Potential immuno-nanomedicine strategies to fight COVID-19 like pulmonary infections

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Abstract

COVID-19, coronavirus disease 2019, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a pandemic. At the time of writing this (October 14, 2020), more than 38.4 million people have become affected, and 1.0 million people have died across the world. The death rate is undoubtedly correlated with the cytokine storm and other pathological pulmonary characteristics, as a result of which the lungs cannot provide sufficient oxygen to the body's vital organs. While diversified drugs have been tested as a first line therapy, the complexity of fatal cases has not been reduced so far, and the world is looking for a treatment to combat the virus. However, to date, and despite such promise, we have received very limited information about the potential of nanomedicine to fight against COVID-19 or as an adjunct therapy in the treatment regimen. Over the past two decades, various therapeutic strategies, including direct-acting antiviral drugs, immunomodulators, a few non-specific drugs (simple to complex), have been explored to treat Acute Respiratory Distress Syndrome (ARDS), Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), influenza, and sometimes the common flu, thus, correlating and developing specific drugs centric to COVID-19 is possible. This review article focuses on the pulmonary pathology caused by SARS-CoV-2 and other viral pathogens, highlighting possible nanomedicine therapeutic strategies that should be further tested immediately.

Keywords: Coronavirus; COVID-19; influenza, pulmonary drug delivery; SARS, SARS-CoV-2; MERS, nanomedicine; nanotherapeutics, pathophysiology

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiological source to cause Coronavirus Disease 2019, or COVID-19. Around 80% of cases of COVID-19 will be mild to moderate, close to 14% have severe disease and around 6% are critical. The possible symptoms include fever, headache, muscle pain, fatigue, and breath shortness followed by the multi-organ failure. Nearly 20% of COVID-19 patients are experiencing Acute Respiratory Distress Syndrome (ARDS) [1,2]. The high mortality rates depend on several clinical factors, such as ARDS, pneumonia, sepsis-induced by the 'cytokine storm' (e.g., an uncontrolled release of cytokines which causes systemic inflammation) followed by lung failure, coagulopathy and thrombotic events, secondary bacterial infections, and vital organ (heart/liver/kidney) injuries followed by failure, and others [1,3-5]. Thus, considering the surge in the new cases and death rate, the World Health Organization announced COVID-19 as a pandemic [6]. The origin of the COVID-19 first arouse in the Wuhan, China on December 16, 2019. Later, its genome was likely correlated with the bat's genome, nearly 96% similar; assuming that it has been transferred from animals, e.g., bats and pangolins (zoonotic disease) [7,8].

SARS-CoV-2 infects both humans and animals, and it has been globally infecting humans with more than 38.4 million cases and more than 1,091,605 deaths (as per October 14, 2020), which is far greater than the SARS and Middle East Respiratory Syndrome (MERS). The infection is spreading through human-human and animal-human transmission via oral-oral and oral-faecal route [9]. Recently, the Advisory Committee on Dangerous Pathogens (ACDP) in the UK categorized the SARS-CoV-2 as a hazard group 3 organism.

Like other SARS-CoV, SARS-CoV-2 also uses angiotensin 1-converting enzyme 2 (ACE2; a close homologue of ACE) for entry into the cells [10,11], in addition to ACE2, it also uses the other cellular protease including transmembrane protease serine 2 (TMPRSS2) [12,13], which is useful in priming of the process of SARS-CoV-2. Recently, one study concluded that SARS-CoV preferentially infects well-differentiated ciliated epithelial cells expressing ACE2. Since ACE2 is also the receptor for the coronavirus NL63, their findings are relevant to the biology of infection with this more common human pathogen [14]. Among other tissues, high expression of ACE2 is found in the lungs and the small intestine [15]. For the SARS-CoV infection, expression of ACE2 in the airway epithelia appears to be both necessary and essential.

The airway epithelial expression ACE2 is complex and correlated with cell differentiation, a finding that may underlie susceptibility to infection [14]; thus, a probable source for the viral loads. Besides, the recent single-cell transcriptome data on *ACE2* and *TMPRSS2* revealed that nasal epithelial cells (goblet/secretory cells and ciliated cells) exhibit high *ACE2* gene expression when compared to other epithelial cells [16]. Equally important, alveolar type II cells exhibit a high expression of *ACE2*. Besides, there is a concern for other associated receptors that act synergistically with infections, i.e., viral scavenging (DMBT1) receptors [17]. On the other hand, high expression of furin, which exposes the binding and fusion domains of SARS-CoV-2, has also been observed in oral epithelial cells [18]. Interestingly, considering the human-human transmission through sneezing and coughing, it is clear that SARS-CoV-2 infects epithelial cells in the upper airway epithelium [16], later the alveoli (type II cells) [19], and oral mucosa [20].

Despite the recent developments in clinical modalities for multiple pulmonary infections, there is an unmet medical need for developing more effective targeted nano-delivery systems. This site-specific local delivery of nano-engineered therapeutic agents with controlled release and stabilization of the therapeutic agent play a crucial role in the management of COVID-19 like pulmonary infections. The foremost challenges with traditional pulmonary drug delivery include lack of penetrability, non-targeted systemic delivery, and interaction with multiple targets at a single therapeutic site. Any advancements in pulmonary nano-drug delivery systems aim to provide maximum therapeutic efficacy with minimal off-targeted side effects, ease of functionalization (chemically, biologically), personalized therapy which together has concomitant benefits. In addition to the potential therapeutic abilities, nanotechnology plays an essential role in bringing cost-effective, quick nano-diagnostics tools to detect pulmonary infection associated biomarkers.

With current approaches to avoid infection and the realization that transmission is the only possible intervention that can be implemented along with complementary medicine and support care, no feasible COVID-19 regimen is available. However, to counter the ongoing outbreaks of COVID-19, a vast number of medications (previously licensed and new drug candidates) targeting various stages of the virus cycle and host immune cells have been considered [21]. In this current review, we discuss the inflammatory response (cytokine storm) in SARS-CoV2 infection, particularly in the lungs. In addition, we also highlight the currently repurposing of drugs and immunotherapeutic targets against COVID-19. Finally, we have placed

a perspective towards nanomedicine by considering the previous investigations ensued in the field of COVID-like infectious diseases.

Inflammatory responses of COVID-19 and lung pathology

The majority of the COVID-19 symptoms and pathological features, at least to some degree, have also been observed in SARS and MERS infections [2,22-24], which also come under the category of zoonotic diseases [25].

A macroscopic examination of COVID-19 post-mortem suggests pleurisy, pericarditis, lung contraction, and pulmonary oedema. [22]. There are also reports suggesting increased lung weight. Computed tomography (CT) scans are considered as the best diagnostic method for COVID-19 infections [26]. The CT scan report of COVID-19 patients showed bilateral lung opacities/infiltrates and hypoxemia with peripheral involvement of lower lobes [22]. Gross pathological observations clearly showed diffuse alveolar damage specifically air sac/alveolar tissue becoming more thickened and scarred permanently due to extensive fibrinogen deposition and associated fibrotic changes (elevated of D-dimer) in the extracellular matrix leading to shortening of breath, pulmonary hypertension and heart failure. Also, numerous pulmonary micro/macrothrombi have been observed, which are repressed by the use of heparin [27,28] and tissue plasminogen activator [29]. These complex reasons cause the lungs to lose their mechanical-stretchable properties, followed by interstitial fibrosis, bronchoconstriction, and other symptoms [2].

Dysfunction of the lungs can be characterized by the shortness of breath, which might be due to several reasons, including lungs occupied with a high amount of interstitial fluid, high content accumulation of collagen, not being able to produce enough surfactant, etc. These typical properties affect the lung tissue elasticity and affects gas transport (pulmonary circulation and diffusion of gas) functions. Lung histology showed the existence of damaged epithelial and endothelial cells, accumulation of exudates, red-blood cells, granulocytes (neutrophils), innate immune cells (monocytes, macrophages, dendritic cells), and lymphocytes (T helper and cytotoxic T cells) in the alveolar spaces that lead to a reduction in gas exchange function. Due to the inflammatory conditions, the rate of diffusion of gas across the alveoli alters, which leads to a hypoventilation condition in the lungs. During a poor gas exchange state, cells experience a high level of stress, which leads to apoptosis and/or necrosis. The role of neutrophils, such as the release of neutrophil elastases, chemokine receptors (CXCR2), and others, on lung epithelial cell

apoptosis, has been documented in the literature [30]. In addition to neutrophil chemokines, type II epithelial cells also express several chemokines in response to the infection [31].

In general, during the infections, the lung epithelium protects the host by providing coordinated innate and adaptive immune responses [32] (**Box 1**). The lung epithelium maintains the dry airspace by regulating the movement of proteins and fluids in and out of the lung. However, in acute infection conditions, like COVID-19, the activation of innate immune cells cross their threshold limit, which results in massive pro-inflammatory cytokine (IL-1β, IL-6, and tumor necrosis factor [TNF]-α) production followed by propagation as severe systemic inflammation. Alveolar macrophages and monocyte-derived macrophages are the primary source for the pro-inflammatory cytokines, especially in SARS, MERS, and ARDS. In addition to alveolar macrophages, type II epithelial cells also produce large amounts of pro-inflammatory cytokines [33,34]. The recurrent inflammatory cascades in the lungs further damage epithelial and endothelial membranes followed by air sac tissue matrix damage. In addition, accumulation of cytokines, chemokines, and reactive oxygen species-rich fluid damage the complete alveoli structure and function, resulting in a low level of oxygen supply to the blood. In this condition, intensive care unit (ICU) patients are supported by artificial air ventilators. Although the use of ventilators is the only option for severely affected COVID-19 patients, it has long been known that the continuous use of ventilation induces lung injury via alveoli cell apoptosis, i.e., ventilator-induced/associated lung injury [35]. Therefore, it is essential to use possible adjunct therapies to overcome treatment-induced injuries.

Cytokine storm in COVID-19

SARS-CoV-2 interacts and induces alveoli tight epithelial cell barrier damage followed by lung injury, which is considered as the primary injury. In addition, infection triggers the further damage of the tight junctions, production of reactive oxygen species and activates innate immune cells (e.g., macrophages) which worsen the condition. This type of immune cell activation induced damage is termed as the secondary injury, i.e., the inflammatory injury. Regardless of the direct primary injury, secondary injury is widely implicated in many other pathogenic infections, including but not limited to influenza, SARS, MERS, and others [24].

Upon infection (including SARS and MERS), the internalized virus uses host intracellular machinery to propagate while destroying host cells. In result, host cells deliver

167 danger signals, i.e., IL-6 and TNF-α. The danger signals recruit the innate immune cells to attack
168 the virus. However, due to the presence of unseen viral signals, the innate immune system
169 delivers copious amounts of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, interferon [IFN]-
170 γ, and others), i.e., called the cytokine storm (**Fig. 2**), which induces non-selective immunogenic
171 cell death followed by organ failure. The above process has been recurrently observed in the
172 lungs of SARS-CoV-2-infected patients, especially patients in the ICU [36]. Hyper activated
173 lymphocytes are the additional culprits in causing the cytokine storm. Patients with severely ill
174 COVID-19 infection show the existence of hyperactivated $CD4^+$ and $CD8^+$ $CD38^+HLA\text{-}DR^+$ T-
175 cells. In vitro stimulation of COVID-19 patients' peripheral blood mononuclear cells with a pool
176 of peptides (resembling SARS-CoV-2 proteome) revealed the existence of SARS-CoV-2-
177 specific $CD4^+$ and $CD8^+$ responses. Upon stimulation with peptides, $CD4^+$ and $CD8^+$ showed
178 activation (CD69 and CD137) and memory ($CD45RA^-$ and $CCR7^+$) responsive marker
179 expression with predominantly Th1 biased cytokine secretion [37].
180 Due to the inflammatory storm that occurs in COVID-19 patients, the development of
181 ARDS results. The initiation of ARDS starts with the recruitment of immature alveolar
182 macrophages at the airspaces in the alveoli; under the influence of growth factors (e.g.,
183 granulocyte-macrophage colony-stimulating factor (GM-CSF), cytokines, etc.) they differentiate
184 into mature alveoli macrophages [38]. Upon maturation, they secrete chemotactic factors, such
185 as IL-8 and other chemokines to attract other inflammatory immune cells [39]. However, each
186 immune cell, e.g., neutrophils, macrophages, megakaryocytes [40], and others, have their own
187 distinct role in infectious conditions. In addition, infection signals the recruitment of neutrophils
188 to the edematous space, across the endothelium, interstitium, and epithelium, whereby they
189 activate and perform regulatory functions, such as phagocytosis of pathogens. Comparably, an
190 increased neutrophil count (i.e., neutrophilia) has been observed in the severely ill COVID-19
191 patients and is accompanied by the severity of the disease [36].
192 Nevertheless, due to the inflammatory microenvironment, the hyperactivation of
193 neutrophils leads to secondary damage to the alveoli. Mediators of neutrophil-induced lung
194 injury include proteases (neutrophil elastase), oxidants (reactive oxygen and nitrogen species),
195 antimicrobial peptides (defensins), and Neutrophil Extracellular Traps (NET). Indeed, the same
196 progression was observed in other lung injury diseases (ARDS, MERS, SARS, and influenza)
197 [30]. Therefore, strategies, which target hyperactivation of neutrophils or their inflammatory

198 mediators, such as CXCR2 (neutrophil chemokine receptor) antagonists, inhibition of neutrophil
199 proteases, inhibitors of peptidylarginine deiminase IV, inhibitors of DNase-1, and others, would
200 be beneficial for COVID-19 (reviewed elsewhere [30]).

201 In addition to neutrophils, macrophages have also been implicated in lung injury during
202 pathogenic infections, e.g., influenza. Blocking monocyte-derived macrophage recruitment to the
203 inflammatory site has attenuated secondary injury induced by macrophages [39,41]. More
204 importantly, pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and others, have a
205 significant role in both primary and secondary injuries. Interestingly, monocyte-derived
206 macrophage releasing of TRAIL (TNF-related apoptosis-inducing ligand) is one of the causative
207 factors for epithelial cell apoptosis; infected epithelial cells express surface death receptors. It
208 has been reported that SARS-CoV-2-induced lung injury consequently degenerates the spleen
209 and lymph nodes. Among the clinical abnormalities, lymphopenia, or lymphocytopenia, is a
210 primary significant condition observed in COVID-19 patients. Recent tissue histopathological
211 studies of the spleen and lymph nodes from COVID-19 patients revealed that macrophages,
212 especially ACE2 expressing $CD169^+$ tissue-resident macrophages, are also directly infected by
213 SARS-CoV-2. Probably, viruses envisage the capability of macrophages that deals with death
214 [42]. However, unlike MERS-CoV [43], these viruses do not directly infect T and B cells [44].
215 Upon infection, these macrophages produce pro-inflammatory cytokines (IL-6, TNF-α) and
216 activate lymphocytes. Subsequently, IL-6 has been used, along with other markers, as a potential
217 inflammatory biomarker in COVID-19 to assess the severity and to provide a prognosis [45].
218 Historically, fatal cases of ARDS, influenza, MERS, and SARS are associated with IL-6 and IL-
219 1β followed by macrophage-activation, and a similar trend has also been observed in COVID-19
220 [46]. Consequently, the activated lymphocytes exhaust [47] and express a high content of Fas
221 receptor, which mediates the Fas/FasL signaling and induces activation-induced cell death,
222 causing lymphocytopenia [44].

223 In addition to neutrophil and macrophage induced secondary injuries, recruited dendritic
224 cells, and antigen presentation to the cytotoxic $CD8^+$ T cells also accelerate the inflammatory
225 process. Dendritic cells recruited to the inflammatory site access the virus and process the
226 antigen presentation to $CD8^+$ T cells. Viral antigen-experienced $CD8^+$ T cells clear the viral load
227 by killing the infected epithelial cells through the release of perforin/granzyme-mediated or the
228 Fas ligand process.

