The long and winding road to Covid-19 treatments and vaccines

Part 2: from cardiovascular disease treatment to developing vaccines

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Note: highlighted in italic are information for non-biologists

Introduction

In addition to drug repurposing, more specific treatments are currently explored to approach Covid-19. Cardiovascular disease (CVD) is one of the most prevalent and deadly condition clinicians have to face. Therefore, therapies supporting the cardiovascular system represent one of the first line of treatment of Covid-19 patients. Immunotherapy represents another option, but collides with the massive production of synthetic antibodies and immunoglobulin purification. Conversely, convalescent plasma is a valid alternative, which deserves thorough investigations. Stem cell therapy has been also considered, with a quite huge number of clinical trials ongoing. Finally, a large number of vaccines are currently being tested, with promising first results, suggesting that we are on our way to defeat SARS-CoV-2 (CoV-2) infection.

CVD treatment

CVD is a major comorbidity of Covid-19, *that is CVD is a pre-existing, concurrent pathology of CoV-2 infection*. However, CoV-2 may also induce CVD as a consequence of the cytokine storm or direct infection of the heart (figure 1). The cardiovascular manifestations of Covid-19 span from coagulopathy to acute myocardial infarction (MI)[1,2]. The key element is the Angiotensin Converting Enzyme 2 (ACE2) receptor. This is expressed not only in airway and pulmonary epithelial cells, but also in the heart, where it counteracts angiotensin II (Ang II), by the conversion in angiotensin 1-7, in the case of excessive renin angiotensin aldosterone system (RAAS) activation[3].

Figure 1

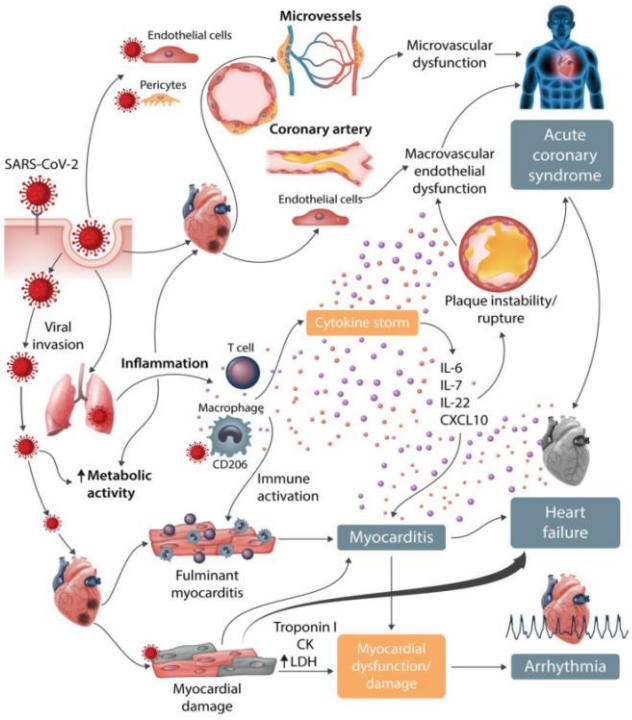


Figure 1. Hypothetical mechanism of cardiovascular involvement in COVID-19. CoV-2 anchors on transmembrane ACE2 to enter the host cells, including endothelial cells, pericytes, and cardiomyocytes, leading to inflammation and multiorgan failure. The infection of endothelial cells or pericytes may be responsible of severe microvascular and macrovascular dysfunction. Moreover, together with the immune hyper-reactivity, it can potentially destabilize atherosclerotic plaques and favor the development of acute coronary syndromes. Upon the occurrence of the cytokine storm and immune cells overactivation, activated T cells and macrophages may infiltrate infected myocardium, leading to myocarditis and severe cardiac damage. Similarly, the viral invasion could directly cause cardiac myocyte damage, leading to myocardial dysfunction and contribute to the development of arrhythmia. Abbreviations: IL=interleukin; CXCL10=C-X-C motif chemokine ligand 10; CK=creatin kinase; LDH=lactate dehydrogenase. (*From: Guzik et al., Cardiovasc Res, 2020*).

- <u>Coagulation abnormalities</u>

  Covid-19 may be defined a thromboinflammatory syndrome, characterized by sepsis-induced coagulopathy (SIC) and disseminated intravascular coagulopathy (DIC), especially in severe cases. Coagulation abnormalities are defined as decreased platelet counts and fibrinogen and increase in pro-coagulant factors, such as fibrinogen degradation products (D-dimer)[4]. Venous thromboembolic events (VTE) have been also reported in a number of patients and although the use of heparin has been suggested to treat this typical coagulation disorder[5], VTE did not take advantage from low molecular weight heparin[6]. Ang II may be responsible for endothelial dysfunction (ED) and increased vascular permeability[7]. Although the pathogenesis is still poorly understood, recently it has been suggested that an imbalance between pro-angiogenic and anti-angiogenic factors may contribute to

CoV-2-dependent coagulopathy. In particular, in Covid-19 patients a high ratio of soluble fms-like tyrosine kinase 1 (sFlt-1)/Placental Growth Factor (PlGF) has been detected[8]. CoV-2 represses ACE2 expression, increasing Ang II levels which, in turn, promotes the growth of sFlt-1 levels. sFlt-1 acts as a decoy for PlGF and impairs nitric oxide production, leading to ED.

