in critically ill patients group and appeared to reflect the illness severity. Further more, the inflammatory cytokine IL-6 levels were significantly elevated in critically ill patients, which is almost 10-folds higher than those in other patients. More importantly, the extremely high IL-6 level was closely correlated with the incidence of RNAaemia (R=0.902) and the vital signs of COVID-19 patients (R= -0.682). [**Source**]

IL-6 and SARS-COV2 RNAemia could also be very good biomarkers of future serious clinical presentation.

Therapeutic Approaches for acute phase

Breathing support

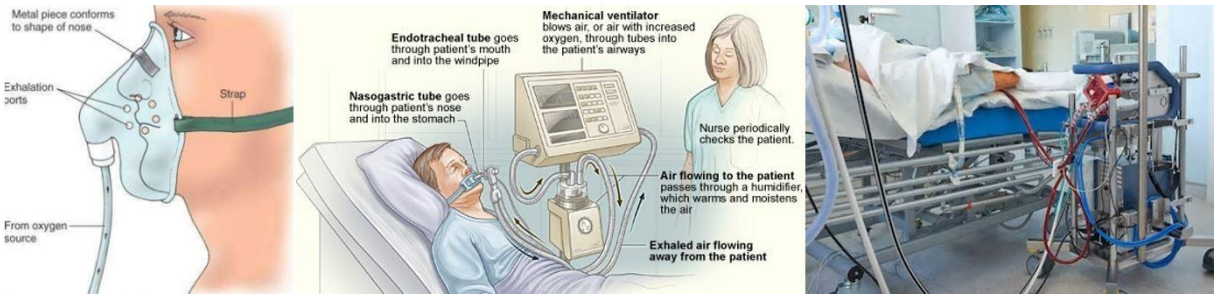
Three levels of oxygenation assistance are used:

1. Supplemental oxygen therapy should be given immediately to patients with hypoxemia. Oxygen therapy can be started at a flow rate of 5 L/min, and the target oxygen saturation is pulse oxygen saturation ≥90% in non-pregnant adult patients, ≥92-95% in pregnant patients, and ≥94% in patients who are critically ill with severe respiratory distress, shock, or coma.

2. If standard oxygen therapy fails, mechanical ventilation should be considered; high flow nasal catheter oxygen or non-invasive ventilation (for example, bilevel positive airway pressure mode) can be used.

3. If no improvement is seen within one hour of non-invasive mechanical ventilation, invasive mechanical ventilation should be used.

The last level of oxygenation assistance to be discussed is extracorporeal membrane pulmonary oxygenation according to their evaluation of the patient's situation (ECMO). You divert the blood flow to an external oxygenation membrane (a kind of artificial lun). This is a heavy clinical procedure and there are only a very limited number of ECMO machines. Furthermore, to run an ECMO machine you need more Health Care Professionals to take care of the patient.



Manage the Sepsis

As written above, some patients do exhibit serious clinical presentations like the Acute Distress Respiratory Syndrome (ADRS) which requires oxygenation. But things can go even worse, with the development of a general inflammation, *i.e.* sepsis, which might lead to multi-visceral organ failure, septic shock and death. Sepsis in a very critical clinical situation and Intensive Care Units all around the world tried to develop a consensus about the best protocols to manage it. Since your blood oxygenation is impaired by ADRS, things can go worse very quickly as all organs depend on oxygen to perform their function, and durable impaired oxygenation can lead to decompensation, a cascade of physiopathological events in which the global homeostasis (all functions that concur to the maintenance of your biological balance within optimal life compatible ranges) derails...so fast.

" [...] *Sepsis remains a common and deadly public health issue in modern medicine. Severe sepsis has been estimated to affect three quarters of a million patients in the United States (US) each year and carries a mortality rate of approximately 30%* [...]" [**Source**]. This percentage of casualties is alike the one observed for COVID19 patients exhibiting sepsis.