In addition to the mentioned irregularities and inflammatory injuries, another factor that causes lung tissue injury is the hypoxia condition. Due to the edematous condition of the alveoli, epithelial cells are more vulnerable to apoptosis, which allows for impaired tissue integrity and their functions (e.g., gas exchange). Lung epithelial cell apoptosis is mediated by several pathways, including a Fas ligand pathway, toll-like receptor (TLR) pathways, intracellular stress-induced, and others [48].

Current repurposed drugs and immunotherapeutic targets available against COVID-19

Unfortunately, at present, there are no newly-developed approved drugs and vaccines for the treatment of COVID-19. However, knowing about the uncertainty of the efficacy of new drugs/vaccines and the lengthy discovery and developmental timeline, special efforts have been made to repurpose existing drugs including antiviral drugs, immunomodulators, and other adjunct therapies (**Fig. 2**) [49,50]. The proposed drugs and their existing targets may or may not work in a similar fashion with SARS-CoV-2. Nevertheless, detailed studies need to be conducted to confirm their possible mechanism of action.

Virus attachment inhibitors

In addition to structural homology, SARS-CoV and SARS-CoV-2 spike (S) proteins exhibit high similarity sequences (nearly 76.5%) [51,52], which preferentially bind to human ACE2 receptors. Earlier studies on SARS-CoVs have shown that overexpression of ACE2 and the injection of spike proteins exacerbated disease severity and lung injury, respectively. As mentioned-above, SARS-CoV-2 also uses ACE2 for its entry into cells [53]. Henceforward, strategies that hinder the virus-ACE2 interaction have been explored (**Table 1**), such as serine protease (TMPRSS2) inhibitors (camostat mesylate) [12], the use of monoclonal antibodies against ACE2, the use of recombinant ACE2 protein (APN01, GSK2586881), and drugs that act on the renin-angiotensin system [52]. Along a similar line, the S protein has gained much attention as a target to develop monoclonal antibodies [54].

Virus entry/fusion inhibitors

Although the spike protein in SARS-CoV and SARS-CoV-2 appears to be similar in homology, SARS-CoV-2 binds to different amino acids and shows high-affinity binding towards

human ACE2 receptors; glutamine residue (at 394 position) in the SARS-CoV-2 receptor-binding domain (RBD) interacts with the lysine (at 31 position) on the human ACE2 receptor [11,52,55]. On the other hand, SARS-CoV entry is facilitated by host cell proteases, such as elastase, cathepsin B, L and/or TMPRSS2. In addition to the proteases as mentioned above, SARS-CoV-2 exhibits a peculiar furin-like cleavage site, which is processed by the furin at the cell surface and trans-Golgi network (TGN) for entry and egress, respectively [18,56,57]. By using molecular modelling, diversified furin inhibitors have been proposed [57].

Antiviral therapeutics for COVID-19

A majority, but not all antiviral drugs come under the class of nucleoside analogues, which mimic the features of nucleic acid and disrupt the virus replication. A list of antiviral drugs tabulated here is explored in for many infections, even sometimes in human studies, such as HIV, influenza, hepatitis B (HBV), hepatitis C (HCV), and others. Several bioinformatic and/or *in vitro* studies on cells have been confirmed and proposed to use known antiviral drugs as anti-SARS-CoV-2 inhibitors (**Table 1**) [58-60].

Immunomodulation for COVID-19

Despite several reasons for mortality, mortality induced by the immune-compromised conditions is confessed, especially in the older age population (~60 years). Various immunoregulatory functions are altered in COVID-19 like pathologies and in addition, the cytokine storm incurs severe damage to the host tissue. Thus, modulation of COVID-19-associated immune responses is mandatory.

Immunostimulatory drugs

A murine model of infection confirmed that CoVs modulate host immune responses by delaying type I interferon (IFN-I) to avoid the detection process [61], e.g., after infection, non-structural proteins (nsp; particularly nsp15) play a significant role in the inhibition of IFN-I responses, which is essential for the innate immune system to clear the virus-infected cells [62,63]. Interestingly, the highest similarity (95%) of this protein was observed in SARS-CoV-2 as well with that of SARS-CoV [64], which raises the possibility to use novel approaches including TLR agonists, IFN agonists, and others (**Table 1**).

Immunotherapies that target inflammatory cytokines or hyperactivated immune cells

Antagonists to inflammatory mediators are widely used in autoimmune diseases in which hyperactivated immune cells damage different organs in the body. From the past few decades, the autoimmune disease associated therapeutic interventions are accompanied by the inhibition of cytokines, including TNF, IL-17A, IL-6, IL-23, and others or blockade their receptors like IL-6R [65,66]. Through this strategic targeting, the infiltration of monocytes, macrophages, and lymphocytes is regulated. Similarly, as explained earlier, COVID-19 infection also has shown hyperimmune activation; thus, the aforementioned strategies are useful as an adjunct therapy [67,68]. Several clinical studies identified that pro-inflammatory cytokines are the causative factors for the severe lung damage and coagulopathy observed in COVID-19 patients, particularly, predominant levels of IL-6 and TNF (**Table 1**) [4]. IL-6 and TNF-α antagonists have been used in the treatment of cytokine release syndrome. Thus, many clinical studies (**Table 1**) have been initiated with the same hypothesis.

Many other possible immunotherapeutic strategies (**Table 1**), such as passive immunotherapy to neutralize SARS-CoV-2, regulatory T cell-targeted therapies, cellular immunotherapies, immunomodulatory therapies and others, for COVID-19 have been recently reviewed [67,68].

Other adjunct therapies for COVID-19

Renin-angiotensin system inhibitors

As explained above, ACE2 is expressed in many tissues. ACE2 plays a vital role in the renin-angiotensin system (RAS), especially in controlling electrolyte balance and blood pressure. Further ACE2 also extends its role in regulating ARDS and virus-induced lung infections [69]. It is believed that SARS-CoV-2 infection reduces the expression of ACE2, thereby disrupts the RAS. A study conducted on the use of ACE inhibitors and angiotensin-II type 1 receptor antagonists either alone or in combination with other antihypertensive drugs against COVID-19 have shown beneficial effects, such as a reduction in IL-6, and an increase in the CD3 and CD8 population, and also a reduction in the overall viral load. On top of that, the said results have not been observed in other anti-hypertensive drugs treatments [70]. However, a detailed mechanism of the efficacy has yet to be investigated. Considering the same mechanism, a few clinical trials are ongoing (NCT04353596, NCT04330300, NCT04367883, NCT04345406).

Apart from the therapeutics/drugs mentioned in the table, other diversified drugs including antimalarial drugs, antibiotics, antioxidants (α-Lipoic acid) [71], angiotensin-II type 1 receptor antagonists, vasodilators, and anti-coagulants are being explored in clinical trials (reviewed elsewhere [72]).

Nanomedicine

Despite the wide availability of therapeutics, the use of various nanomedicine strategies has been successful in treating many ailments; precisely, however, its use in pulmonary drug/therapeutic targeting as an adjunct therapy awaits. Nanoengineering with potential drugs opens up the possibilities for improved treatment strategies in lung infections. Pulmonary nano-drug delivery systems offer unique physicochemical properties including mucosal penetrability, ease of ligand functionalization, enhanced permeation due to small size, increased local concentrations of drugs and high adjuvant properties for vaccine applications, which make them ideal drug delivery systems for the treatment of COVID-19-like pulmonary infections (**Fig. 3**).

Do nanomedicine interventions play a vital role in the management of COVID-19-like pulmonary infections?

Regardless of the latest advances in therapeutic modalities for various pulmonary infections, there is still an unmet medical need to bring advanced nano-targeted therapeutics to the clinic. ARDS is one of the significant causes of morbidity and mortality across the world, characterized by acute, diffuse and inflammatory lung injury, which ultimately leads to refractory hypoxemia [73,74]. Patients with ARDS combat multiple organ failure as they do not tolerate off-target side effects of various drugs. Besides, ARDS-associated infections are allied with heterogeneous pathophysiological lung environments [75,76]. The main drawbacks of conventional pulmonary drug delivery are the lack of alveoli mucosa penetrability, lack of effectiveness of non-targeted delivery by a systemic route and association of multiple targets at a single therapeutic site. The past decade's rise in nano-research is now transforming into considerable commercialization attempts around the globe, and an increasing number of nanomedical therapies are now approved by the food and drug administration (FDA) and other agencies [77], from the first approved nano-pharmaceutical (Doxil® liposomal form of doxorubicin) in 1995 [78]. Nanoengineering with potential drugs opens up possibilities for

353 improved treatment strategies in lung infections. Besides, the unique and/or abnormal signaling
354 features of the pulmonary endothelium, including vasodilatory molecules (NO, prostacyclins)
355 and enhanced expression of leukocyte-binding molecules (like ICAM and VCAM) [79] may play
356 a potential role in nanocarrier binding for reducing ARDS-associated pulmonary embolisms (PE)
357 and pulmonary arterial hypertension (PAH) diseases.

358 Nanoparticles have shown significant potential advantages for the delivery of antiviral
359 molecules [80,81]. In addition, nanoparticles display direct antiviral activity. Up to date, various
360 forms of nanoparticle systems have been reported to exhibit antiviral activity including silver
361 nanoparticles, functional gold nanoparticles, and quantum dots [82]. For instance, the widely
362 used strategy by most of the 'antiviral' nanoparticles include blocking the viral attachment or
363 viral entry into the host cells. Unfortunately, at the latter stage, the 'antiviral' nanoparticles have
364 no suppressive effect on the progeny of the virus in their late phase of replication. In addition,
365 despite challenges [83,84], numerus studies have reported on nanoparticle based drug therapies
366 against respiratory tract infections, e.g. selenium-, sliver-, PEG-PLGA-nanoparticles against the
367 influenza (H1N1) virus. These nanoparticle based systems have shown an increased therapeutic
368 efficacy and reduced associated toxicity compared to free drugs (reviewed in [85]). Interestingly,
369 among these features, their mucoadhesive property presents a significant priority in designing
370 nanoparticle-based therapies against respiratory infections; this could delineate the capacity of
371 targeted delivery to the lungs. To achieve this high mucoadhesive property, several modifications
372 of nanomaterials via various functionalization mechanisms have been developed (**Fig. 3**) [81].

373 Development of the disease-responsive nano delivery system originated by considering
374 their unique interactions, such as hydrogen bonding, hydrophobicity, ionic bonding, and others.
375 Also, selective functionalization, including thiols, catechols, acrylates, and others, have proven
376 their increased covalent interaction with mucin glycoproteins. Furthermore, polymers, such as
377 cellulose, chitosan, pectin, alginate, and others have also bound in a noncovalent manner [84].
378 Among these polymers, chitosan nanoparticles have shown encouraging results for targeted drug
379 delivery. Bioavanta-Bosti, a pioneer company in chitosan nanoparticle science, has begun a
380 proof-of-concept study on a chitosan-based aerosol system namely “Novochizol aerosol". This
381 system is used for the delivery of drugs like losartan, valsartan, telmisartan, and digoxin, which
382 may have a potential role in the battle against the coronaviruses [86] Chitosan can be seen as a
383 major player since it has unique mucoadhesive properties due to the interaction between

positively charged chitosan and negatively charged mucus [87]. This is an effective way to improve the retention time of the drugs in the lungs, and hence might have enhanced clinical efficacy through prolonged lung retention.

Rationale applications of nanomedicine against Coronaviruses

One of the possible strategies to fight against SARS-CoV-2 could implicate the prevention of viral entry into the host cell. It is well known that blockage of viral surface proteins can lead to virus inactivation, so that targeted nanoparticles specific to virus-expressed proteins, could minimize viral internalization. In our latter portion, we have unfolded the role of metallic and metallic-based nanoparticles in blocking viral proteins and, thus, preventing possible viral entry. Another way to deliver antivirals (such as zidovudine, acyclovir, dapivirine, and efavirenz) is to enhance drug bioavailability by using organic nanoparticles (such as albumin and gelatin) and virus-specific targeting proteins (reviewed elsewhere [85]). This approach would provide a dual benefit as it can lead to the successful delivery of drugs along with the targeted antiviral activity.

Nanomedicine in immunotherapy, vaccines and vaccine adjuvants

Many flu (influenza) vaccines, such as Inflexal® V, Influvac® Plus, and others, were approved for clinical use against influenza infections [85,88]. However, the use of vaccines is the most excellent strategy to combat COVID-19 like diseases, their discovery time-line, stability, and delayed mechanism of action (production of immunological memory against target antigens) making them a second choice of therapies. Nevertheless, therapeutic and prophylactic strategies are under development to address current and potential future coronavirus infections, including effective vaccines. As previously discussed, the essential genomic match between SARS-CoV-2 and other coronaviruses help the pharma industry and academic institutions design appropriate vaccine candidates.

The development of COVID-19 vaccine candidates relyies on several high-tech platforms including attenuated and inactivated viruses, replicating and non-replicating viral vectors, DNA and mRNA, virus-like particles, and recombinant protein-based approaches. Some platforms offer vital advantages, such as viral vectors, which offer strong immune responses, superior protein expression, and prolonged stability. On the other hand, DNA or mRNA offer antigen

manipulation flexibility [89]. In contrast, the recombinant protein-based development approach is more comfortable for scaling up vaccine doses using existing production capabilities. To date, 213 vaccines are in development, and 35 are in clinical testing [90]. The main strategy for most of the vaccine candidates is to induce antibodies against the viral S protein, averting the ACE2-mediated host uptake. In the case of SARS-CoV vaccine development, higher antigen-specific antibody titers and better protection were reported with the S protein subunit vaccines when compared to any other target strategy. SARS/MERS vaccine development research has suggested that S protein subunits and RBD of the S1 subunit as the most preferred target sites [91]. As complete knowledge on the SARS-CoV-2 specific antigens are limited, traditional vaccinology methods in combination with nanotechnology would be beneficial.

Recent data from a team of researchers at UC San Diego highlighted the role of nanotechnology in COVID-19 vaccine development [90]. From a vaccine engineers' point of view, nanomedicine offers them an ideal platform for the delivery of antigens, serving as adjuvant platforms, and mimicking viral structures. As cellular immune responses are essential to combat viral infections, vaccines and vaccine adjuvants, which elicit Th1-biased immune responses, are preferred in COVID-19 like diseases. It is important to note here that in most of the cases humoral immune responses have been observed for most adjuvants and delivery platforms while only viral vectors, immune stimulating complexes (ISCOMs) and Montanide™ have shown cytotoxic T cell responses in the clinic [92]. Recent reports indicate that nanoparticles allow multivalent antigen presentation and stabilization of antigens upon administration. They can also serve as adjuvants for boosting immune responses and as carriers for targeted antigen delivery [93]. Indeed, an mRNA vaccine delivered by a liposomal nanoparticle is amongst the candidates currently in clinical trials against SARS-CoV-2 [94].

Among the different types of vaccines, subunit and DNA/RNA vaccines have gained much attention, due to their fewer side-effects, low cost, and ease of preparation [95]. Often subunit vaccines require vaccine adjuvants and substances/compositions that increase the immunogenicity of the targeted antigens. More information could be found in our recent review [92]. Further, stability and tissue-specific targeting issues are limiting their success [93]. Therefore, the use of nanomedicine has become an attractive and quick strategy to address these limitations, mainly needed for COVID-19 like pandemics. For example, we have used an oleic acid nanoemulsion system to increase the antigen uptake capacity of mucosa (in the nasal

446 epithelial). These featured nanomaterials would be advantageous to use as targeted delivery
447 vehicles for the respiratory tract [96].