- Myocardial injury

  Myocardial injury (figure 2) in Covid-19 patients has been reported to follow two distinct patterns. The first is probably related to the Cov-2–dependent cytokine storm and presents elevated levels of high-sensitivity cardiac troponin I (hs-cTnI), which constantly increases in non-survivors and tracks other pro-inflammatory cytokines and markers - interleukin 6 (IL-6), D-dimer, lactate dehydrogenase, ferritin - and of N-terminal pro B-type natriuretic peptide (NT-proBNP). However,

Figure 2

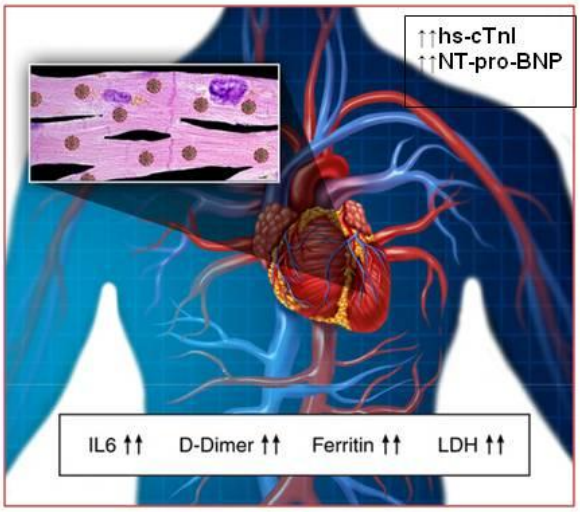


Figure 2. Covid-19 and myocardial injury. Myocardial injury, manifested by increased hs-cTnI and NT-pro-BNP, can result from the cytokine storm characterized by elevated levels of IL-6, ferritin, LDH, and D-dimer or from the direct effect of CoV-2 infection on the heart. Abbreviations: hs-cTnI= high sensitivity-cardiac troponin I; NT-proBNP= N-terminal pro B-type natriuretic peptide; LDH=lactate dehydrogenase; IL-6=interleukin-6. (*Adapted from: Clerkin et al., Circulation, 2020*)

some patients presented predominantly cardiac manifestation, with severe cardiogenic shock, suggesting the onset of viral myocarditis or stress cardiomyopathy. This condition predispose to cardiac arrhythmia, which includes conduction block, atrial and ventricular fibrillation and ventricular tachycardia and which is the second most frequent complication in Covid-19 patients after acute respiratory distress syndrome (ARDS)[9]. Treatment of these patients required steroids (mainly glucocorticoids), intravenous immunoglobulins administration, antiviral therapy, till extracorporeal membrane oxygenation (ECMO)[10,11]. The late onset of myocardial injury (about 15 days after the first symptoms)[2] supports the hypothesis of a T-cell response-mediated cardiac damage, as demonstrated by an

increase in the level of $CCR6^{+}Th17$ $CD4^{+}$ T lymphocytes[2], which are mediators of myocarditis[12], but the precise mechanism of CoV-2-dependent myocardial injury remains still unknown. Heart failure (HF) was a common outcome in Covid-19 patients. About 52% of non-survivors had HF compared to 12% of survivors[2].

- Hypertension

  The role of ACE2 in the RAAS system is the link between Covid-19 and hypertension. ACE inhibitors (ACEi) or blockers (ARBs) enhance ACE2 levels in rats[13]. Therefore, it has been postulated that anti-hypertensive therapies may be detrimental for Covid-19 patients. However, no evidences have been reported of an increase of ACE2 in human tissues upon ACEi or ARBs administration[14]. Indeed, the European Society of Hypertension, the International Society of Hypertension, and the European Society of Cardiology have discouraged to stop anti-hypertensive therapies in Covid-19 patients[15]. Of note, ARBs have been repurposed for Covid-19 therapy. Losartan impairs ACE2 internalization and degradation, therefore may be protective against CoV-2 infection[15]. Irbesartan, another ARBs, has been suggested as beneficial in Covid-19 treatment for it role on potassium metabolism. Indeed, hypokalemia – that is low plasma potassium levels – has been reported as a common features of Covid-19 patients. Hypokalemia is difficult to manage, depends on the severity of the disease and on RAAS activation[16]. Therefore, ACEi or ARBs has been proposed to be advantageous in this setting. Another relationship between Covid-19 and hypertension is the immune system. Poor control of blood pressure leads to a dysregulation of the immune system, in particular to a $CD8^{+}$ T cells dysfunction, which are no more able to efficiently counteract viral infections[17]. Therefore, hypertension therapy may also recover, at least partially, this immune dysregulation.

CVD treatment in Covid-19 patients includes heart failure, arrhythmias and anti-coagulation therapy, where appropriate. Recently, a phase II clinical trial started with APN01, a recombinant form of the human ACE2 (hrACE2; ClinicalTrials.gov Identifier: NCT04335136). Indeed, it has been demonstrated that a recombinant ACE2 can prevent CoV-2 infection not only of Vero-E6 cells, but also of capillary (figure 3) and kidney organoids[18]. Nevertheless, the neutralizing activity of hrACE2 is not complete, indicating alternative routes for CoV-2 to the infect target cells[18].