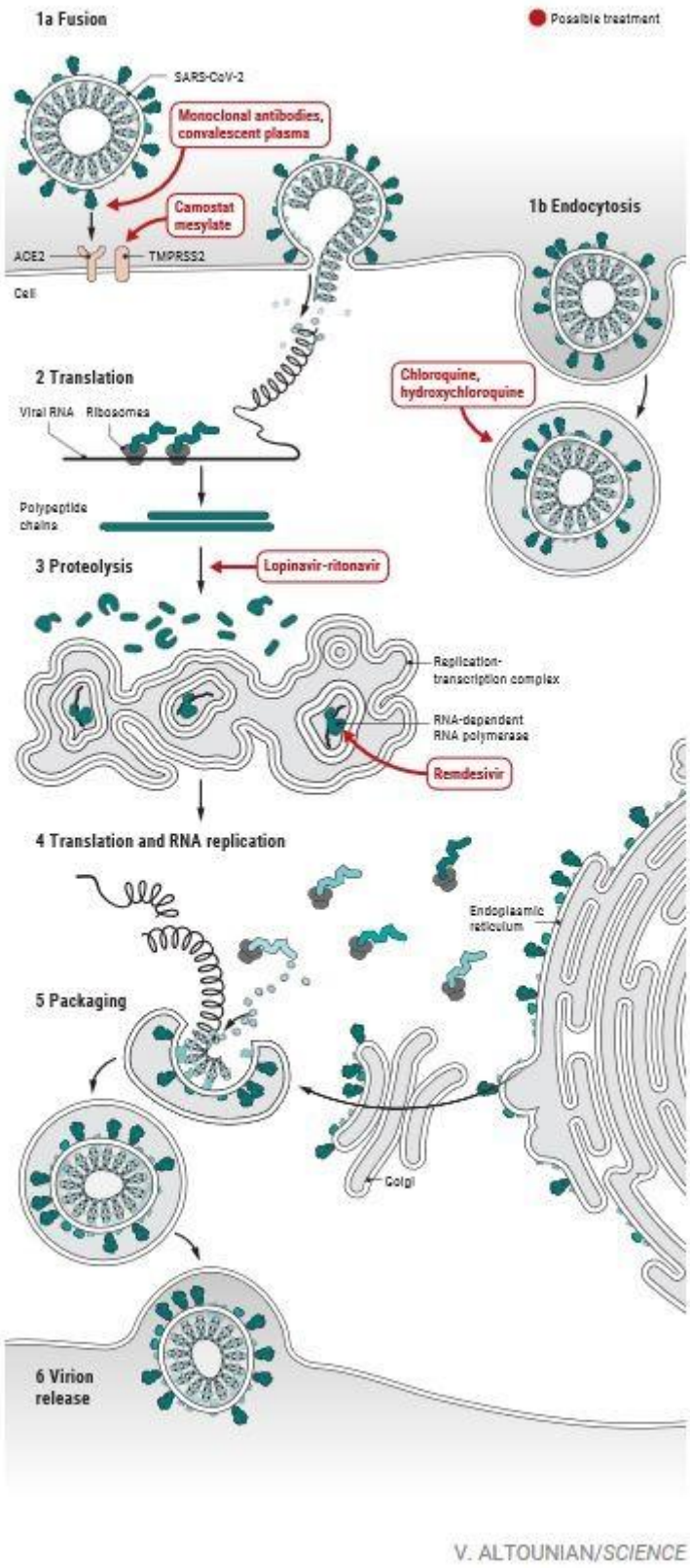
Usually it is managed through "bundles" of standardized operation procedures (SOP).To simplify things, as written patients with a septic shock often present multi-visceral organ failure. These organs cannot sustain "nominal physiological functions". Hence, Intensive Care Unit (ICU) teams have to somehow do the job of maintaining "homeostatis" with heavy treatments and very tight monitoring of patients' clinical conditions. You need to maintain blood pressure (vasopressor), the volume of body liquids (crystalloid), to provide oxygen (endotracheal intubation and ventilation), to support heart contracting activity (Dobutamine), maintain glycemia (insuline), prevent inflammation (hydrocortisone) and blood clot formation (recombinant human activated protein C - rhAPC) by limiting the coagulation cascade by inactivating FVa and FVIIIa, directly and indirectly limiting systemic inflammatory response syndrome (SIRS), and improved the fibrinolysis process and of course you need antibio-therapy. [**Source1**][**Source2**]

Other therapeutic approaches

Prevent the virus from infecting cells

In **Episode1**, we have seen that the virus needs to bind onto a specific receptor to be able to infect the cell. At the beginning of this Episode3, we discovered that ACE2 receptor was the surface protein target by SARS-COV2 Spike protein, with TMPRSS2 furin protein as an "activator" of SARS-COV2 Spike protein.

In this Episode3, we also had a look to the virus replication cycle which provides different kinds of therapeutic strategy targets. The World Health Organization is currently managing a clinical trial named SOLIDARITY to test 3 "drugs" in order to slow the infection or even cure it. Here is a lovely diagram published by "ScienceMag" which summarizes the drugs and the molecular targets.