448 Few exciting studies have been documented on nanotechnology-based vaccine
449 developments against SARS-CoV-2. Erasmus et al. developed an *Alphavirus*-derived replicon
450 RNA vaccine candidate (repRNA-CoV2S), which is composed of the SARS-CoV-2 spike
451 protein replicons in the squalene-based emulsion of lipid inorganic nanoparticles (LION) [97].
452 Superparamagnetic iron oxide (Fe_3O_4) and cationic lipid 1,2-dioleoyl-3-trimethylammonium
453 propane were used as inorganic and lipid source in the nanoparticles, respectively. Immunization
454 of this vaccine in mice and macaques produced antigen-specific antibody responses, which were
455 comparable to the convalescent response from COVID-19 [97]. The superiority of the vaccine
456 composition is that it elicited robust long-term antibody responses against SARS-CoV-2 upon
457 single-site intramuscular injection. Having squalene, a gold standard oil-based vaccine adjuvant,
458 in the nanoparticle composition, it is anticipated to induce Th1-mediated cellular and robust
459 antibody responses [92]. Besides, taking into consideration that older people are more prone to
460 SARS-CoV-2 infections, the authors have studied the vaccine candidate responses in young and
461 old mice. Vaccine candidates produced SARS-CoV-2 neutralizing antibodies effectively with
462 and without multiple injection schedules. Furthermore, their two-vial approach, which contains a
463 LION in one vial and repRNA in another vial, are easier to scale up than the complexed
464 formulations [97].

465 In addition, carbon-nanomaterials have also been explored recently as novel anti-viral
466 agents owing to their unique physicochemical properties. Garg et al. proposed conceptual
467 insights into a series of bioisosteres derived from triazole functionalized heteroatom co-doped
468 carbon quantum dots (TFH-CQDs) and design of peptide inhibitors to combat human
469 coronavirus either by blocking the viral entry or inhibiting the viral enzymes for replication, such
470 as helicase and 3CLpro [98].

471 NVX-CoV2373 is another COVID-19 nanoparticle vaccine candidate [99], which is
472 composed of trimeric full-length recombinant spike glycoproteins of SARS-CoV-2 and Matrix-
473 M1 adjuvant (the combination of different purified fractions of saponins from the tree *Quillaja*
474 *Saponaria* Molina with cholesterol and phospholipid) [100]. Preclinical studies have shown its
475 potency by eliciting anti-spike IgG antibodies with hACE2 receptor blocking, and virus
476 neutralization capacity. Besides, it induced both T and B cell responses [99]. By considering the

above merits, the vaccine candidate has been taken for clinical evaluation (Phase 1-2; NCT04368988). The safety and immunogenicity studies of this vaccine was conducted on 131 healthy adults [101]. Results revealed that the vaccine is safe and induced optimal immune responses. Preferably, the said adjuvant should enhance a Th1 polarized response. The vaccine benefited from the adjuvant in terms of dose sparing and enhanced immune response [101].

Huan et al. studied the CoVaccine HT™ adjuvant effect against the SARS-CoV-2 spike S1 protein in mice [102]. The CoVaccine HT™ is an oil-in-water emulsion type, which is composed of negatively-charged sucrose fatty acid sulphate ester and squalene (source: plant-derived) [103]. Importantly, this vaccine adjuvant has proven its potency and efficacy in different vaccine formulations, including malaria, ebola, Zika, and others [104-106]. For comparison of adjuvant activity against SARS-CoV-2, alum (a gold standard in the family of adjuvants), and Th2 adjuvant, were used in the study. The CoVaccine HT™ induced high antigen-specific antibody titers, class switching response, cell-mediated immune responses and virus-neutralizing antibody titers, which are more significant than the alum. One of the recent studies by Rao L et al., utilized the nano biotechnological approach to formulate nanodecoys with cellular membrane nanovesicles derived from genetically edited 293T/ACE2 and THP-1 cells. The nanodecoys with biological properties have abundant ACE2 and cytokine receptors, which compete with host cells and significantly inhibited viral replication and infection [107]. Of note, a detailed discussion of nanotechnology-based vaccine development against COVID-19 has been covered in recent reviews [108-112].

Nanomedicine as therapeutic agents

As explained earlier, being the prime receptor of SARS-CoV-2, ACE2 has become a vibrant target for most therapeutic and prophylactic strategies. Recently, Wang et al., have developed a biocompatible, cell membrane derived nano-antagonist particulate system prepared from ACE2-rich cells by a simple, cost-effective membrane extrusion method. These synthesized particles termed as HEK-293T-hACE2 NPs contained 265.1 ng/mg of ACE2 on the surface of the particles and acted as a plug to trap SARS-CoV-2 S1 in a competitive dose-dependent manner. Being highly interactive to ACE2, it inhibited the SARS-CoV-2 pseudovirus entry into renal tubular cells [113]. Thus, it is clear that nanotechnology could support the conception of therapeutic tools for inhibiting viral and host interactions.

In an effort to improve the next generation of nano-engineered delivery mechanisms, research has been focused on incorporating therapeutic benefits in order to provide safe and efficient nano-platforms to combat COVID-19-like and other types of pulmonary infections. The success of nanomedicine as an antiviral therapy has been already proven in COVID-19-like pulmonary infections. One such example is Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which has been identified as an infective virus with high pathogenicity and mortality rate [114]. MERS-CoV can cause severe respiratory illnesses and is recognized as a severe threat to public health. Currently, there has been no vaccine or effective treatment for MERS, and its treatment mainly relies on supportive measured and combination therapy of traditional antiviral drugs, such as interferon and ritonavir [115]. However, neither of them showed good antiviral effects in patients, leading to limited therapeutic applications. One of the possible reasons for an increasing number of MERS-CoV infections was due to spike protein (S protein)-mediated membrane fusion between MERS-CoV and host cells [116-118]. Recently, Ning and his team developed a novel gold nanorod-based peptide inhibitor to fight against MERS. The peptide showed good inhibitory activity against the S protein-mediated membrane fusion, and more importantly the designed gold nanorods enhanced inhibitory viral activity. The peptide-functionalized gold nanorods improved biostability and biocompatibility and had better physical and pharmaceutical profiles than those of the peptide alone, endowing potential clinical applications for the treatment of MERS (see **Table 2** for more information, **Fig. 4**) [119]. Though nanotechnology offers a wide range of diagnostics techniques, SARS-CoV-2 specific diagnostic methods (e.g., biosensors) have yet to be fully developed [120,121].

To deliver antibiotics locally with increased therapeutic efficacy, various nano-engineered approaches, such as liposomal formulations, have been reported for lung delivery [122-124], which are intended to increase permeation and intracellular drug delivery. Such strategies provide insights into future clinical targeting systems with improved therapeutic responses to fight pulmonary infections.

Interestingly, the MERS-CoV spike (S) protein is responsible for receptor binding and entry into cells. It is an immunodominant antigen and induces neutralizing antibodies in the host. Both of these characters make the S protein a perfect candidate for anti-MERS-CoV vaccines. Coleman et al. reported that MERS-CoV S nanoparticle-based vaccination in mice induced a higher neutralizing antibody response to the S protein and protected the mice against a viral

challenge [125]. Such functionalized nanoparticle formulation systems could help to pave the way to develop new platform strategies to combat COVID-19-like respiratory diseases.

In addition to the liposomal strategies, polymeric nanosystems [126,127] also enhance the localization of the drug in the lung regions and boost the therapeutic response. In an attempt to improve immunization approaches to strengthen immune responses, hollow viruses, such as particulate matter constructs (virus-like particles-VLPs), deficient in genetic material could induce a robust immune response or improve the response to lung-related infections and are worth exploring in the immediate future for specific pulmonary infections [128,129].

Semiconductor nanocrystals such as quantum dots (QD) have also offered a wide range of peculiar properties attractive for treating viruses, including size-dependent optical and electronic properties [130]. Due to their distinguished luminescent properties, such as broad excitation spectroscopy, narrow and bright emission spectroscopy, long fluorescence lifetime, and size-dependent emission wavelengths, QDs have indeed shown hopes for virus-cell labelling, detection and image tracking. By using a pig coronavirus porcine epidemic diarrhoea virus (PEDV) as a model, recently Du et al. have demonstrated that Ag_2S nanocrystals (NC) have an excellent viral inhibitory ability with a different mechanism of action from other functional nanoparticles [82]. In addition to the inhibition of viral negative-strands generation and viral budding, Ag_2S NC has also induced IFN-stimulated genes and the expression of several pro-inflammatory cytokines [82]. Moreover, the Ag_2S NC were also shown to have comparable virus inhibitory effects on other RNA viruses, such as porcine reproductive and respiratory syndrome virus (PRRSV). Therefore, Ag_2S NC has been identified to have broad-spectrum antiviral properties against RNA viruses [82]. Recent studies have shown that silver nanoparticles display antiviral activity against influenza A virus, hepatitis B virus, human parainfluenza virus, herpes simplex virus, and human immunodeficiency virus [131], which provide other types of therapeutic opportunities for combating pulmonary-associated infections. Recent reports describe the antiviral activity of silver or gold nanoparticles against DNA or RNA viruses. The possible mechanism of silver and other metal plasmonic nanoparticles is shown in fig. 4. Apart from functionalization and targeting groups present on the nanoparticle surface, the mode of action is also dependent on the shape and size, which could interact with virus particles with a well-defined spatial arrangement. In recent years, scholars have paid more attention to the use of traditional medicine for fighting viruses [21]. The antiviral effects of traditional medicine

might be associated with inhibiting the replication of viruses. Besides, they might improve respiratory virus-mediated lung damage [132].

Over the years, textile industries have been using different inorganic/metal nano-composites (alginate, copper, gold, zinc, magnesium, silver, titanium, and others), which would protect textiles from diversified microbes [133]. Although inorganic reagents have been used as broad-spectrum antimicrobial compounds, the drawback of their instability limits their use, e.g., silver vigorously reacts with Cl^-, HS^-, and SO_4^{2-}, and others lose antimicrobial capacity [133]. To overcome this, a nanotechnology-based strategy, which increases the stability of the silver has gained much attention. Recently, silver nanoparticles prepared with the acrylic-based polymers in polycotton fabrics have shown a significant antimicrobial effect, including SARS-CoV-2 (SARS-CoV-2/human/BRA/SP02cc/2020-MT350282) [134]. The possible mechanism behind the antimicrobial, and in particular antiviral effect is explained by two pathways. First, sliver nanoparticles prevent the viral attachment to the host cells by interacting with virus surface glycoproteins via a sulfur linkage. Second, these nanoparticles interact wit transcription factors, which are required for virus replication. However, the exact mechanism is yet to be established [134].

Du et al. have developed a unique one-step method in which they have used curcumin to prepare uniform and stable cationic carbon dots (CCM-CDs) with antiviral properties [135,136]. Curcumin has already proven its effectiveness against various infections and exhibits antiviral activity by reducing viral RNA expression, protein synthesis, and virus titers. In addition, it was found to have a protective effect on cells against virus-induced apoptosis and cytopathic activity [137]. The authors studied the inhibitory performance of CCM-CDs on viral replication. In their study, they have selected PEDV as a coronavirus model. Most importantly, their findings suggest that the developed CCM-CDs inhibit the proliferation of PEDV with much higher efficiency than non-curcumin modified carbon dots (**Fig. 5**) [136].

Besides, nanotechnology applications have advanced to develop personalized patient management, including preparation of naso-delivery vehicles (for the easy administration of drugs by an intranasal route), insufflations (to deliver the drug directly to lungs), and others. The use of inorganic-polycotton fabrics (explained earlier) has been one of the best strategies for contagious diseases [134]. In order to combat the spread of coronavirus, the use of adequate personal protective equipment (PPE), including masks, gloves, medical aprons and kits, are also

necessary [138]. Antimicrobial activity is imparted by the use of inorganic based nanoparticle coatings, which could be used in various types of fibers or materials, and also fabricating such cloths with anti-viral agents could be an interesting approach to minimize the spread of the virus. Moreover, there is a wide scope to consider the novel nano-strategies to combat emerging microbes, e.g., development of face/mouth masks, which are composed of stable and safety antimicrobial inorganic composites (with antibiotics/antiviral coatings, e.g., silicon nitride) [139,140]. Although these nano-inorganic composites exhibit less allergic responses, detailed toxicity parameters should be sensibly evaluated. As composites are in the nano-range, there is a chance of ingestion and toxicity due to the accumulated composites.

Future nanocarrier research with specific ligand functionalization to target the afore-mentioned molecules are likely to enhance their therapeutic efficacy by avoiding off-targeted drug side effects. The advantages of their large surface-to-volume ratios, the high surface area activity and the size-dependent optical and electronic properties of nanocarriers, which are widely explored in the field of biosensors and biomedicine, and their viricidal activity, have also been systematically investigated. All of these properties make nanoparticles a strong potential weapon to fight against COVID-19-like pulmonary infectious diseases in the near future.

Conclusion and future outlook

The complete genome sequencing of SARS-CoV-2 revealed a close homology with other CoVs, including SARS-CoV and MERS-CoV. In particular, viral enzymes such as 3CL protease, helicase, papain-like protease, and RNA-dependent RNA polymerase and their active sites are highly conserved across other CoVs. However, a better understanding of the SARS-CoV-2 tropism would benefit for therapeutic purposes. In addition, the controversial effects of immune cells on secondary injuries warrant further investigation. Finally, many other endogenous protective factors that help in preventing tissue injury and innate repair functions are all unanswered questions that need to be explored further. The use of immunomodulatory therapies may not clear the virus load but have the potential to reduce the severity of the disease caused by the cytokine storm, details that we emphasized on the 'cytokine storm' should also support future vaccine research on COVID-19.

Until today, a few questions remain unclear including the best suitable therapeutic strategy to fight against COVID-19 and associated lung infections. The past decade, however,

has witnessed the use of several combination strategies during numerous pandemics and other viral infections like HIV and influenza. Therefore, the use of a broad-spectrum antiviral agents in combination is advisable against COVID-19 and nanomedicine is primed to be central for any solutions due to its attractive properties as mentioned above. Currently, the scientific community is globally juggling many drugs to find suitable and/or manageable therapeutic options against COVID-19. Despite initial encouraging preliminary results in short cohorts, a tremendous effort is needed for targeted approaches, however, the time spent now will help with all future pandemics as nanomedicines can be a platform for numerous viruses, requiring simply to be functionalized with different regions that target different viruses. Worthwhile bioinformatic studies have come up with several druggable proteins and repurposing drugs, and rationalization of these drugs for the future use would be prudent [58,59].

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Contributions

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Editing and Reviewing, T.J.W, Y.R, and J.B.

Final Approval, All the authors.

Declaration of interests

None

References

[1] H.L. Ji, R. Zhao, S. Matalon, M.A. Matthay, Physiol. Rev. 100 (2020) 1065-1075, https://doi.org/10.1152/physrev.00013.2020

[2] Z. Xu, L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, et al., Lancet Respir. Med. 8 (2020) 420-422, https://doi.org/10.1016/S2213-2600(20)30076-X

[3] F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, et al., Lancet 395 (2020) 1054-1062, https://doi.org/10.1016/S0140-6736(20)30566-3

[4] B. Zhang, X. Zhou, Y. Qiu, Y. Song, F. Feng, J. Feng, et al., PLoS One 15 (2020) e0235458, https://doi.org/10.1371/journal.pone.0235458

[5] Q. Ruan, K. Yang, W. Wang, L. Jiang, J. Song, Intensive Care Med. 46 (2020) 846-848, https://doi.org/10.1007/s00134-020-05991-x

[6] WHO, http://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic, 2020.

[7] P. Liu, W. Chen, J.P. Chen, Viruses 11 (2019) 979, https://doi.org/10.3390/v11110979

[8] D. Cyranoski, Nature, https://www.nature.com/articles/d41586-020-00364-2, 2020.