Figure 3

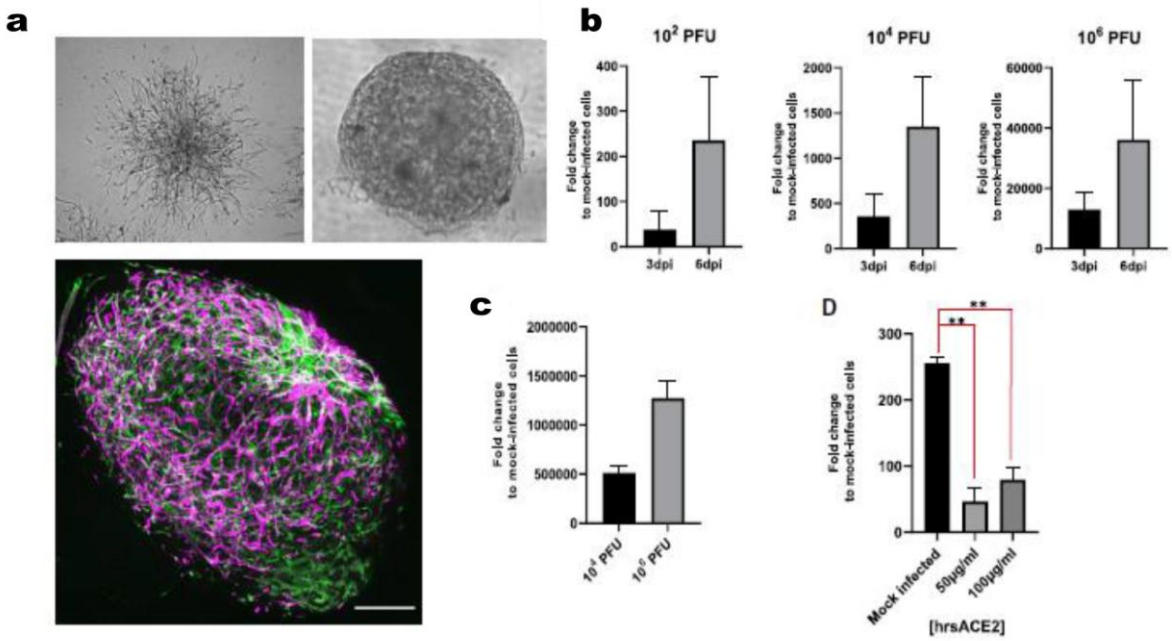


Figure 3. CoV-2 can infect capillary organoids. a) Upper panels. Light microscopy showing representative vascular organoids. Lower panel. Immunostaining of vascular organoids using anti-CD31 to detect endothelial cells and anti-PDGFRβ to detect pericytes b) Viral RNA from blood vessel organoids at day 3 and 6 post- CoV-2 infection (dpi). c) Determination of progeny virus. Supernatants of CoV-2 infected vascular organoids were collected 6 dpi and used to infect Vero E6 cells, at different concentrations, defined as plaque forming units (PFU). After 48 hours, viral RNA was assessed by qRT-PCR. The data show that infected vascular organoids produce CoV-2 progeny, depending on the initial level of infection. d) Vascular organoids were exposed to a mix of 10^6 infectious viral particles and hrsACE2 for 1 hour. 3 dpi, viral RNA was assessed by qRT-PCR. hrsACE2 decreased the level of CoV-2 infection in vascular organoids. Abbreviations: hrsACE2= human recombinant soluble ACE2. (*From: Monteil et al., Cell, 2020*)

Immunotherapy

Boosting the immune response is one of the current strategy to treat Covid-19. The most promising therapeutic routes are represented by synthetic monoclonal neutralizing antibodies and convalescent plasma.

- Monoclonal antibodies

  A number of antibodies have been isolated and proved to neutralize CoV-2 binding to the host cells. Importantly, some of them have been demonstrated to cross-react with SARS-CoV. Indeed, the 47D11 antibody impairs both SARS-CoV and CoV-2 infection “in vitro” (figure 4) and formation of syncithia. This specific antibody has

been isolated from supernatants of mouse hybridomas from mice immunized with SARS-CoV Spike (S). *A hybridoma is a hybrid cell resulting from the fusion of a B cell, producing a specific antibody, and a myeloma cell, which confer to the hybrid cell the property to indefinitely propagate in culture and produce high antibodies quantity.* The 47D11 antibody has affinity for a conserved epitope in the $S1_B$ subdomain of SARS-CoV and CoV-2 S protein (figure 4b), whereas it does not affect S binding to ACE 2 (figure 4c). Therefore, another mechanism has to be evoked to explain the