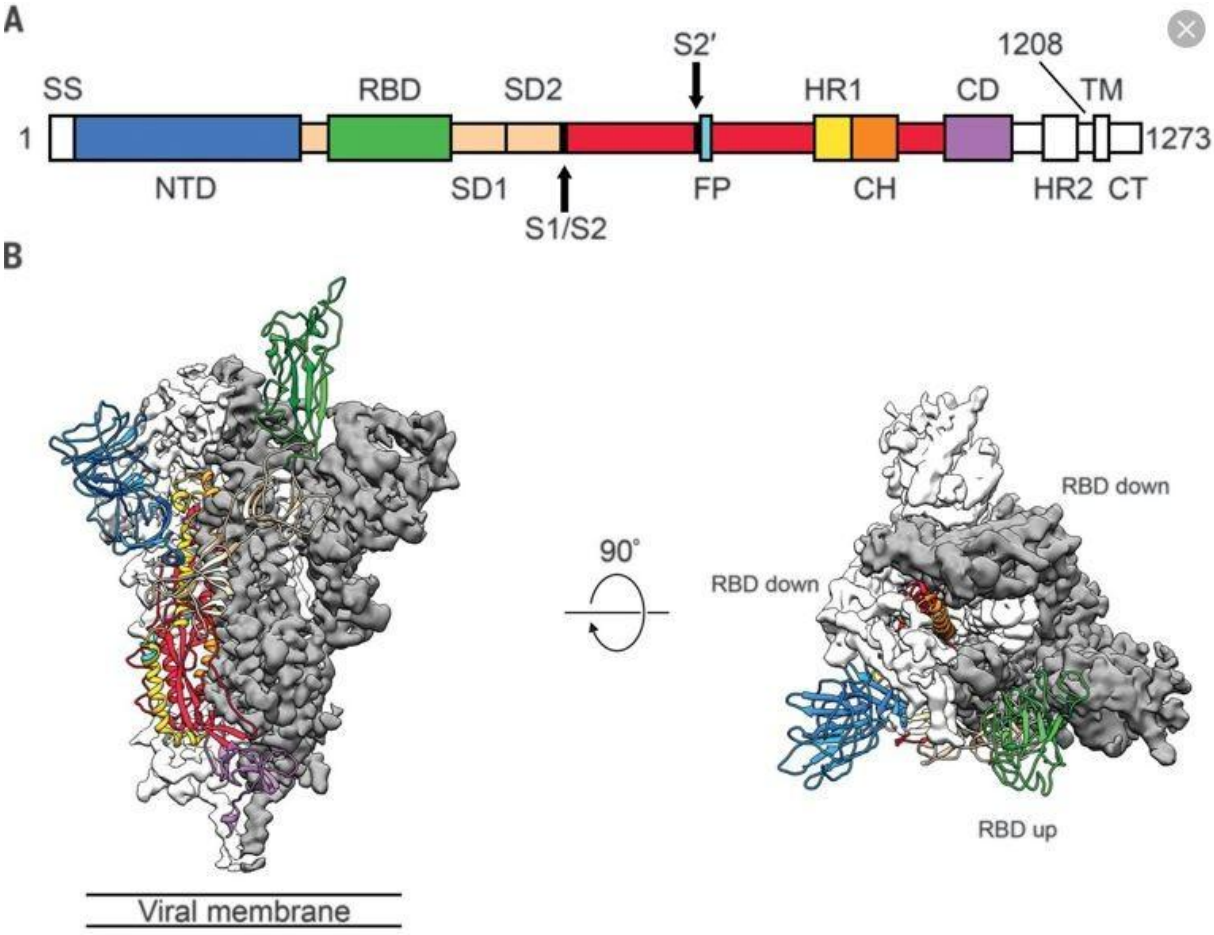


[Image Source]

In this category there are specifically designed recombinant monoclonal antibodies targeting either the SARS-COV2 Spike protein, ACE2 receptor, or TMPRSS2 furin activator.

As written in **Episode1** targeting human cells receptor is not a good idea as they are often used for normal/functional intercellular communication. ACE2 is slicing Angiotensin2 (a strong vasoconstrictor into Angiotensin1 (1-7) which is a vasodilator. Same logic applies for TMPRSS2 furin protein, although we saw that "Nafamostat", "Camostat Mesylate" could strongly inhibate TMPRSS2-mediated Spike protein activation and hence prevent endocytosis.