[9] J. Hindson, Nat. Rev. Gastroenterol. Hepatol. 17 (2020) 259, https://doi.org/10.1038/s41575-020-0295-7

[10] M. Yuan, N.C. Wu, X. Zhu, C.D. Lee, R.T.Y. So, H. Lv, et al., Science 368 (2020) 630-633, https://doi.org/10.1126/science.abb7269

[11] Y. Wan, J. Shang, R. Graham, R.S. Baric, F. Li, J. Virol. 94 (2020), https://doi.org/10.1128/JVI.00127-20

[12] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, et al., Cell 181 (2020) 271-280 e278, https://doi.org/10.1016/j.cell.2020.02.052

[13] T. Meng, H. Cao, H. Zhang, Z. Kang, D. Xu, H. Gong, et al., bioRxiv (2020) 2020.2002.2008.926006, https://doi.org/10.1101/2020.02.08.926006

[14] H.P. Jia, D.C. Look, L. Shi, M. Hickey, L. Pewe, J. Netland, et al., J. Virol. 79 (2005) 14614-14621, https://doi.org/10.1128/JVI.79.23.14614-14621.2005

[15] I. Hamming, W. Timens, M.L. Bulthuis, A.T. Lely, G. Navis, H. van Goor, J. Pathol. 203 (2004) 631-637, https://doi.org/10.1002/path.1570

[16] W. Sungnak, N. Huang, C. Becavin, M. Berg, R. Queen, M. Litvinukova, et al., Nat. Med. 26 (2020) 681-687, https://doi.org/10.1038/s41591-020-0868-6

[17] G. Han, A. Sinjab, W. Treekitkarnmongkol, P. Brennan, K. Hara, K. Chang, et al., bioRxiv (2020) 2020.2004.2016.045617, https://doi.org/10.1101/2020.04.16.045617

[18] M. Zhong, B.-p. Lin, H.-b. Gao, A.J. Young, X.-h. Wang, C. Liu, et al., bioRxiv (2020) 2020.2004.2018.047951, https://doi.org/10.1101/2020.04.18.047951

[19] Y. Zhao, Z. Zhao, Y. Wang, Y. Zhou, Y. Ma, W. Zuo, Am. J. Respir. Crit. Care Med. (2020) 2020.2001.2026.919985, https://doi.org/10.1164/rccm.202001-0179LE

[20] H. Xu, L. Zhong, J. Deng, J. Peng, H. Dan, X. Zeng, et al., Int. J. Oral Sci. 12 (2020) 8, https://doi.org/10.1038/s41368-020-0074-x

[21] E. Callaway, Nature 580 (2020) 576-577, http://doi.org/10.1038/d41586-020-01221-y

[22] B. Hanley, S.B. Lucas, E. Youd, B. Swift, M. Osborn, J. Clin. Pathol. 73 (2020) 239-242, https://doi.org/10.1136/jclinpath-2020-206522

[23] Y. Yi, P.N.P. Lagniton, S. Ye, E. Li, R.H. Xu, Int. J. Biol. Sci. 16 (2020) 1753-1766, https://doi.org/10.7150/ijbs.45134

[24] L. Lin, L. Lu, W. Cao, T. Li, Emerg. Microbes Infect. 9 (2020) 727-732, https://doi.org/10.1080/22221751.2020.1746199

[25] P.I. Lee, P.R. Hsueh, J. Microbiol. Immunol. Infect. 53 (2020) 365-367, https://doi.org/10.1016/j.jmii.2020.02.001
[26] C. Hani, N.H. Trieu, I. Saab, S. Dangeard, S. Bennani, G. Chassagnon, et al., Diagn. Interv. Imaging. 101 (2020) 263-268, https://doi.org/10.1016/j.diii.2020.03.014
[27] N. Tang, H. Bai, X. Chen, J. Gong, D. Li, Z. Sun, J. Thromb. Haemost. 18 (2020) 1094-1099, https://doi.org/10.1111/jth.14817
[28] E.M. Negri, B. Piloto, L.K. Morinaga, C.V.P. Jardim, S.A.E.-D. Lamy, M.A. Ferreira, et al., medRxiv (2020) 2020.2004.2015.20067017, https://doi.org/10.1101/2020.04.15.20067017
[29] H.D. Poor, C.E. Ventetuolo, T. Tolbert, G. Chun, G. Serrao, A. Zeidman, et al., medRxiv (2020) 2020.2004.2017.20057125, https://doi.org/10.1101/2020.04.17.20057125
[30] T. Narasaraju, B.M. Tang, M. Herrmann, S. Muller, V.T.K. Chow, M. Radic, Front. Pharmacol. 11 (2020), https://doi.org/10.3389/Fphar.2020.00870
[31] P. Puneet, S. Moochhala, M. Bhatia, Am. J. Physiol. Lung Cell Mol. Physiol. 288 (2005) L3-15, https://doi.org/10.1152/ajplung.00405.2003
[32] S. Hippenstiel, B. Opitz, B. Schmeck, N. Suttorp, Respir. Res. 7 (2006) 97, https://doi.org/10.1186/1465-9921-7-97
[33] A.J. Thorley, P.A. Ford, M.A. Giembycz, P. Goldstraw, A. Young, T.D. Tetley, J. Immunol. 178 (2007) 463-473, https://doi.org/10.4049/jimmunol.178.1.463
[34] S. Tian, W. Hu, L. Niu, H. Liu, H. Xu, S.-Y. Xiao, Preprints (2020), https://doi.org/10.20944/preprints202002.0220.v1
[35] S. Dhanireddy, W.A. Altemeier, G. Matute-Bello, D.S. O'Mahony, R.W. Glenny, T.R. Martin, et al., Lab. Invest. 86 (2006) 790-799, https://doi.org/10.1038/labinvest.3700440
[36] J. Liu, S. Li, J. Liu, B. Liang, X. Wang, H. Wang, et al., EBioMedicine 55 (2020) 102763, https://doi.org/10.1016/j.ebiom.2020.102763
[37] D. Weiskopf, K.S. Schmitz, M.P. Raadsen, A. Grifoni, N.M.A. Okba, H. Endeman, et al., Sci. Immunol. 5 (2020) 2020.2004.2011.20062349, https://doi.org/10.1126/sciimmunol.abd2071
[38] R.L. Zemans, J.S. Hagood, Nat. Immunol. 21 (2020) 112-114, https://doi.org/10.1038/s41590-019-0585-9
[39] M.A. Matthay, R.L. Zemans, G.A. Zimmerman, Y.M. Arabi, J.R. Beitler, A. Mercat, et al., Nat. Rev. Dis. Primers 5 (2019) 18, https://doi.org/10.1038/s41572-019-0069-0
[40] G. Li, S.E. Fox, B. Summa, B. Hu, C. Wenk, A. Akmatbekov, et al., bioRxiv (2020) 2020.2004.2011.037473, https://doi.org/10.1101/2020.04.11.037473
[41] K.L. Lin, S. Sweeney, B.D. Kang, E. Ramsburg, M.D. Gunn, J. Immunol. 186 (2011) 508-515, https://doi.org/10.4049/jimmunol.1001002
[42] G. Lemke, Nat. Rev. Immunol. 19 (2019) 539-549, https://doi.org/10.1038/s41577-019-0167-y
[43] H. Chu, J. Zhou, B.H. Wong, C. Li, J.F. Chan, Z.S. Cheng, et al., J. Infect. Dis. 213 (2016) 904-914, https://doi.org/10.1093/infdis/jiv380
[44] y. chen, Z. Feng, B. Diao, R. Wang, G. Wang, C. Wang, et al., medRxiv (2020) 2020.2003.2027.20045427, https://doi.org/10.1101/2020.03.27.20045427
[45] F. Zeng, Y. Huang, Y. Guo, M. Yin, X. Chen, L. Xiao, et al., Int. J. Infect. Dis. 96 (2020) 467-474, https://doi.org/10.1016/j.ijid.2020.05.055
[46] D. McGonagle, K. Sharif, A. O'Regan, C. Bridgewood, Autoimmun. Rev. 19 (2020) 102537, https://doi.org/10.1016/j.autrev.2020.102537
[47] B. Diao, C. Wang, Y. Tan, X. Chen, Y. Liu, L. Ning, et al., Front. Immunol. 11 (2020) 827, https://doi.org/10.3389/fimmu.2020.00827
[48] Y. Koizumi, H. Nagase, T. Nakajima, M. Kawamura, K. Ohta, Allergol. Int. 65 Suppl (2016) S30-37, https://doi.org/10.1016/j.alit.2016.05.006

[49] T. Pillaiyar, S. Meenakshisundaram, M. Manickam, Drug Discov. Today 25 (2020) 668-688, https://doi.org/10.1016/j.drudis.2020.01.015
[50] S. Ekins, M. Mottin, P. Ramos, B.K.P. Sousa, B.J. Neves, D.H. Foil, et al., Drug Discov. Today 25 (2020) 928-941, https://doi.org/10.1016/j.drudis.2020.03.019
[51] X. Xu, P. Chen, J. Wang, J. Feng, H. Zhou, X. Li, et al., Sci. China Life Sci. 63 (2020) 457-460, https://doi.org/10.1007/s11427-020-1637-5
[52] H. Zhang, J.M. Penninger, Y. Li, N. Zhong, A.S. Slutsky, Intensive Care Med. 46 (2020) 586-590, https://doi.org/10.1007/s00134-020-05985-9
[53] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, et al., Nature 579 (2020) 270-273, https://doi.org/10.1038/s41586-020-2012-7
[54] I. Mercurio, V. Tragni, F. Busto, A. De Grassi, C.L. Pierri, Cell. Mol. Life Sci. (2020) 2020.2004.2017.046185, https://doi.org/10.1007/s00018-020-03580-1
[55] K. Wu, G. Peng, M. Wilken, R.J. Geraghty, F. Li, J. Biol. Chem. 287 (2012) 8904-8911, https://doi.org/10.1074/jbc.M111.325803
[56] B. Coutard, C. Valle, X. de Lamballerie, B. Canard, N.G. Seidah, E. Decroly, Antiviral Res. 176 (2020) 104742, https://doi.org/10.1016/j.antiviral.2020.104742
[57] W. Canrong, Y. Yueying, L. Yang, Z. Peng, W. Yali, W. Qiqi, et al., chinaXiv, https://doi.org/10.12074/202002.00062
[58] A.A. Elfiky, Life Sci. 253 (2020) 117592, https://doi.org/10.1016/j.lfs.2020.117592
[59] D.E. Gordon, G.M. Jang, M. Bouhaddou, J. Xu, K. Obernier, M.J. O'Meara, et al., bioRxiv (2020) 2020.2003.2022.002386, https://doi.org/10.1101/2020.03.22.002386
[60] C. Ma, M.D. Sacco, B. Hurst, J.A. Townsend, Y. Hu, T. Szeto, et al., bioRxiv (2020) 2020.2004.2020.051581, https://doi.org/10.1101/2020.04.20.051581
[61] R. Channappanavar, A.R. Fehr, R. Vijay, M. Mack, J. Zhao, D.K. Meyerholz, et al., Cell Host Microbe 19 (2016) 181-193, https://doi.org/10.1016/j.chom.2016.01.007
[62] X. Deng, M. Hackbart, R.C. Mettelman, A. O'Brien, A.M. Mielech, G. Yi, et al., Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E4251-E4260, https://doi.org/10.1073/pnas.1618310114
[63] X. Liu, P. Fang, L. Fang, Y. Hong, X. Zhu, D. Wang, et al., Mol. Immunol. 114 (2019) 100-107, https://doi.org/10.1016/j.molimm.2019.07.003
[64] D. Kim, J.Y. Lee, J.S. Yang, J.W. Kim, V.N. Kim, H. Chang, Cell 181 (2020) 914-921 e910, https://doi.org/10.1016/j.cell.2020.04.011
[65] G. Schett, M. Sticherling, M.F. Neurath, Nat. Rev. Immunol. 20 (2020) 271-272, https://doi.org/10.1038/s41577-020-0312-7
[66] C. Galeotti, J. Bayry, Nat. Rev. Rheumatol. 16 (2020) 413-414, https://doi.org/10.1038/s41584-020-0448-7
[67] S.R. Bonam, S.V. Kaveri, A. Sakuntabhai, L. Gilardin, J. Bayry, Cell Rep. Med. 1 (2020) 100016, https://doi.org/10.1016/j.xcrm.2020.100016
[68] E. Stephen-Victor, M. Das, A. Karnam, B. Pitard, J.F. Gautier, J. Bayry, Eur. Respir. J. (2020) 2002182, https://doi.org/10.1183/13993003.02182-2020
[69] K. Kuba, Y. Imai, J.M. Penninger, Curr. Opin. Pharmacol. 6 (2006) 271-276, https://doi.org/10.1016/j.coph.2006.03.001
[70] J. Meng, G. Xiao, J. Zhang, X. He, M. Ou, J. Bi, et al., Emerg. Microbes Infect. 9 (2020) 757-760, https://doi.org/10.1080/22221751.2020.1746200
[71] M. Zhong, A. Sun, T. Xiao, G. Yao, L. Sang, X. Zheng, et al., medRxiv (2020) 2020.2004.2015.20066266, https://doi.org/10.1101/2020.04.15.20066266
[72] M.P. Lythgoe, P. Middleton, Trends Pharmacol. Sci. 41 (2020) 363-382, https://doi.org/10.1016/j.tips.2020.03.006

[73] E.R. Johnson, M.A. Matthay, J. Aerosol Med. Pulm. Drug Deliv. 23 (2010) 243-252, https://doi.org/10.1089/jamp.2009.0775
[74] G.D. Rubenfeld, E. Caldwell, E. Peabody, J. Weaver, D.P. Martin, M. Neff, et al., N. Engl. J. Med. 353 (2005) 1685-1693, https://doi.org/10.1056/NEJMoa050333
[75] M.A. Matthay, Proc. Am. Thorac. Soc. 5 (2008) 297-299, https://doi.org/10.1513/pats.200708-141DR
[76] E. Fan, D. Brodie, A.S. Slutsky, JAMA 319 (2018) 698-710, https://doi.org/10.1001/jama.2017.21907
[77] C.L. Ventola, P & T : a peer-reviewed journal for formulary management 42 (2017) 742-755,
[78] Y. Barenholz, J. Control. Release 160 (2012) 117-134, https://doi.org/10.1016/j.jconrel.2012.03.020
[79] P. Crosswhite, Z. Sun, Mol. Med. 20 (2014) 191-201, https://doi.org/10.2119/molmed.2013.00165
[80] D. Lembo, M. Donalisio, A. Civra, M. Argenziano, R. Cavalli, Expert Opin. Drug Deliv. 15 (2018) 93-114, https://doi.org/10.1080/17425247.2017.1360863
[81] L. Singh, H.G. Kruger, G.E.M. Maguire, T. Govender, R. Parboosing, Ther. Adv. Infect. Dis. 4 (2017) 105-131, https://doi.org/10.1177/2049936117713593
[82] T. Du, J. Liang, N. Dong, J. Lu, Y. Fu, L. Fang, et al., ACS Appl. Mater. Interfaces 10 (2018) 4369-4378, https://doi.org/10.1021/acsami.7b13811
[83] J.S. Nguyen-Van-Tam, C. Sellwood, Curr. Opin. Virol. 3 (2013) 192-198, https://doi.org/10.1016/j.coviro.2013.02.004
[84] R. Shaikh, T.R. Raj Singh, M.J. Garland, A.D. Woolfson, R.F. Donnelly, J. Pharm. Bioallied Sci. 3 (2011) 89-100, https://doi.org/10.4103/0975-7406.76478
[85] F.D. Cojocaru, D. Botezat, I. Gardikiotis, C.M. Uritu, G. Dodi, L. Trandafir, et al., Pharmaceutics 12 (2020) 171, https://doi.org/10.3390/pharmaceutics12020171
[86] https://www.swissbiotech.org/listing/bioavanta-bosti-announces-immediate-availability-of-its-chitosan-nanoparticle-technology-to-formulate-aerosol-anit-covid-19-drugs/, 2020.
[87] R.C.F. Cheung, T.B. Ng, J.H. Wong, W.Y. Chan, Mar. Drugs 13 (2015) 5156-5186, https://doi.org/10.3390/md13085156
[88] C. Herzog, K. Hartmann, V. Kunzi, O. Kursteiner, R. Mischler, H. Lazar, et al., Vaccine 27 (2009) 4381-4387, https://doi.org/10.1016/j.vaccine.2009.05.029
[89] S.P. Kaur, V. Gupta, Virus Res. 288 (2020) 198114, https://doi.org/10.1016/j.virusres.2020.198114
[90] H.F. Florindo, R. Kleiner, D. Vaskovich-Koubi, R.C. Acúrcio, B. Carreira, E. Yeini, et al., Nat. Nanotechnol. 15 (2020) 630-645, https://doi.org/10.1038/s41565-020-0732-3
[91] M. Jeyanathan, S. Afkhami, F. Smaill, M.S. Miller, B.D. Lichty, Z. Xing, Nat. Rev. Immunol. 20 (2020) 615-632, https://doi.org/10.1038/s41577-020-00434-6
[92] S.R. Bonam, C.D. Partidos, S.K.M. Halmuthur, S. Muller, Trends Pharmacol. Sci. 38 (2017) 771-793, https://doi.org/10.1016/j.tips.2017.06.002
[93] R. Pati, M. Shevtsov, A. Sonawane, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.02224
[94] M.D. Shin, S. Shukla, Y.H. Chung, V. Beiss, S.K. Chan, O.A. Ortega-Rivera, et al., Nat. Nanotechnol. 15 (2020) 646-655, https://doi.org/10.1038/s41565-020-0737-y
[95] D. Hobernik, M. Bros, Int. J. Mol. Sci. 19 (2018) 3605, https://doi.org/10.3390/ijms19113605
[96] V. Sravanthi, M.C.P. Pallavi, S.R. Bonam, S. Sathyabama, H.M.S. Kumar, J. Drug Deliv. Sci. Technol. 28 (2015) 56-63, https://doi.org/10.1016/j.jddst.2015.05.007
[97] J.H. Erasmus, A.P. Khandhar, M.A. O'Connor, A.C. Walls, E.A. Hemann, P. Murapa, et al., Sci. Transl. Med. 12 (2020) eabc9396, https://doi.org/10.1126/scitranslmed.abc9396