Figure 4

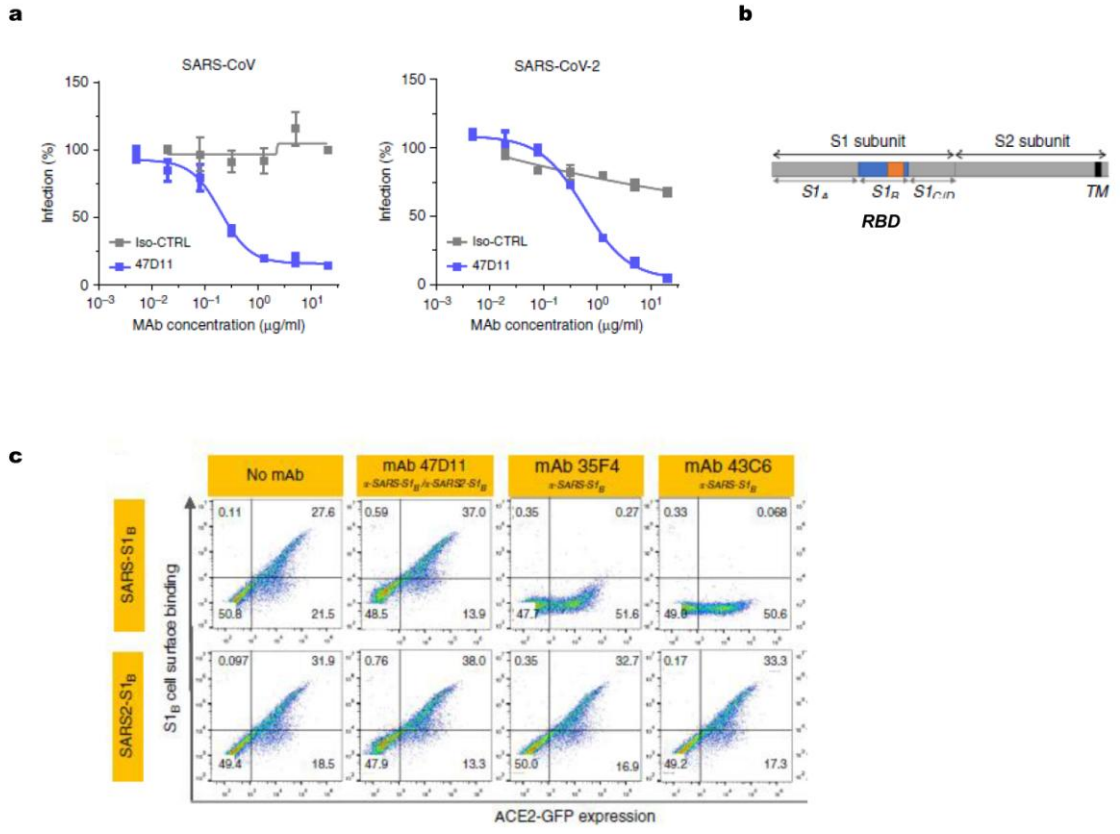


Figure 4. a) Inhibition of SARS- CoV (left) and CoV-2 (right) infectivity by 47D11 antibody (blu line) compared to a control antibody (grey line). b) Schematic structure of S protein. c) FACS analysis of the interference of different antibodies with SARS-CoV (upper panels) and CoV-2 (lower panels) $S1_B$ subunit binding to ACE2 conjugated to the GFP protein and transfected in Vero cells. Abbreviations: ACE2=Ace Converting Enzyme 2; GFP=green fluorescent protein. mAb=monocolonal antibody; RBD=receptor binding domain. (*From: Wang et al., Nat Commun, 2020*).

neutralizing activity of this antibody[19]. Another screening of B cells from a SARS-CoV infected patient, identified an antibody, called S309, which potently inhibited both SARS-CoV and CoV-2 infection, engaging S RBD domain and limiting ACE2 recognition (figure 5a)[20]. The same line of evidence has been obtained from other antibodies[21,22]. One of them, named CB6, isolated from the serum of a Covid-19 patient, has shown to possess prophylactic and therapeutic efficacy in rhesus monkeys (figure 5b)[21].

Figure 5

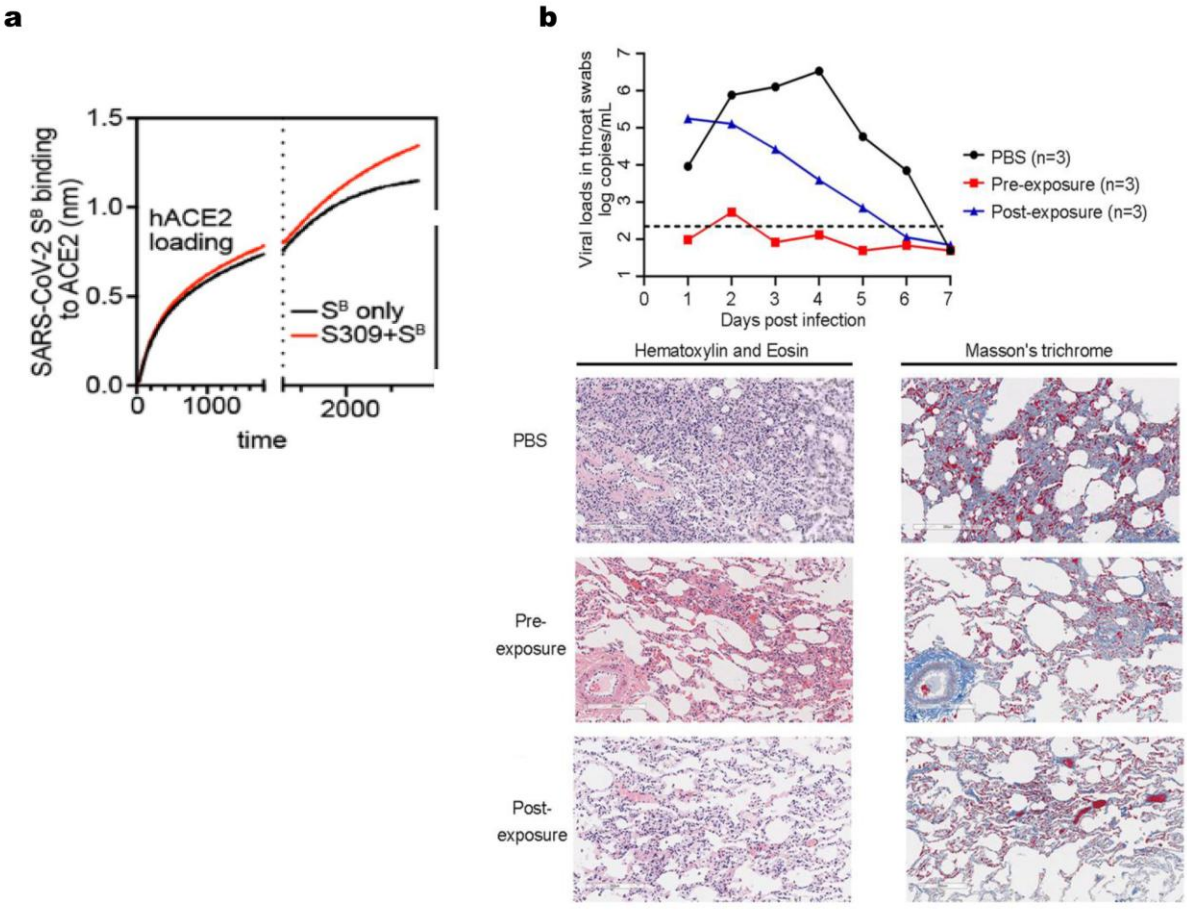


Figure 5. a) Competition between the S309 antibody and ACE2 for CoV-2 S_B Spike domain binding. The dashed vertical line indicates the beginning of the association of S309 to S_B or of free S_B to ACE2. b) Upper panel. Effect of CB6 antibody on CoV-2 viral load, determined by real time PCR, in 6 monkeys treated before (red) or after (blue) infection. Other 3 monkeys were treated with phosphate buffered saline (PBS) after infection. Lower panel. Histological and immunohistochemical analyses of PBS-treated monkey-derived lungs, treated before (pre-exposure) or after (post-exposure) infection. Hematoxylin and eosin staining shows the inflammatory infiltrate. Masson trichrome staining shows lung fibrosis. In both analyses, lungs are more damaged in control tissues than in CB6-treated. Abbreviations: PBS=phosphate buffered saline; hACE2=human ACE2 (*From: Shi et al., Science, 2020*).