Another way is to create a monoclonal antibody that binds onto the Spike Protein, recovering the SARS-COV2 "molecular key" and prevent it to be inserted in ACE2 "lock". To do so, it is important to have an accurate 3D structure of SARS-COV2 Spike protein.



*(**A**) Schematic of 2019-nCoV S primary structure colored by domain. Domains that were excluded from the ectodomain expression construct or could not be visualized in the final map are colored white. SS, signal sequence; S2', S2' protease cleavage site; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail. Arrows denote protease cleavage sites. (**B**) Side and top views of the prefusion structure of the 2019-nCoV S protein with a single RBD in the up conformation. The two RBD down protomers are shown as cryo-EM density in either white or gray and the RBD up protomer is shown in ribbons colored corresponding to the schematic in (A)* [**Image Source/Source**]

To do so, sequencing of SARS-COV2 RNA is needed, and mathematical models are used to determine the 3D frame of the Spike Protein from its amino-acid sequence. High Performance Computing and Augmented/Virtual reality could assist researcher in doing this job faster than before. Given this 3D structure, Artificial Intelligence algorithms can be used to screen all existing drugs, and identify candidates that can bind to this Spike 3D structure. Same to identify new monoclonal antibody by measuring in a virtual proteomic platform the bind-affinity of these antibodies to specific areas of the SARS-COV2 Spike protein.

As drawn on the diagram above, you can also use IgM and IgG found in the plasma of cured patients to block the spike protein (blood derived products/drugs).

Prevent the endocytosis and injection of genetic payload

The so famous now "HydroxyChloroquine" (HCQ) (and Chloroquine and alike molecules) appears to be active on this specific step.

"[…] ***Chloroquine can inhibit a pre-entry step of the viral cycle*** *by interfering with viral particles binding to their cellular cell surface receptor. Chloroquine was shown to inhibit quinone reductase 2, a structural neighbour of UDP-N-acetylglucosamine 2-epimerases that are involved in the biosynthesis of sialic acids. The sialic acids are acidic monosaccharides found at the extremity of sugar chains present on cell transmembrane proteins and are critical components of ligand recognition. The possible interference of chloroquine with sialic acid biosynthesis could account for the broad antiviral spectrum of that drug since viruses such as the human coronavirus HCoV-O43 and the orthomyxoviruses use sialic acid moieties as receptors. The potent anti-SARS-CoV-1 effects of chloroquine in vitro were considered attributable to a deficit in the glycosylation of a virus cell surface receptor, the angiotensin-converting enzyme 2 (ACE2) on Vero cells.* […]". [**Source**]

But Chloroquine can (and First of all) also act on the immune system through cell signalling and **regulation of pro-inflammatory cytokines**. Chloroquine is a well-known immunomodulatory agent capable of mediating an anti-inflammatory response. Therefore, there are clinical applications of this drug in inflammatory diseases such as rheumatoid arthritis, lupus erythematosus and sarcoidosis [**Source**].

In other words, (Hydroxy)Chloroquine is able to slow down the "cytokin storm" which is responsible of the acute worsening of clinical conditions leading to sepsis. We better understand that the sooner it is given, the smaller the cytokine storm, and possibly the smaller the virus replication would be. We also understand that if it is given to patients exhibiting sepsis, it might also be too late, as at that stage, it is too late to achieve the regulation of pro-inflammatory cytokines.

Prevent the assembly of the virion

Some genes of the RNA payload code for the envelope of the virus which is composed of the same kind of proteins (repeated frame). Instead of producing N copies of this monomer, the virus makes the infected cell produce a long single protein made of (N) monomers (yes a polymer^^ - like a perl necklace). Then, specific enzyms named "viral proteases", slice this "polymer" into monomers, which is critical for virion's assembly.

Abbott developed (lopinavir) sold under the brand name Kaletra to treat HIV infections (in 2000). Lopinavir specifically inhibits the protease of HIV, an important enzyme that cleaves a long protein chain into peptides during the assembly of new viruses. Because lopinavir is quickly broken down in the human body by our own proteases, it is given with low levels of ritonavir, another protease inhibitor, that lets lopinavir persist longer. The combination can inhibit the protease of other viruses as well, specifically coronaviruses. [**Source**]

Prevent the replication of the virus RNA

Remdesivir is a molecule developed by Gilead Sciences to combat Ebola and related viruses, remdesivir shuts down viral replication by inhibiting a key viral enzyme, the RNA-dependent RNA polymerase. It is a nucleosidic-analog, *i.e.* something that is like the A, T, C, G (nucleotides) that are the basic bricks of genetic information. The RNA-polymerase, assemble those to rebuild a copy of the SARS-COV2 genetic information.