[98] P. Garg, S. Sangam, D. Kochhar, S. Pahari, C. Kar, M. Mukherjee, Nano Today 35 (2020) 101001, https://doi.org/10.1016/j.nantod.2020.101001
[99] J.-H. Tian, N. Patel, R. Haupt, H. Zhou, S. Weston, H. Hammond, et al., bioRxiv (2020) 2020.2006.2029.178509, https://doi.org/10.1101/2020.06.29.178509
[100] S.E. Magnusson, A.F. Altenburg, K.L. Bengtsson, F. Bosman, R.D. de Vries, G.F. Rimmelzwaan, et al., Immunol. Res. 66 (2018) 224-233, https://doi.org/10.1007/s12026-018-8991-x
[101] C. Keech, G. Albert, I. Cho, A. Robertson, P. Reed, S. Neal, et al., New Engl. J. Med. (2020), https://doi.org/10.1056/NEJMoa2026920
[102] B.K. Haun, C.-Y. Lai, C.A. Williams, T.A. Wong, M.M. Lieberman, L. Pessaint, et al., bioRxiv (2020) 2020.2007.2024.220715, https://doi.org/10.1101/2020.07.24.220715
[103] N. Lakhan, N.E. Stevens, K.R. Diener, J.D. Hayball, J. Immunol. Methods 439 (2016) 37-43, https://doi.org/10.1016/j.jim.2016.09.008
[104] L.O. Medina, A. To, M.M. Lieberman, T.A.S. Wong, M. Namekar, E. Nakano, et al., Front. Immunol. 9 (2018) 2464, https://doi.org/10.3389/fimmu.2018.02464
[105] A.T. Lehrer, T.-A.S. Wong, M.M. Lieberman, T. Humphreys, D.E. Clements, R.R. Bakken, et al., Vaccine 36 (2018) 3090-3100, https://doi.org/10.1016/j.vaccine.2017.01.068
[106] K.A. Kusi, E.J. Remarque, V. Riasat, V. Walraven, A.W. Thomas, B.W. Faber, et al., Malar. J. 10 (2011) 182, https://doi.org/10.1186/1475-2875-10-182
[107] L. Rao, S. Xia, W. Xu, R. Tian, G. Yu, C. Gu, et al., Proceedings of the National Academy of Sciences (2020) 202014352, https://doi.org/10.1073/pnas.2014352117
[108] C. Weiss, M. Carriere, L. Fusco, I. Capua, J.A. Regla-Nava, M. Pasquali, et al., ACS Nano 14 (2020) 6383-6406, https://doi.org/10.1021/acsnano.0c03697
[109] G. Chauhan, M.J. Madou, S. Kalra, V. Chopra, D. Ghosh, S.O. Martinez-Chapa, ACS Nano 14 (2020) 7760-7782, https://doi.org/10.1021/acsnano.0c04006
[110] G. Nikaeen, S. Abbaszadeh, S. Yousefinejad, Nanomedicine (Lond) 15 (2020) 1501-1512, https://doi.org/10.2217/nnm-2020-0117
[111] M. Nasrollahzadeh, M. Sajjadi, G.J. Soufi, S. Iravani, R.S. Varma, Nanomaterials (Basel) 10 (2020) 1072, https://doi.org/10.3390/nano10061072
[112] M.A. Heinrich, B. Martina, J. Prakash, Nano Today 35 (2020) 100961, https://doi.org/10.1016/j.nantod.2020.100961
[113] C. Wang, S. Wang, Y. Chen, J. Zhao, S. Han, G. Zhao, et al., bioRxiv (2020) 2020.2008.2012.247338, https://doi.org/10.1101/2020.08.12.247338
[114] R.J. de Groot, S.C. Baker, R.S. Baric, C.S. Brown, C. Drosten, L. Enjuanes, et al., J. Virol. 87 (2013) 7790-7792, https://doi.org/10.1128/JVI.01244-13
[115] A. Assiri, A. McGeer, T.M. Perl, C.S. Price, A.A. Al Rabeeah, D.A. Cummings, et al., N. Engl. J. Med. 369 (2013) 407-416, https://doi.org/10.1056/NEJMoa1306742
[116] Z.A. Memish, A.I. Zumla, A. Assiri, N. Engl. J. Med. 369 (2013) 884-886, https://doi.org/10.1056/NEJMc1308698
[117] A.M. Zaki, S. van Boheemen, T.M. Bestebroer, A.D. Osterhaus, R.A. Fouchier, N. Engl. J. Med. 367 (2012) 1814-1820, https://doi.org/10.1056/NEJMoa1211721
[118] A. Assiri, J.A. Al-Tawfiq, A.A. Al-Rabeeah, F.A. Al-Rabiah, S. Al-Hajjar, A. Al-Barrak, et al., Lancet Infect. Dis. 13 (2013) 752-761, https://doi.org/10.1016/S1473-3099(13)70204-4
[119] X. Huang, M. Li, Y. Xu, J. Zhang, X. Meng, X. An, et al., ACS Appl. Mater. Interfaces 11 (2019) 19799-19807, https://doi.org/10.1021/acsami.9b04240
[120] G. Palestino, I. García-Silva, O. González-Ortega, S. Rosales-Mendoza, Expert Rev. Anti Infect. Ther. (2020) 1-16, https://doi.org/10.1080/14787210.2020.1776115
[121] S. Talebian, G.G. Wallace, A. Schroeder, F. Stellacci, J. Conde, Nat. Nanotechnol. 15 (2020) 618-621, https://doi.org/10.1038/s41565-020-0751-0

[122] D. Cipolla, J. Blanchard, I. Gonda, Pharmaceutics 8 (2016) 6, https://doi.org/10.3390/pharmaceutics8010006
[123] M. Shirley, Drugs 79 (2019) 555-562, https://doi.org/10.1007/s40265-019-01095-z
[124] K.N. Olivier, D.E. Griffith, G. Eagle, J.P. McGinnis, 2nd, L. Micioni, K. Liu, et al., Am. J. Respir. Crit. Care Med. 195 (2017) 814-823, https://doi.org/10.1164/rccm.201604-0700OC
[125] C.M. Coleman, T. Venkataraman, Y.V. Liu, G.M. Glenn, G.E. Smith, D.C. Flyer, et al., Vaccine 35 (2017) 1586-1589, https://doi.org/10.1016/j.vaccine.2017.02.012
[126] J.A. Wiley, L.E. Richert, S.D. Swain, A. Harmsen, D.L. Barnard, T.D. Randall, et al., PLoS One 4 (2009) e7142, https://doi.org/10.1371/journal.pone.0007142
[127] N. Vij, T. Min, R. Marasigan, C.N. Belcher, S. Mazur, H. Ding, et al., J. Nanobiotechnology 8 (2010) 22, https://doi.org/10.1186/1477-3155-8-22
[128] W. Shang, Y. Yang, Y. Rao, X. Rao, NPJ vaccines 5 (2020) 18, https://doi.org/10.1038/s41541-020-0170-0
[129] T.T. Le, Z. Andreadakis, A. Kumar, R.G. Rom?n, S. Tollefsen, M. Saville, et al., Nat. Rev. Drug Discov. 19 (2020) 305-306, https://doi.org/10.1038/d41573-020-00073-5
[130] T. Du, J.G. Liang, N. Dong, L. Liu, L.R. Fang, S.B. Xiao, et al., Carbon 110 (2016) 278-285, https://doi.org/10.1016/j.carbon.2016.09.032
[131] T.Q. Huy, N.T. Hien Thanh, N.T. Thuy, P.V. Chung, P.N. Hung, A.T. Le, et al., J. Virol. Methods 241 (2017) 52-57, https://doi.org/10.1016/j.jviromet.2016.12.015
[132] Y. Li, X. Liu, L. Guo, J. Li, D. Zhong, Y. Zhang, et al., Syst. Rev. 9 (2020) 75, https://doi.org/10.1186/s13643-020-01343-4
[133] M. Rai, A. Yadav, A. Gade, Biotechnol. Adv. 27 (2009) 76-83, https://doi.org/10.1016/j.biotechadv.2008.09.002
[134] G.C. Tremiliosi, L.G.P. Simoes, D.T. Minozzi, R.I. Santos, D.C.B. Vilela, E.L. Durigon, et al., bioRxiv (2020) 2020.2006.2026.152520, https://doi.org/10.1101/2020.06.26.152520
[135] H.B. Liu, Y.L. Bai, Y.R. Zhou, C.C. Feng, L.Z. Liu, L.R. Fang, et al., RSC Adv. 7 (2017) 28016-28023, https://doi.org/10.1039/c7ra03167j
[136] D. Ting, N. Dong, L.R. Fang, J. Lu, J. Bi, S.B. Xiao, et al., ACS Appl. Nano Mater. 1 (2018) 5451-5459, https://doi.org/10.1021/acsanm.8b00779
[137] S.Z. Moghadamtousi, H.A. Kadir, P. Hassandarvish, H. Tajik, S. Abubakar, K. Zandi, Biomed Res. Int. 2014 (2014) 186864, https://doi.org/10.1155/2014/186864
[138] E.V.R. Campos, A.E.S. Pereira, J.L. de Oliveira, L.B. Carvalho, M. Guilger-Casagrande, R. de Lima, et al., J. Nanobiotechnology 18 (2020) 125, https://doi.org/10.1186/s12951-020-00685-4
[139] G. Pezzotti, E. Ohgitani, M. Shin-Ya, T. Adachi, E. Marin, F. Boschetto, et al., bioRxiv (2020) 2020.2006.2019.159970, https://doi.org/10.1101/2020.06.19.159970
[140] M. Riediker, Nanotoxicology 14 (2020) 866-868, https://doi.org/10.1080/17435390.2020.1786185
[141] H. Kim, M. Park, J. Hwang, J.H. Kim, D.R. Chung, K.S. Lee, et al., ACS Sens. 4 (2019) 1306-1312, https://doi.org/10.1021/acssensors.9b00175
[142] C.Y. Cheng, Y.L. Lee, C.P. Chen, Y.C. Lin, C.E. Liu, C.H. Liao, et al., J. Microbiol. Immunol. Infect. 53 (2020) 488-492, https://doi.org/10.1016/j.jmii.2020.03.032
[143] R.R. Wang, Q.H. Yang, R.H. Luo, Y.M. Peng, S.X. Dai, X.J. Zhang, et al., PLoS One 9 (2014) e105617, https://doi.org/10.1371/journal.pone.0105617
[144] D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, et al., JAMA 323 (2020) 1061-1069, https://doi.org/10.1001/jama.2020.1585
[145] M.K. Lo, R. Jordan, A. Arvey, J. Sudhamsu, P. Shrivastava-Ranjan, A.L. Hotard, et al., Sci. Rep. 7 (2017) 43395, https://doi.org/10.1038/srep43395

[146] T.K. Warren, R. Jordan, M.K. Lo, A.S. Ray, R.L. Mackman, V. Soloveva, et al., Nature 531 (2016) 381-385, https://doi.org/10.1038/nature17180
[147] T.P. Sheahan, A.C. Sims, R.L. Graham, V.D. Menachery, L.E. Gralinski, J.B. Case, et al., Sci. Transl. Med. 9 (2017) eaal3653, https://doi.org/10.1126/scitranslmed.aal3653
[148] J.S. Morse, T. Lalonde, S. Xu, W.R. Liu, ChemBioChem 21 (2020) 730-738, https://doi.org/10.1002/cbic.202000047
[149] L.J. Stockman, R. Bellamy, P. Garner, PLoS Med. 3 (2006) e343, https://doi.org/10.1371/journal.pmed.0030343
[150] M.E. Morra, L. Van Thanh, M.G. Kamel, A.A. Ghazy, A.M.A. Altibi, L.M. Dat, et al., Rev. Med. Virol. 28 (2018) e1977, https://doi.org/10.1002/rmv.1977
[151] https://www.fujifilm.com/jp/en/news/hq/3211?_ga=2.98370583.926946726.1587470916-273032141.1587470916, 2020.
[152] J. Blaising, S.J. Polyak, E.I. Pecheur, Antiviral Res. 107 (2014) 84-94, https://doi.org/10.1016/j.antiviral.2014.04.006
[153] Q. Liu, X. Fang, L. Tian, X. Chen, U. Chung, K. Wang, et al., medRxiv (2020) 2020.2004.2011.20056523, https://doi.org/10.1101/2020.04.11.20056523
[154] R. O'Hanlon, M.L. Shaw, Curr. Opin. Virol. 35 (2019) 14-18, https://doi.org/10.1016/j.coviro.2019.01.006
[155] R.S. Cvetkovic, G. Keating, Biodrugs 16 (2002) 303-311; discussion 313-304, https://doi.org/10.2165/00063030-200216040-00005
[156] R. Cauchois, M. Koubi, D. Delarbre, C. Manet, J. Carvelli, V.B. Blasco, et al., Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 18951-18953, https://doi.org/10.1073/pnas.2009017117
[157] G. Cavalli, G. De Luca, C. Campochiaro, E. Della-Torre, M. Ripa, D. Canetti, et al., Lancet Rheumatol 2 (2020) e325-e331, https://doi.org/10.1016/S2665-9913(20)30127-2
[158] V. Bronte, S. Ugel, E. Tinazzi, A. Vella, F. De Sanctis, S. Cane, et al., J. Clin. Invest. (2020), https://doi.org/10.1172/JCI141772
[159] B.K. Titanji, M.M. Farley, A. Mehta, R. Connor-Schuler, A. Moanna, S.K. Cribbs, et al., Clin. Infect. Dis. ciaa879 (2020), https://doi.org/10.1093/cid/ciaa879
[160] G. Lapadula, A. Marchesoni, A. Armuzzi, C. Blandizzi, R. Caporali, S. Chimenti, et al., Int. J. Immunopathol. Pharmacol. 27 (2014) 33-48, https://doi.org/10.1177/03946320140270S103
[161] C. Harrison, A.M. Vannucchi, Ther Adv Hematol 3 (2012) 341-354, https://doi.org/10.1177/2040620712459746
[162] F. Kazazi-Hyseni, J.H. Beijnen, J.H. Schellens, Oncologist 15 (2010) 819-825, https://doi.org/10.1634/theoncologist.2009-0317
[163] C.J. Parker, S. Kar, P. Kirkpatrick, Nat. Rev. Drug Discov. 6 (2007) 515-516, https://doi.org/10.1038/nrd2369
[164] V. Giudice, P. Pagliano, A. Vatrella, A. Masullo, S. Poto, B.M. Polverino, et al., Front. Pharmacol. 11 (2020) 857, https://doi.org/10.3389/fphar.2020.00857
[165] F. Diurno, F.G. Numis, G. Porta, F. Cirillo, S. Maddaluno, A. Ragozzino, et al., Eur. Rev. Med. Pharmacol. Sci. 24 (2020) 4040-4047, https://doi.org/10.26355/eurrev_202004_20875
[166] P. Zheng, Y. Liu, H.G. Chen, M. Devenport, P. Reddy, S.S. Farag, et al., Biol. Blood Marrow Transplant. 26 (2020) S180-S181, https://doi.org/10.1016/j.bbmt.2019.12.741
[167] G. Giovannoni, C. Hawkes, J. Lechner-Scott, M. Levy, E. Waubant, J. Gold, Mult. Scler. Relat. Disord. 39 (2020) 102073, https://doi.org/10.1016/j.msard.2020.102073
[168] Z. Meng, T. Wang, C. Li, X. Chen, L. Li, X. Qin, et al., medRxiv (2020) 2020.2004.2011.20061473, https://doi.org/10.1101/2020.04.11.20061473
[169] H. Bian, Z.-H. Zheng, D. Wei, Z. Zhang, W.-Z. Kang, C.-Q. Hao, et al., medRxiv (2020) 2020.2003.2021.20040691, https://doi.org/10.1101/2020.03.21.20040691