- Convalescent plasma

  Passive antibody therapy is a well consolidated procedure, consisting in the administration of purified antibodies – which, however, have more prophylactic than therapeutic outcomes – immunoglobulins, which contains the antibodies against the specific pathogen of interest, and convalescent plasma, which is employed in emergency situations. In fact, immunoglobulins purification and production is time consuming; therefore, convalescent plasma, in the absence of efficient treatments, represents the only therapeutic option for novel occurring diseases, such as Covid-19. Figure 6 shows a standardized protocol for convalescent plasma collection[23].

  More than 100 clinical trials are ongoing by using convalescent plasma. Early reports, on small numbers of patients, simultaneously undergoing pharmacological therapy, reported an improvement in the clinical conditions of treated patients[24,25], raising optimism regarding this passive immune therapy. However, very recently, the results of the first randomized clinical trial on 103

Figure 6

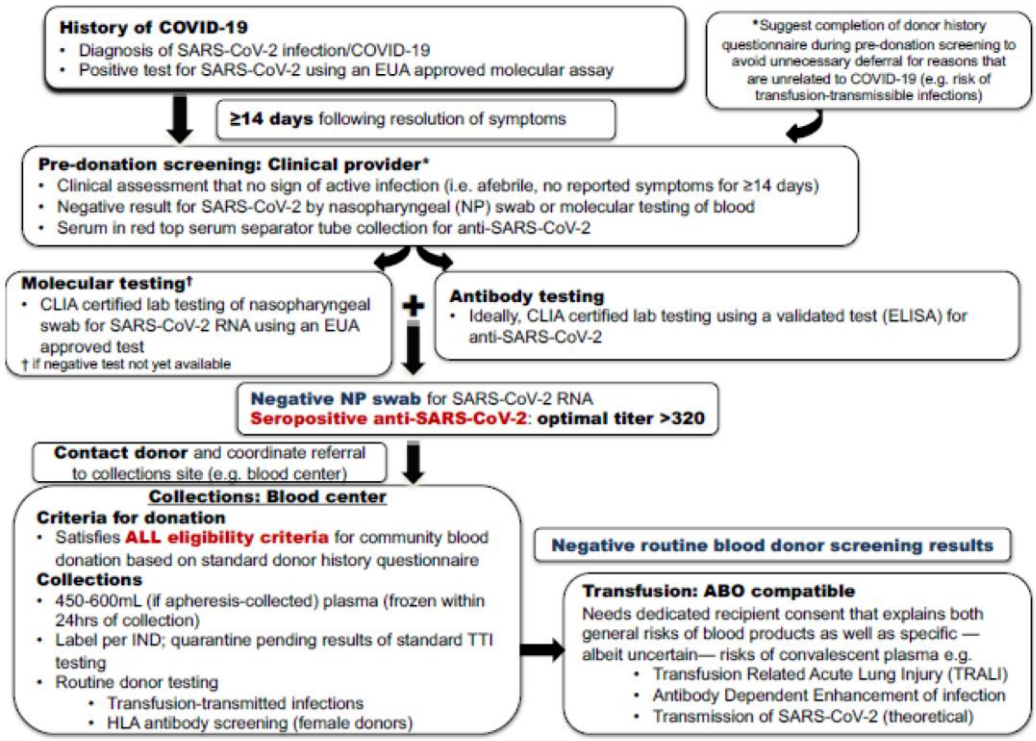
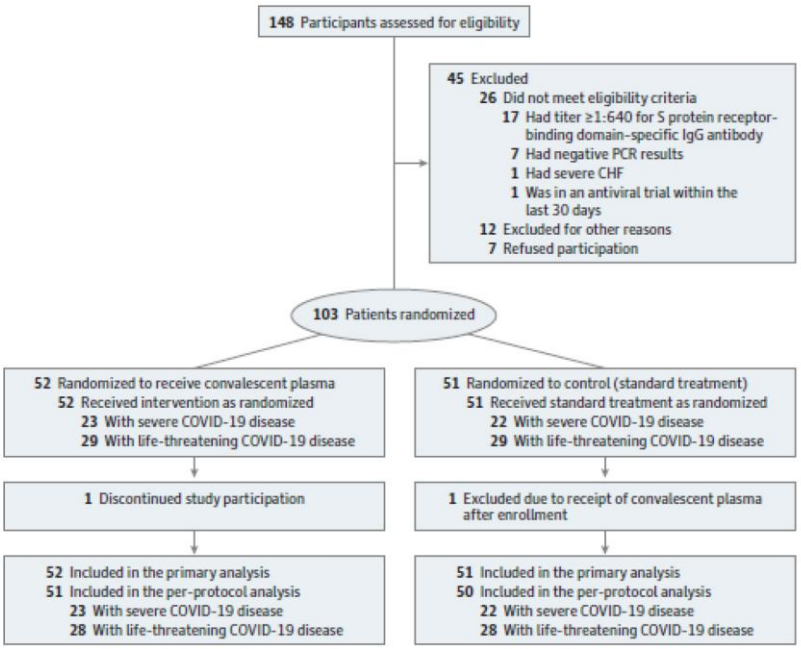


Figure 6. Protocol for the collection of convalescent plasma, as defined by *Bloch et al., JCI, 2020*[135].