Nucleosidic analog on one side looks like a genuine nucleotides, so the RNA-Polymerase can "use" them as regular bricks of genetic information to rebuild the SARS-COV2 genetic information… But on the other side, is might present a huge molecular structure (occupying molecular volume/space) which prevent the RNA-polymerase to add additional nucleotides to the viral RNA… stopping the replication.

Researchers tested remdesivir last year during the Ebola outbreak in the Democratic Republic of the Congo, along with three other treatments. It did **not show any effect**. (Two others did.) But the enzyme it targets is similar in other viruses, and in 2017 researchers at the University of North Carolina, Chapel Hill, showed in test tube and animal studies that the drug **can inhibit the coronaviruses that cause SARS and MERS**. [**Source**]

Conclusion

Despite the impatience of people, and partial scientific articles spread through social network, we need to do state of the art clinical assays to be able to draw clinically relevant conclusions. It is not because (HydroxyChloroquine) was given to 500 patients in New York, 24 then 70 in France, to hundreds in China with apparently good results, that it is the one fits all miracle cure of COVID19.

My point is that its pro-inflammatory cytokine regulation properties are key to avoid sepsis when given in the early stages of the COVID19. Its anti-inflammatory/immuno-

modulation properties are clinically used as a treatment of some auto-immune diseases... And this is the 1st reason why we need to be extra careful not to create the conditions of a supply chain disruption due to the "fear of the crowd".

- HydroxyChloroquine is not toilet paper... it is used to cured chronic diseases and shortage of supply for these patients should not be tolerated because some others might think it could save their lives (even if they are not COVID19+ at that time, since some people even said it should be used as a COVID10 prophylactic treatment).
- HydroxyChloroquine can also have hard-to-anticipate-iatrogenic-effects as its "half life" is very long... meaning your body accumulates successive doses, and its iatrogenic effects are dose-dependent and show cardiac and renal toxicity. Self-medication must never be an option, HydroxyChloroquine must be given under medical monitoring !
- Hydroxychloroquine is one option of the WHO led clinical assay "SOLIDARITY", and European clinical assay "DISCOVERY". Of course, things never go fast enough when you are in Intensive Care Units and compassionate use of Hydroxychloroquine still remains an option at the hospital... Possibly, it should be used a few-day prior to possible sepsis for greater clinical benefits. Some investigators refused to enroll patients in the "non-hydroxychloroquine" arm of the assay, because it could reduce their clinical outcomes. One way to be respectful to medical ethics and statistics would be to enroll patients in early phases, when we have little time to adjust the treatment.

Another point relates to the massification of COVID19 detection. I have already given some insights about this [**Part1**], [**Part2**], and [**basic maths**] (sorry it is in French, but you can use your favorite translator). I don't think that it is the right solution for a "targeted" confinement, especially during the acceleration phase of the Pandemic... because it is not as "massive" as people may think... because we target mild-symptomatic patients and not the asymptomatic patients (that ignore themselves that they are contaminating people). I think a better approach to this issue, would be to use a probabilistic confinement:

- if it looks like COVID19, even with mild-symptoms, or few symptoms.. then it is COVID.
- if it is COVID, then you should put yourself in self-confinement, use Paracetamol to manage pain and fever (and NOT aspirin, and NOT ibuprofen).
- We'd need non-ambiguous confirmation of COVID19 for serious presentation to ensure differential diagnosis.

If we step-back and have a look to the pandemic. The core and genuine issue is the ability to identify contaminated people who will develop severe clinical conditions in the coming days (Day0 + 7 ~ 10). Because these are the people who are at risk of dying. Because these are the people who put the highest pressure on our health system. In this Episode3 we identified possible "biomarkers" of a probable/possible worsening of near future clinical conditions (CD4/CD8 lymphocytes decrease, IL-6, SARS-COV2 circulating RNA load).

Probabilistic self-confinement would prevent the identification of these early biomarkers… and as people would have to wait for severe clinical presentation to be admitted in Intensive Care Unit (possibly too late).. in parallel of this massification of detection, in an ideal world, we should also perform evaluation of these biomarkers. But alike the massification of RT-PCR, it has a cost and logistic issue.

Comments

As I shall write new articles to shed some light on what's going on, do not hesitate to comment is you need to correct what's been shared here or to ask additional questions.

Do not hesitate to share, if you think such information is worth spreading.

In case you miss them: [**Episode1**], [**Episode2**]