[170] A. Markham, S.J. Keam, Drugs 79 (2019) 1355-1361, https://doi.org/10.1007/s40265-019-01167-0
[171] V. Casanova, F.H. Sousa, P. Shakamuri, P. Svoboda, C. Buch, M. D'Acremont, et al., Front. Immunol. 11 (2020) 85, https://doi.org/10.3389/fimmu.2020.00085
[172] B. Liu, M. Li, Z. Zhou, X. Guan, Y. Xiang, J. Autoimmun. 111 (2020) 102452, https://doi.org/10.1016/j.jaut.2020.102452
[173] M. Roumier, R. Paule, M. Groh, A. Vallee, F. Ackermann, medRxiv (2020) 2020.2004.2020.20061861, https://doi.org/10.1101/2020.04.20.20061861
[174] L.M. Canziani, S. Trovati, E. Brunetta, A. Testa, M. De Santis, E. Bombardieri, et al., J. Autoimmun. (2020) 102511, https://doi.org/10.1016/j.jaut.2020.102511
[175] T. Klopfenstein, S. Zayet, A. Lohse, P. Selles, H. Zahra, N.d.J. Kadiane-Oussou, et al., Int. J. Infect. Dis. (2020), https://doi.org/10.1016/j.ijid.2020.08.024
[176] N. Mohtadi, A. Ghaysouri, S. Shirazi, A. sara, E. Shafiee, E. Bastani, et al., Virology 548 (2020) 1-5, https://doi.org/10.1016/j.virol.2020.05.006
[177] W. Cao, X. Liu, T. Bai, H. Fan, K. Hong, H. Song, et al., Open Forum Infect. Dis. 7 (2020) ofaa102, https://doi.org/10.1093/ofid/ofaa102
[178] C. Sheridan, Nat. Biotechnol. 38 (2020) 655-658, https://doi.org/10.1038/d41587-020-00011-1
[179] C. Shen, Z. Wang, F. Zhao, Y. Yang, J. Li, J. Yuan, et al., JAMA 323 (2020) 1582-1589, https://doi.org/10.1001/jama.2020.4783
[180] K. Duan, B. Liu, C. Li, H. Zhang, T. Yu, J. Qu, et al., Proc. Natl. Acad. Sci. USA (2020) 202004168, https://doi.org/10.1073/pnas.2004168117
[181] G. Rada, J. Corbalan, P. Rojas, medRxiv (2020) 2020.2004.2013.20064162, https://doi.org/10.1101/2020.04.13.20064162
[182] C.D. Russell, J.E. Millar, J.K. Baillie, Lancet 395 (2020) 473-475, https://doi.org/10.1016/S0140-6736(20)30317-2
[183] Y. Xiong, Y. Liu, L. Cao, D. Wang, M. Guo, A. Jiang, et al., Emerg. Microbes Infect. 9 (2020) 761-770, https://doi.org/10.1080/22221751.2020.1747363
[184] F.C. Breedveld, J.M. Dayer, Ann. Rheum. Dis. 59 (2000) 841-849, https://doi.org/10.1136/ard.59.11.841
[185] J. Fantini, C. Di Scala, H. Chahinian, N. Yahi, Int. J. Antimicrob. Agents 55 (2020) 105960, https://doi.org/10.1016/j.ijantimicag.2020.105960
[186] M. Mahevas, V.T. Tran, M. Roumier, A. Chabrol, R. Paule, C. Guillaud, et al., BMJ 369 (2020) m1844, https://doi.org/10.1136/bmj.m1844
[187] J. Magagnoli, S. Narendran, F. Pereira, T. Cummings, J.W. Hardin, S.S. Sutton, et al., medRxiv (2020) 2020.2004.2016.20065920, https://doi.org/10.1101/2020.04.16.20065920
[188] A. Shamshirian, A. Hessami, K. Heydari, R. Alizadeh-Navaei, M.A. Ebrahimzadeh, G.W. Yip, et al., medRxiv (2020) 2020.2004.2014.20065276, https://doi.org/10.1101/2020.04.14.20065276
[189] L. Caly, J.D. Druce, M.G. Catton, D.A. Jans, K.M. Wagstaff, Antiviral Res. 178 (2020) 104787, https://doi.org/10.1016/j.antiviral.2020.104787
[190] G. Momekov, D. Momekova, Biotechnol Biotec Eq 34 (2020) 469-474, https://doi.org/10.1080/13102818.2020.1775118
[191] K.N. Olivier, P.A. Shaw, T.S. Glaser, D. Bhattacharyya, M. Fleshner, C.C. Brewer, et al., Ann Am Thorac Soc 11 (2014) 30-35, https://doi.org/10.1513/AnnalsATS.201307-231OC
[192] M. Moreno-Sastre, M. Pastor, A. Esquisabel, E. Sans, M. Vinas, A. Fleischer, et al., Int. J. Pharm. 498 (2016) 263-273, https://doi.org/10.1016/j.ijpharm.2015.12.028
[193] E. Alton, D.K. Armstrong, D. Ashby, K.J. Bayfield, D. Bilton, E.V. Bloomfield, et al., Lancet Respir. Med. 3 (2015) 684-691, https://doi.org/10.1016/S2213-2600(15)00245-3

[194] A.L. Galloway, A. Murphy, J.M. DeSimone, J. Di, J.P. Herrmann, M.E. Hunter, et al., Nanomedicine 9 (2013) 523-531, https://doi.org/10.1016/j.nano.2012.11.001
[195] A.M. Manicone, Expert Rev. Clin. Immunol. 5 (2009) 63-75, https://doi.org/10.1586/177666X.5.1.63
[196] M. Zietz, N.P. Tatonetti, medRxiv (2020) 2020.2004.2008.20058073, https://doi.org/10.1101/2020.04.08.20058073
[197] J. Zhao, Y. Yang, H. Huang, D. Li, D. Gu, X. Lu, et al., medRxiv (2020) 2020.2003.2011.20031096, https://doi.org/10.1101/2020.03.11.20031096
[198] P. Guillon, M. Clément, V. Sébille, J.-G. Rivain, C.-F. Chou, N. Ruvoën-Clouet, et al., Glycobiology 18 (2008) 1085-1093, https://doi.org/10.1093/glycob/cwn093
[199] A. Fischer, Mucosal Immunol. 13 (2020) 563-565, https://doi.org/10.1038/s41385-020-0303-9
[200] H. Salje, C. Tran Kiem, N. Lefrancq, N. Courtejoie, P. Bosetti, J. Paireau, et al., Science 369 (2020) 208-211, https://doi.org/10.1126/science.abc3517
[201] R. Carsetti, C. Quintarelli, I. Quinti, E. Piano Mortari, A. Zumla, G. Ippolito, et al., The Lancet Child & Adolescent Health 4 (2020) 414-416, https://doi.org/10.1016/S2352-4642(20)30135-8
[202] K.W. Ng, N. Faulkner, G.H. Cornish, A. Rosa, R. Harvey, S. Hussain, et al., bioRxiv (2020) 2020.2005.2014.095414, https://doi.org/10.1101/2020.05.14.095414
[203] H. Lv, N.C. Wu, O.T.-Y. Tsang, M. Yuan, R.A.P.M. Perera, W.S. Leung, et al., Cell Rep. 31 (2020) 107725, https://doi.org/10.1016/j.celrep.2020.107725
[204] J. Mateus, A. Grifoni, A. Tarke, J. Sidney, S.I. Ramirez, J.M. Dan, et al., Science (2020) eabd3871, https://doi.org/10.1126/science.abd3871
[205] T. Sekine, A. Perez-Potti, O. Rivera-Ballesteros, K. Strålin, J.-B. Gorin, A. Olsson, et al., Cell (2020), https://doi.org/10.1016/j.cell.2020.08.017
[206] A. Grifoni, D. Weiskopf, S.I. Ramirez, J. Mateus, J.M. Dan, C.R. Moderbacher, et al., Cell 181 (2020) 1489-1501.e1415, https://doi.org/10.1016/j.cell.2020.05.015
[207] J. Braun, L. Loyal, M. Frentsch, D. Wendisch, P. Georg, F. Kurth, et al., medRxiv (2020) 2020.2004.2017.20061440, https://doi.org/10.1101/2020.04.17.20061440
[208] N.L. Bert, A.T. Tan, K. Kunasegaran, C.Y.L. Tham, M. Hafezi, A. Chia, et al., bioRxiv (2020) 2020.2005.2026.115832, https://doi.org/10.1101/2020.05.26.115832
[209] M. Ujike, F. Taguchi, Viruses 7 (2015) 1700-1725, https://doi.org/10.3390/v7041700

Figures

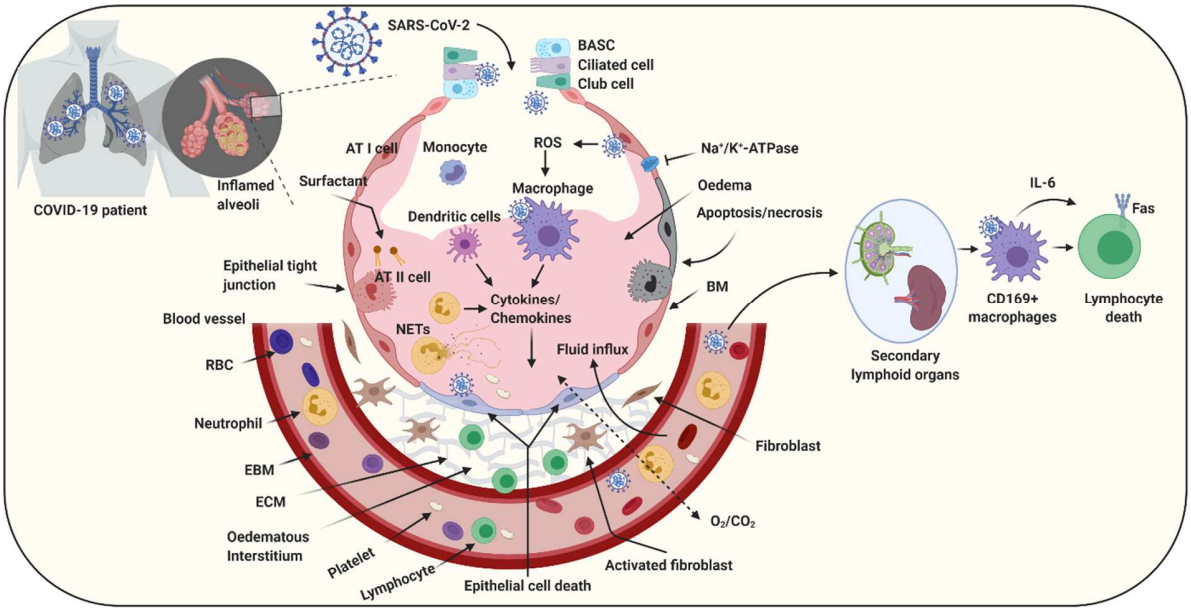

Fig. 1. Lung pathology in COVID-19. The lungs of severely ill COVID-19 patients appear to be opaque in the CT scan. SARS-CoV-2 enters via the nose, mouth, or eyes and reaches the alveoli, where a high expression of ACE2 receptors are present. Alveoli exist in the form of balloon-shaped structures. In any lung infection, different cells and substances are involved in protective immunity as well as inflammation. Invading SARS-CoV-2 interacts with, especially, tracheobronchial and alveolar epithelium and subsequently induces damage (apoptosis/necrosis) to the cells. The damage affects the tight barrier integrity of both the endothelium and epithelium layers. The epithelium is composed of a monolayer of alveolar type I and alveolar type II cells, which perform gas exchange and the production of surfactant functions, respectively. These functions keep the air space dry in the lungs. The damaged cells produce danger signals, such as reactive oxygen/nitrogen species, which recruit the innate immune cells, such as monocytes, immature macrophages, neutrophils, and dendritic cells. Upon uncontrolled activation, immune cells, epithelial cells, and fibroblast cells secrete copious amounts of pro-inflammatory cytokines and chemokines, which in turn act as a causative factor for epithelial cell death. In addition, they block the functional Na^+/K^+-ATPase pump, which keeps the osmotic equilibrium in the alveolus. The impaired tight junctions lose their fluid resistance nature and allow the fluids into the alveolus leading to edematous inflammation, which obstructs the vital gas exchanges process.
Note: This hypothetical figure illustration is based on the output obtained from different non-peer reviewed publications and in comparison of other lung diseases, such as ARDS, SARS, MERS, influenza.
Abbreviations: ATI, alveolar type I cell; ATII, alveolar type II cell; BASC, bronchioalveolar stem cell; BM, basement membrane; EBM, endothelial basement membrane; ECM, extracellular matrix, NETs, neutrophil extracellular traps; RBC, red blood cell; ROS, reactive oxygen species.