Figure 7

a



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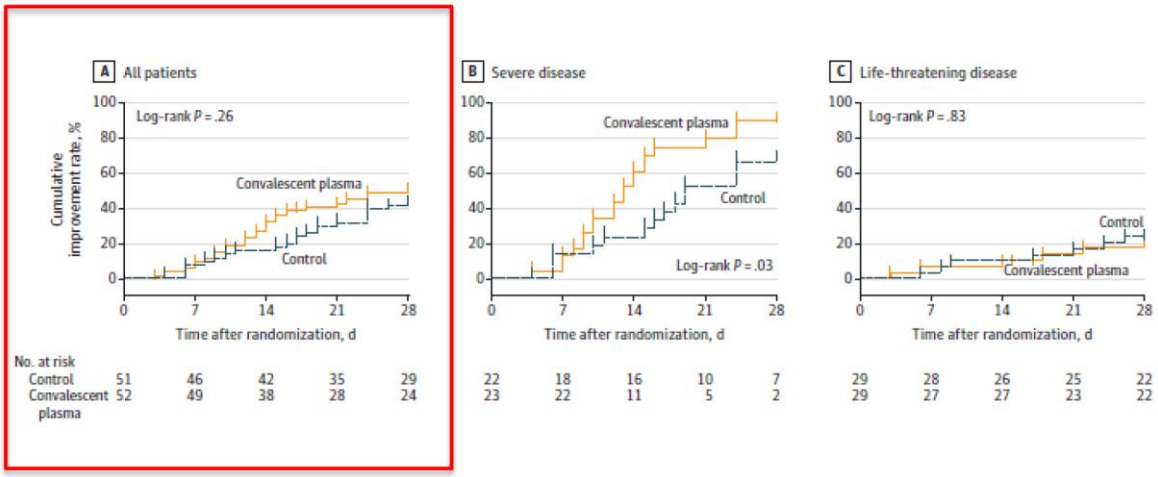

Figure 7. a) Graphical representation of patients inclusion/exclusion criteria and subsequent randomization into convalescent plasma-treated and control groups. b) Cumulative improvement, defined as percentage of discharged patients or that have shown a 2 points reduction in a scale of severity from 1 to 6 as established by Wang et al., Lancet, 2020. Overall (red box), patients did not take significant advantages from convalescent plasma treatment. (*From: Li et al., Lancet, 2020*).

severe Covid-19 patients, employing convalescent plasma plus standard treatment with respect to standard treatment alone, showed no statistically significant improvement in the treated group, considering combined clinical conditions (figure 7)[26]. Nevertheless, patients treated with convalescent plasma resulted negative for viral RNA after 24, 48 and 72 hours. Moreover, this study has many limitations, including late randomization (30 days after the onset of symptoms), the presence of concomitant, not protocolized, standard therapy, and the fact it was open-label. Therefore, clinical outcomes could be dependent on physician's decisions during the management of patients[26]. For these reasons, despite these discouraging results, convalescent plasma therapy deserves further rigorous investigations. A recent report discussed the presence of low neutralizing antibody titer in convalescent plasma from 149 infected individuals. However, some clones of memory B lymphocytes have been observed to produced recurrent anti-S-RBD antibodies with potent neutralizing activity, indicating vaccines inducing this particular type of antibodies as the most promising[27].

Stem cell-based therapy

The interest for a cell therapy approach in treating Covid-19 is demonstrated by the number of clinical trials currently ongoing. A variety of stem cells are employed, including bone marrow mesenchymal stem cells (BM-MSCs), adipose-derived MSCs (Ad-MSCs), dental pulp and cord blood stem

Figure 8

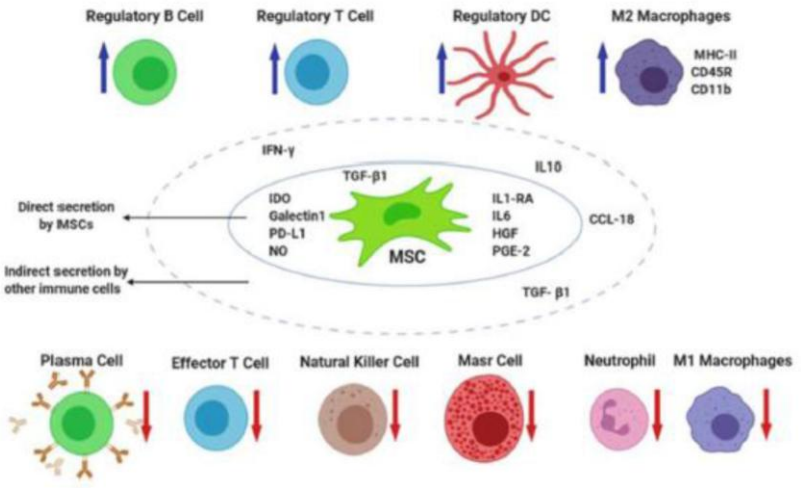

Figure 8. Mesenchymal stem cells and their immunomodulatory activity on immune cells. Abbreviations: MSCs= Mesenchymal Stem Cells; IL=Interleukin; IL-R= IL-Receptor; TGF–β1=transforming growth factor-β1; PD-L1= Programmed Death-Ligand 1; IDO=Indole amine 2,3-Diossigenase; NO=Nitric Oxide; CCL-18=Chemokine 18; HGF= Hepatocyte Growth Factor;PGE-2=Prostaglandin 2; MHC= Major Histocompatibility Complex. (*From*: *Golchin et al., Stem Cell Rev, 2020*).

cells and cardiospheres derived cells (CDCs) from the human heart tissue. MSCs are particularly relevant for human stem cell therapy as they are easily accessible, expand rapidly in culture, are poorly immunogenic and, most importantly, are safe[28,29]. MSCs immunomodulatory activity is at the basis of the therapeutic protocols used to treat Covid-19[30]. In fact, MSCs produce a number of cytokines and may regulate immune cells number and activity (figure 8)[30]. Furthermore, they are multipotent and easily differentiate into tissue-specific cells repairing damaged tissues. Intriguingly, one of the problem related to MSCs infusion, that is lung entrapment, turns favorable in Covid-19, as MSCs may improve pulmonary conditions, repair alveolar epithelial cells and recover Covid-19 pneumonia. Results for MSCs-based treatment and Covid-19 suffer from the low number of patients treated. However, a number of studies reported an improvement in the clinical conditions of affected individuals after MSCs transplantation[31,32]. A study related to the compassionate use of CDCs reported the safety and efficacy of this treatment, although only 6 patients were treated and were undergoing other pharmacological treatments[32].