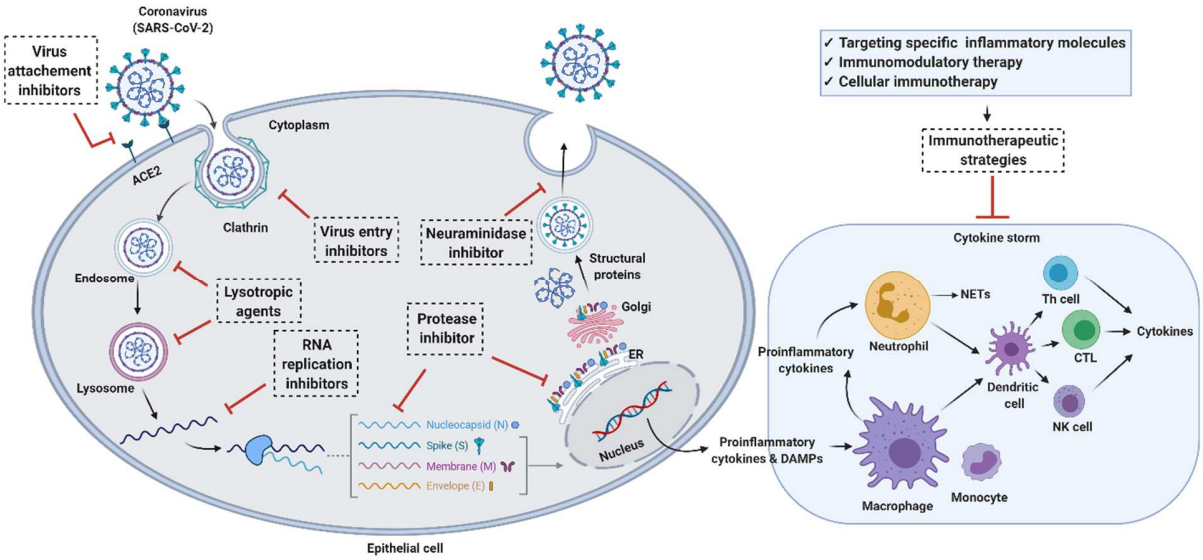

Fig. 2. Therapeutic strategies for COVID-19. SARS-CoV-2 uses epithelial cells, particularly lung epithelial cells, for their propagation. During the replication process, virus particles induce cell death signals to release pro-inflammatory cytokines and DAMPs, which in turn are sensed by the macrophages, monocytes, and neutrophils followed by the activation of other bystander cells and the development of the systemic cytokine storm. Based on the life-cycle of SARS-CoVs, the above-proposed drugs have been used in clinical trials. Note: As detailed, immunological responses of SARS-CoV-2 have not been established yet; thus, studies illustrated are in comparison with SARS and MERS. More details can be found in the text and table 1.
Abbreviations: ACE2, angiotensin 1-converting enzyme 2; CTL, cytotoxic T lymphocytes; DAMPs, danger-associated molecular patterns; ER, endoplasmic reticulum; NETs, neutrophil extracellular traps; RNA, ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

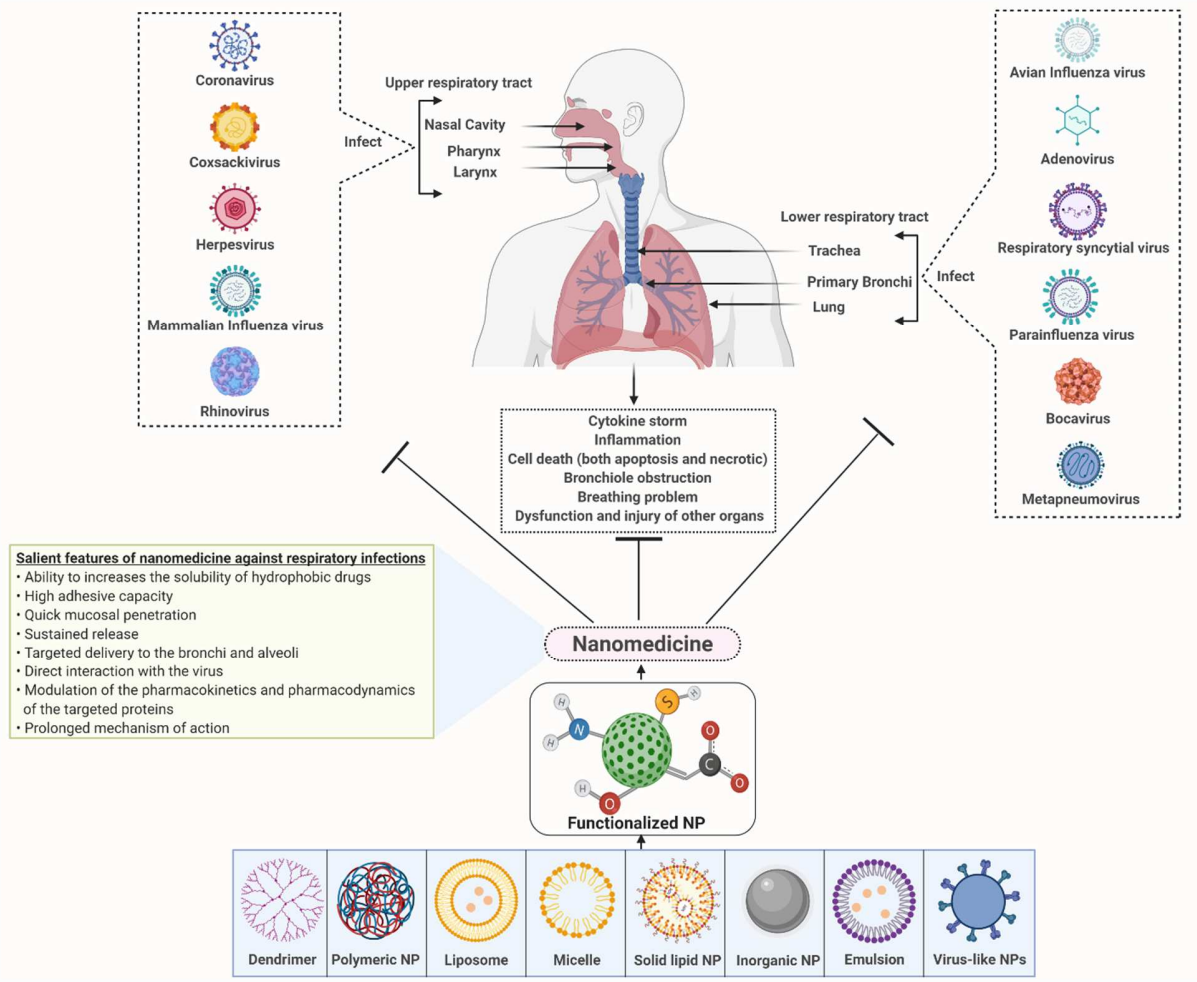

Fig. 3. Nanomedicine based strategies to prevent the pathologies associated with respiratory infections. A variety of viruses from different sources are responsible for respiratory infections. Few viruses, such as rhinovirus, parainfluenza virus, coronaviruses, adenoviruses, coxsackievirus, respiratory syncytial virus, herpesvirus, bocavirus, and others, particularly infect the upper respiratory tract. On the other hand, influenza virus, parainfluenza virus, respiratory syncytial virus, bocavirus, adenoviruses, metapneumovirus, and others infect the lower respiratory tract. In both the cases, they cause the common cold, bronchitis, bronchiolitis, and sometimes-severe pneumonia. Furthermore, the infection results in the dysfunction and damage to the other vital organs. Nanomedicine-based strategies that are explained above target the pathologies and reduce the severity of the disease.
Abbreviations: NP, nanoparticle

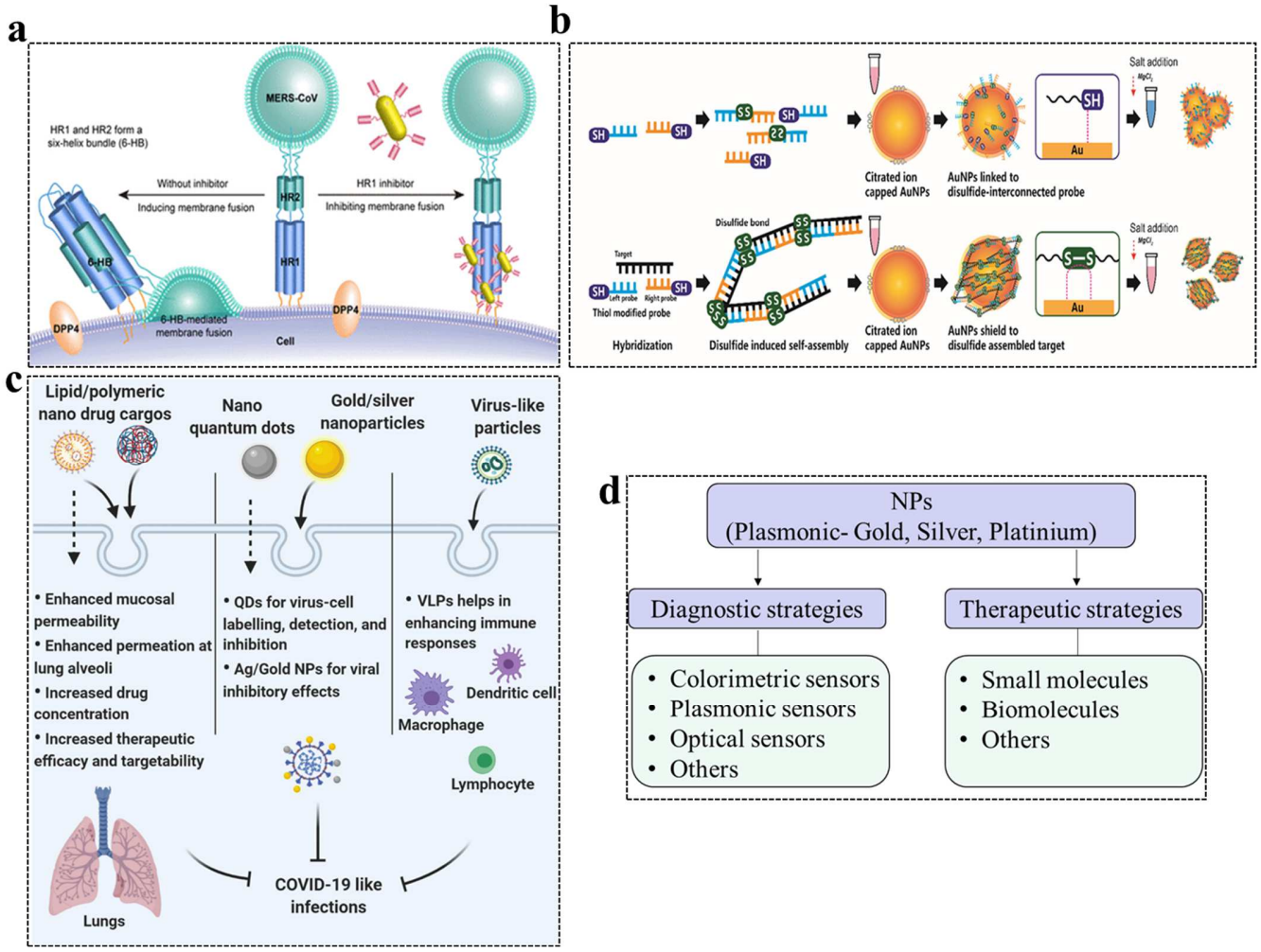

Fig. 4. Diversified application of nanomedicine in combating respiratory infections. a) Schematic diagram of the inhibition of MERS-CoV S2 subunit-mediated membrane fusion with HR1 inhibitors. HR1 inhibitors can inhibit HR1/HR2 complex (6-HB)-mediated membrane fusion and prevent MERS-CoV infections. Adapted from ref [119]. Printed with permission from "Copyright 2019 American Chemical Society". b) Strategy to detect the corona infection by colorimetric detection of double stranded DNA based on disulfide-induced self-assembly and shielding of AuNPs from salt-induced aggregation. In the absence of targets (virus), salt induces aggregation of AuNPs. Adapted from ref [141]. Printed with permission from "Copyright 2019 American Chemical Society". c) Mechanisms of action of different nanoparticles. The design and use of nanomedicine approaches help in enhancing the delivery system targetability and therapeutic efficacy in lung-associated infections. The drug loaded nano vehicles can be passively or actively targeted to the pulmonary epithelium to enhance the permeation and localized drug release thereby reducing associated side effects. Inorganic nano-systems are useful in the diagnosis of virus infection and also have inhibitory effects on the virus. The virus like particle systems (VLPs) enhance the immune response to combat lung-associated infections. d) Strategies to combat the COVID-19-like respiratory infectious diseases. Nanomedicine can

play a potential role in the diagnostic and therapeutic of COVID-19 like diseases. NPs are useful for the development of different sensors to detect SARS-CoV-2-like infections and, thus, can be used for an early real-time detection of virus with precession. A therapeutics approach at the moment is based upon the post functionalization strategies by using different biomolecules and small molecule inhibitors to prevent the entry of the viruses inside the host cells and to block viral replication.

Abbreviations: AuNPs, gold nanoparticles; COVID-19, coronavirus disease 2019; DDP4, dipeptidyl peptidase four receptors; 6-HB, 6-helix bundle; HR, heptad repeat; QDs, quantum dots; MERS-CoV, Middle East respiratory syndrome-related coronavirus; NPs, nanoparticles; VLPs, virus-like particles

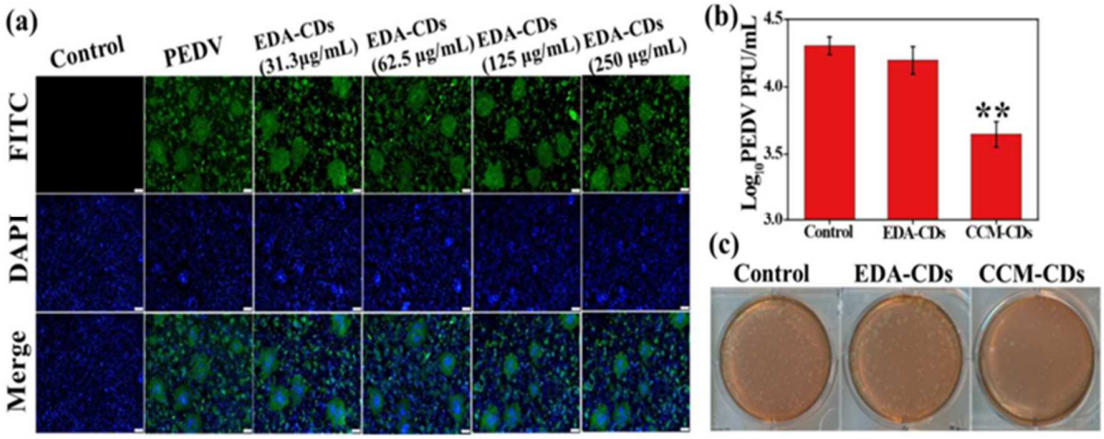

Fig. 5. The effect of EDA-CDs on PEDV. (a) The effect of different concentrations of EDA-CDs on PEDV-infected Vero cells by indirect immunofluorescence assay. Scale bar: 100 μm. (b) The titers of PEDV when exposed or unexposed to 125 μg/mL EDA-CDs or CCM-CDs. All error bars were determined according to the three replicate experiments. ** $p < 0.01$ and indicates superior antiviral activity of CCM-CDs to EDA-CDs treated and untreated, against PEDV. (c) Virus titers were calculated in the presence and absence of EDA-CDs or CCM-CDs. Pictures were taken at 12 hpi. Figure is reproduced from ref [136]. Copyright 2018 American Chemical Society.