Fighting the virus: vaccines against CoV-2

Figure 9a shows the current strategies to obtain vaccines. 130 clinical trials are currently ongoing testing the safety and efficacy of vaccines against CoV-2 infection, based on different methods/platforms (figure 9a)[33].

- <u>Vaccine platforms</u>

a. RNA and DNA vaccines are based on the mRNA expression of the antigene "in vivo" after the injection of nanoparticles-containing the mRNA (for RNA vaccines) or plasmids (for DNA vaccines). These vaccines stimulate humoral and T-cell specific immune responses.
b. Vaccines based on recombinant proteins have the advantage to avoid virus manipulation. They require adjuvants to generate robust memory immune humoral responses.
c. Adenoviral-based vaccines (Ad-vaccines) exploit the ability of genetically modified adenoviruses *(that is, the adenovirus is deprived of all its dangerous genes)* to elicit an innate immune response together with a specific response against the encoded antigen carried by the vector. However, adenoviruses circulate with high frequency within most populations, creating a pre-existing immunogenic environment, reducing Ad-vaccines efficacy.

Figure 9

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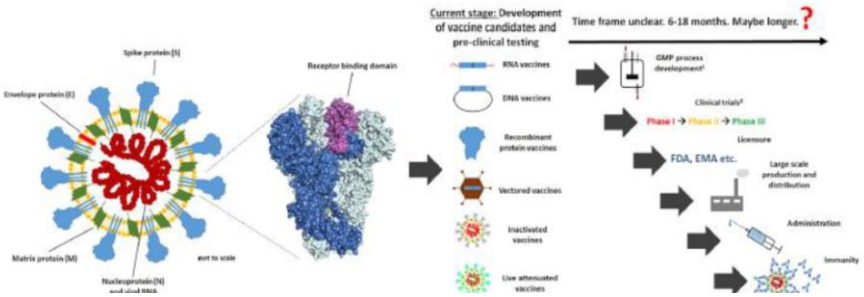

b

Platform	Target	Existing, Licensed Human Vaccines Using the Same Platform	Advantages	Disadvantages
RNA vaccines	Spike	No	No infectious virus needs to be handled. Vaccines are typically Immunogeni. Rapid production possible.	Safety issues with reactogenicity have been reported.
DNA vaccines	Spike	No	No infectious virus needs to be handled. Easy scale up, low production costs, high heat stability. Tested in humans for SARS-CoV-1, rapid production possible.	Vaccine needs specific delivery devices to reach good immunogenicity.
Recombinant protein vaccines	Spike	Yes for baculovirus (influenza, HPV) and yeast expression (HBV, HPV)	No infectious virus needs to be handled, adjuvants can be used to increase immunogenicity.	Global production capacity might be limited. Antigen and/or epitope integrity needs to be confirmed. Yields need to be high enough.
Viral vector-based vaccines	Spike	Yes for VSV (Ervebo), but not for other viral vectored vaccines	No infectious virus needs to be handled. Excellent preclinical and clinical data for many emerging viruses.	Vector immunity might negatively affect vaccine effectiveness.
LAV	Whole Virion	Yes	Straightforward process used for several licensed human vaccines, existing infrastructure can be used.	Creating infectious clones for attenuated coronavirus vaccine seeds takes time because of large genome size. Safety testing will need to be extensive.
Inactivated vaccines	Whole Virion	Yes	Straightforward process used for several licensed human vaccines, existing infrastructure can be used, has been tested in humans for SARS-CoV-1, adjuvants can be used to increase immunogenicity.	Large amounts of infectious virus need to be handled. Antigen and/or epitope integrity needs to be confirmed.

Figure 9. a) Strategies for CoV-2 vaccines production. b) The table shows the platform used, including advantages and disadvantages. Abbreviations: GMP=good manufacturing practice; FDA=Food and Drug Administration; EMA= European Medicine Agency. (*From: Amanat & Krammer, Cell, 2020*).

d. Live attenuated vaccines (LAV) and inactivated vaccines are produced after many passages in culture (LAV), which render the virus incompetent to replication, or by exposure to chemical agents or heat (inactivated vaccines). The first are very immunogenic, stimulating a response highly similar to the living virus, while the second are less immunogenic and require multiple injections. It is evident that these types of vaccines, which also require special safety measures when prepared, are not easily translated into the human clinical practice for highly pathogenic viruses.

- Developing CoV-2 vaccines

  Below, a list of top runners vaccines undergoing clinical trials is shown.

a. BNT162 (BioNTech, Pfizer, and Fosun Pharma): potential first-in-class mRNA vaccine designed to induce immunity and prevent COVID-19 infection.

b. Recombinant Novel Coronavirus Disease Vaccine incorporating the Adenovirus Type 5 Vector (Ad5-nCoV) (CanSino Biologicals). Very recently, the result of an open-labeled, non-randomized, first-in-human clinical trial (ClinicalTrials.gov Identifier: NCT04313127) by using and Ad-based vaccine have been reported. All the 108 volunteer selected showed no severe adverse events, which were mild/moderate. Importantly, humoral and cell-mediated immune responses, peaking at 28 and 14 days post injection respectively, were observed (figure 10)[34].

c. INO-4800 (Inovio Pharmaceuticals and Beijing Advaccine Biotechnology). This is a DNA vaccine adjuvated by CELLECTRA hand-held device for intradermal delivery

Figure 10

a

	Day 14				Day 28			
	Low dose group (n=36)	Middle dose group (n=36)	High dose group (n=36)	p value	Low dose group (n=36)	Middle dose group (n=36)	High dose group (n=36)	p value
ELISA antibodies to the receptor binding domain								
GMT	76·5 (44·3–132·0)	91·2 (55·9–148·7)	132·6 (80·7–218·0)	0·29	615·8 (405·4–935·5)	806·0 (528·2–1229·9)	1445·8 (935·5–2234·5)	0·016
≥4-fold increase	16 (44%)	18 (50%)	22 (61%)	0·35	35 (97%)	34 (94%)	36 (100%)	0·77
Neutralising antibodies to live SARS-CoV-2								
GMT	8·2 (5·8–11·5)	9·6 (6·6–14·1)	12·7 (8·5–19·0)	0·24	14·5 (9·6–21·8)	16·2 (10·4–25·2)	34·0 (22·6–50·1)	0·0082
≥4-fold increase	10 (28%)	11 (31%)	15 (42%)	0·42	18 (50%)	18 (50%)	27 (75%)	0·046

Data are mean (95% CI) or n (%). The p values are the result of comparison across the three dose groups. If the difference was significant across the three groups, the differences between groups were estimated with 95% CIs. SARS-CoV-2= severe acute respiratory syndrome coronavirus 2. GMT=geometric mean titre.

b

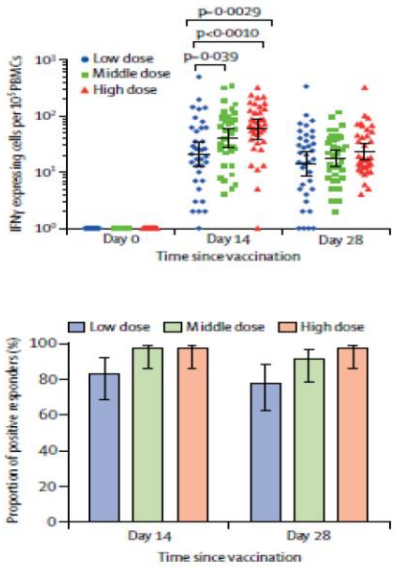

Figure 10. a) Production of antibodies directed against Spike RBD with neutralizing activity after 14 and 28 days, in three different groups of volunteers, receiving low, middle or high of the Ad5-nCoV vaccine. b) Upper panel. Number of cells producing interferon γ after 14 and 28 days from vaccine injection at low, middle or high doses. Lower panel. Percentage of individuals who showed an immune response to the vaccine after 14 and 28 days post-injection. (*From: Zhu et al., Lancet ,2020*).

by electroporation. (ClinicalTrials.gov Identifier: NCT04336410)

d. mRNA-1273 (Moderna). Novel lipid nanoparticle (LNP)-encapsulated mRNA vaccine encoding for a prefusion stabilized form of the S protein. This is one of the most advanced vaccines. Recently, Moderna announced that after the two doses tested (25 μg and 100 μg) all participants seroconverted by day 15 after the first dose. Data on neutralizing antibodies titers are currently available only for first 4 participants belonging to the 25 μg and 100 μg groups. mRNA-1273 stimulated the production of neutralizing antibody titer levels in all eight initial participants, reaching or exceeding neutralizing antibody titers generally seen in convalescent sera. Further, mRNA-1273 was generally safe and well tolerated. (ClinicalTrials.gov Identifier: NCT04283461 and NCT04405076). This vaccine is undergoing phase III human trial.

e. Pittsburgh Coronavirus Vaccine (PittCoVacc, University of Pittsburgh). Microneedle array (MNA)-delivered recombinant protein subunit vaccine targeting CoV-2. The MNA is a fingertip-sized patch of 400 small needles made of sugar and proteins, designed to deliver the S protein pieces into the skin, where the needles dissolve. This vaccine stems for a similar vaccine produced against MERS-CoV S and has been proven to elicit antigen-specific antibody response in 2 weeks in mice[35].

f. ChAdOx1 nCoV-19 (University of Oxford, Advent and partners). Vaccine based on an chimpanzee adenoviral vector to encode CoV-2-S. ChAdOx1 is derived from the chimpanzee adenovirus isolate Y25 and has been tested in many pre-clinical and clinical trials, demonstrating safety with robust humoral and cellular immunogenicity. Currently, a clinical trial (ClinicalTrials.gov Identifier: NCT04324606) is ongoing on 900 volunteers. Vaccine safety and efficacy are the primary objectives of this study. Humoral and cellular immune response will be evaluated as secondary outcomes[36].

g. Covid-eVax (Takis Biotech and Rottapharm). This is an intramuscular electroporation-assisted DNA vaccine, encoding for a portion of CoV-2 S protein. No published results are available. However, Takis announced very promising results in animal models in terms of immunogenicity and safety and the translation to human trial in autumn.

h. Three clinical trials utilizing inactivated vaccines are underway (ClinicalTrials.gov Identifier: NCT04383574; NCT04352608; NCT04412538). Recent results in rhesus macaques, after three immunization at two doses of an inactivated vaccine

(piCoVacc), have reported protection against CoV-2 challenge and production of neutralizing antibodies also in mice and rats[37].

Conclusions

We will likely assist to CoV-2 eradication when an efficient and safe vaccine will be massively produced. However, the improvement of therapies and, above all, the adoption of containment countermeasures have allowed a decrease of infected patients and deaths, at least in some countries. Furthermore, it has to be highlighted again that, without the extraordinary effort and cooperation among researchers, physicians and industries, an acceleration in tenable and effective therapeutic and prophylactic approaches would have not occurred. Although we are still far to solve the problem, we have now more tools to face Covid-19 pandemic and to vanquish CoV-2.

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