Abbreviations: CCM-CDs, curcumin carbon dots; EDA-CDs, ethylenediamine carbon dots; PEDV, porcine epidemic diarrhoea virus

1200 **Tables**

1201 **Table 1. Therapeutic strategies for COVID-19*. Below is a list of drugs currently being**

1202 **explored for repurposing against COVID-19.**

Type of therapeutic strategy	Drug candidate	Comment
Antiviral therapy		
Protease inhibitors	Camostat mesilate, lopinavir/ritonavir, darunavir/cobicistat, ASC09, Danoprevir, Boceprevir, GC376	• In a clinical study on a small cohort of Taiwan COVID-19 patients, lopinavir (200 mg)/ritonavir (50 mg) did not shorten the duration of viral shedding [142].
Nucleoside reverse transcriptase inhibitors	Azvudine, Emtricitabine/Tenofovir	• It exhibited high potency anti-HIV-1 activity [143].
Neuraminidase inhibitors	Oseltamivir	• It is licensed for influenza A and B treatment. • It has been used either alone or in combination for the treatment of COVID-19 (n=124). Although many patients have recovered, the doses used in combination had no effective outcome [144].
RNA polymerase inhibitors	Remdesivir, Ribavirin, Favipiravir	• These drugs mainly come under the category of nucleoside analogues. • Although remdesivir was developed for the treatment of Ebola [145] and Marlburg [145,146], it has exhibited antiviral therapy against SARS and MERS [147]. In addition, molecular docking studies also supported their anti-SARS-CoV-2 function [148]. • Interestingly, ribavirin has been listed in the WHO essential medicine list (21st list in 2019). Though meta-analysis data has not shown significant clinical benefit for ribavirin against SARS [149] and MERS [150], the use as a drug candidate against COVID-19 should not be neglected. • Favipiravir is approved to treat influenza infection and currently evaluated in phase III

		clinical trials against COVID-19 [151].
Viral fusion inhibitor	Umifenovir (Arbidol)	• Arbidol is mainly prescribed for the treatment of upper respiratory tract infections, mainly caused by the influenza virus [152]. • A retrospective study on arbidol in COVID-19 patients (n=257) showed high efficacy with reduced mortality rate than other antiviral agents [153].
Viral endonuclease inhibitor	Baloxavir marboxil (Xofluza)	• It acts against the influenza virus and is approved by the FDA [154].
Virus attachment inhibitors	Camostat mesylate, Nafamostat mesylate (Fusan)	• It is used to treat pancreatitis. • Nafamostat mesylate is used to treat acute pancreatitis. • Nafamostat mesylate inhibits SARS-CoV-2 spike protein-interaction with the host cell surface receptors [12].
Immunotherapies and inhibitors of inflammation		
IL-1 receptor antagonist	Anakinra	• It blocks the IL-1-mediated pro-inflammatory effects. • It has shown a beneficial effect in rheumatoid arthritis patients [155]. • Under evaluation at various centers for severe COVID-19 cases [156,157]
Janus kinase inhibitor	Baricitinib	• Baricitinib selectively inhibits Janus kinase (JAK1/JAK2) and gp130 family cytokines, primarily type I IFN-mediated immune responses. • Baricitinib also blocks the viral entry by inhibiting AP2-associated protein kinase one and cyclin G-associated kinases. • Under evaluation at various centers for severe COVID-19 cases [158,159]
TNF inhibitor	Adalimumab (Humira)	• Adalimumab is a recombinant monoclonal antibody against TNF responses, which is approved for the treatment of autoimmune disease, particularly rheumatoid arthritis (RA)

		[160].
Janus kinase inhibitor	Ruxolitinib	• It is approved for the treatment of myelofibrosis [161].
Anti-VEGF	Bevacizumab	• A humanized anti-VEGF monoclonal IgG1 antibody. • It was approved and available for the treatment of different cancers (advanced colorectal cancer, advanced non-small cell lung cancer, metastatic breast cancer, advanced glioblastoma multiforme, and advanced renal cell cancer) either alone or in combination [162].
Complement inhibitor	Eculizumab (Soliris, Elizaria)	• A humanized monoclonal antibody against complement C5. • It is approved and available for the treatment of complement induced paroxysmal nocturnal hemoglobinuria [163]. • Under evaluation for severe COVID-19 cases [164,165].
DAMPs regulators	CD24Fc	• CD24Fc is a fusion protein to selectively inhibit DAMP-associated inflammation. • It is currently under clinical (phase III) evaluation against GvHD [166].
Sphingosine-1-phosphate receptor modulator	Fingolimod (FTY720)	• Acts as a sphingosine-1-phosphate receptor regulator. • It inhibits the egress of lymphocytes into the systemic circulation and approved for the treatment of MS [167].
Viral replication inhibitors	Interferon β1α, Recombinant human interferon α1β, α2β	• Activation of type I IFN signals through JAK–STAT pathway enhances the virus killing capacity of cells. • An open-labelled study conducted on medical staff (n=2944) with recombinant human IFN-α nasal drops with or without thymosin-α1 shown potential preventive effect from COVID-19 infection [168].

Viral entry inhibitors	Meplazumab	• It uniquely binds to the CD147, thereby inhibits the binding of SARS-CoV-2 spike protein to the cell membrane. • A recent clinical trial conducted on COVID-19 (17) patients with meplazumab efficiently improved the recovery of patients with SARS-CoV-2 pneumonia. During the recovery phase, treated patients have shown normalized lymphocyte count without any side-effects [169].
PD-1 antagonist	Camrelizumab	• A monoclonal antibody against Programmed Cell Death Protein 1 (PD-1) and approved for the treatment of Hodgkin lymphoma [170].
Antiviral peptide	CSA0001	• Human cathelicidin LL-37 is a host defense peptide with immunomodulating properties against bacterial and viral infections [171].
TLR agonists	PUL-042, Polyinosinic-polycytidylic acid (poly I:C)	• PUL-042 is a TLR2/6/9 agonist. • Poly I:C is a TLR3 agonist.
IL-6 antagonist	Siltuximab	• Effective in reducing cytokine release syndrome (CRS) [172].
IL-6 receptor antagonist	Sarilumab, Tocilizumab	• Effective in reducing cytokine release syndrome (CRS) [172]. • Use of tocilizumab in COVID-19 patients (n=30) shown reduced mechanical ventilation [173-175].
GM-CSF antagonist	Gimsilumab, TJ003234	• Monoclonal antibodies act as an antagonist to GM-CSF.
Inhibition of SARS-CoV-2 induced inflammatory innate and adaptive immune responses	Intravenous immunoglobulin (IVIG)	Suppresses the inflammatory responses mediated by hyperactivated innate and adaptive immune cells [176,177].

Virus neutralization	Immunoglobulin from recovered patients, Anti-SARS-CoV-2 inactivated convalescent plasma	• Passive immunization of seroconverted antibodies neutralizes the virus particles. • Treatment of COVID-19 patients with plasma that has been collected from the COVID-19 recovered patients has shown reduced viral load with improved clinical symptoms [178-180].
Viral load clearance	NK cell treatment, NKG2D-ACE2 CAR-NK Cells	• This strategy inhibits the surplus release of cytokines and neutralizes the GM-CSF, clear the virus-infected cells [67].
Non-specific immunosuppressors/immunomodulators		
Cellular therapy	Mesenchymal stromal cells	• These cells localize at the site of injury and especially inflammation at which they induce anti-inflammatory. Also, they modulate the function of active T cell to regulatory phenotype [181].
Steroid drugs	Corticosteroids	• Conflicting results have been reported from different treatment centers. • In addition, they develop diversified side effects, including the possibility of secondary infections, non-specific immunosuppression, deferred viral clearance impaired antigen-specific antibody responses, avascular necrosis, and osteoporosis [182,183].
Anti-rheumatics	Leflunomide	• Isoxazole derivative • It inhibits the dihydroorotate dehydrogenase by blocking the pyrimidine synthesis. • Making low availability of the pyrimidine pool, it affects the lymphocyte proliferation and inflammatory responses [184].
Calcium channel blocker	Tetrandrine	• Bisbenzylisoquinoline alkaloid. • It shows immunosuppressant activity by blocking calcium channels.
Anti-parasitic drugs	Chloroquine/ Hydroxychloroquine, Ivermectin	• Aminoquinolines derivatives mainly used for the treatment of malaria infections.

		• Despite their anti-malarial, anti-inflammatory, and immunomodulatory activity, anti-SARS-CoV-2 activity has also been reported in which CQ effectively binds to the sialic acids and gangliosides on the host cell surface thereby inhibiting the virus attachment. However, the later report is based on molecular modelling studies [185]. • Besides, CQ/HCQ is active against COVID-19 when it is in combination with a macrolide antibiotic (azithromycin) but not alone [186-188]. • Ivermectin exhibited in vitro anti-SARS-CoV-2 activity [189]. However, dose-escalation studies showed that a 'massive overdose' is required for antiviral activity in humans, which may raise further concerns about side-effects [190].
Antibiotics	Azithromycin, Carrimycin	• Macrolide antibiotics currently used against COVID-19 as an adjunct therapy.

Abbreviations: CQ/HCQ, (hydroxy)chloroquine; DAMP, Danger-associated molecular patterns; GM-CSF, granulocyte-macrophage colony-stimulating factor; GvHD, graft versus host disease; MS, multiple sclerosis; STAT, signal transducers and activators of transcription; VEGF, vascular endothelial growth factor.

*The above-mentioned drugs are currently evaluated in clinical trials against COVID-19 (for more information https://clinicaltrials.gov/).

1211 **Table 2. Expertise gained in the nanomedicine approaches for pulmonary infections.**

Product/ Formulation type	Indication	Therapeutic observations	Comments
Lipoquin®	Local antibiotic treatment for lung infections	Ciprofloxacin unilamellar nano liposome nebulized delivery system provides the reduction of dose frequency to a once daily treatment while maintaining high local concentration in clinical evaluations compared to larger size liposomes [122].	Commercial nano liposomal products for lung delivery are intended to increase the permeation, intra cellular drug enhancement for pulmonary infections, and these approaches provide insights for the future therapeutics targeting systems with enhanced therapeutic response to fight pulmonary infections.
Arikayce® (liposomal Amikacin)	Nontuberculous Mycobacterial Lung Disease	Liposomal Amikacin system is shown to increase intracellular drug concentration and overall therapeutic improvement [123,191].	
Ambisome® (liposomal amphotericin B)	Allergic bronchopulmonary aspergillosis	Currently in phase 2 clinical trials for Allergic Bronchopulmonary Aspergillosis treatment (ClinicalTrials.gov Identifier: NCT02273661).	
Liposomal amikacin for inhalation (LAI)	Bronchiectasis (Phase II clinical trial)	LAI produces improvement in sputum conversion with limited systemic toxicity in patients with refractory MAC (Mycobacterium avium complex) disease [124].	Strategies with liposomal based nano or emulsified systems had high lung mucosa penetration capabilities. These strategies help in drug deposition with high retention in airways; these investigations provide clues for future COVID-19 like pulmonary infection associated drug delivery applications.
Tobramycin nano lipid carriers (NLCs)	*P. aeruginosa* infections associated with cystic fibrosis	Drug NLCs combat P. aeruginosa infection and also increase *in vivo* mucus penetration [192].	
pGM169 drug with GL67A lipid solution	Cystic fibrosis (Phase IIb clinical trial)	Increased drug deposition and retention in the proximal airways [193].	
MERS-CoV S-containing nanoparticles	Recombinant MERS-CoV S nanoparticle vaccine and Matrix-M1 adjuvant combination as a vaccine	MERS-CoV S nanoparticle vaccine produced high titter anti-S neutralizing antibody and protected mice from MERS-CoV infection *in vivo* [125].	Introduction of vaccinated with MERS-CoV S nanoparticles developed a MERS-CoV neutralizing antibody response targeted to the MERS-CoV S.

Immunoprophylactic strategy with protein cage nanoparticle (PCN)	Pulmonary viral infections including influenza viruses, a mouse-adapted SARS-coronavirus	Pulmonary instillation of PCN dramatically enhanced the subsequent host immune responses to primary viral infections of the lungs [126].	Like liposomal strategies, polymeric nano systems also enhance the localization of drug in the lung regions for a better therapeutic response.
PS-341 loaded PLGA-PEG NPs	Cystic fibrosis (Preclinical study in mice)	Enhanced drug localization with NPs to the lungs, helps in reducing immunosuppressive side effects resulting from PS-341 systemic administration over 11 days [127].	
PRINT® (Particle Replication in Non-wetting Templates) technology	Influenza vaccine	To deliver influenza vaccine antigens poly (lactide-co-glycolide) PRINT particles were designed to bind to a commercial trivalent injectable influenza vaccine electrostatically. [194].	Increased understanding of the delivery of the vaccine antigen. This approach has increased vaccine effectiveness and reduces the amount of antigen necessary to induce an immune response.
Gold nanorod-based heptad repeat (HR1) peptide inhibitor	Middle East respiratory syndrome coronavirus (MERS-CoV)	Pregnancy-induced hypertension (PIH), is a potent HR1 inhibitor and can selectively inhibit MERS-CoV S with an IC50 value of 1.171 μM. The developed pregnancy-induced hypertension (PIH) gold nanorods (PIH-AuNRs) exhibit a 10-fold higher inhibitory activity than PIH and can completely inhibit cell fusion at 1.171 μM with good biostability, excellent targeting ability and minimum off target effects. Therefore, PIH-AuNRs are a promising antiviral agent and may have a huge impact on developing pharmaceuticals in the clinic [119].	HR1 peptide inhibitors have been developed to inhibit HR1/HR2-mediated membrane fusion between MERS-CoV and host cells, which is the major pathway of MERS-CoV-induced host infections.
Virus like nanoparticles (VLPs)	In enhancing or triggering the strong immune response in pulmonary	Virus mimicking empty particulate structures [128,129].	Hollow virus like particulate structures which lack in genetic material will provoke or enhance a strong immune response to combat

	infections		lung associated infections.

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Boxes

Box 1: Lung physiology.

Bronchi start from the trachea and extend to all parts of the lungs via bronchioles. The lung epithelium is comprised of different columns, which consist of mucus-secreting goblet cells, mucus clearance microvilli ciliated cells and cuboidal cells. The alveolar epithelium forms a tight barrier and regulates the movement of fluid and proteins from the circulatory system to the airspaces, and it regulates the entry of pathogenic substance that comes during the inhalation process. The alveolar epithelium contains two types of cells, alveolar type I (principal component) and II cells. Both cell types form a lining to the endothelial basement membrane and primarily type 1 cell perform diverse functions, including barrier function, fluid reabsorption, gas exchange function, and others.

Nevertheless, type I cells are more susceptible to infections, injury, and cell death. Type II cells (pneumocytes) produce surfactants, which exist in the alveolar lining reduce the liquid surface area/surface tension [195]. Type II cells also act as the progenitor for type I cells during injury. This integrity of these two epithelial cells is maintained by the transmembrane proteins, such as claudins, occludins, and others.

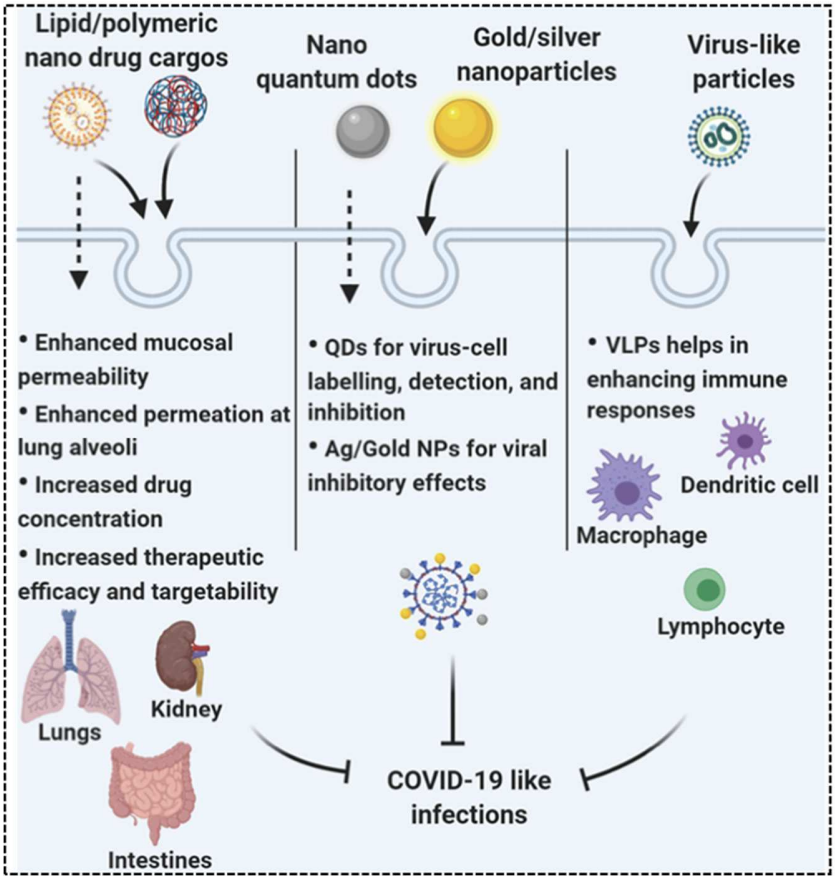
Gas (O_2/CO_2) transport and exchange is the primary function of lungs in the human body. In physiological conditions, the lungs exhibit excellent elastic characteristics of the lung parenchyma and surface tension of alveoli. The elastic properties are acquired by the components, such as elastic fibers (elastin, microfibrils), fibril forming collagens (tensile strength, type I, II, III, V, XI), and geometric arrangement.

In general, during the infections, the lung epithelium senses the pathogens and/or injury and produce pro-inflammatory cytokines, chemokines, and other danger signals, which will recruit leukocytes [195]. Recruited antigen-presenting cells (APCs) also recognize the invading pathogens, and pathogen-associated molecular patterns (PAMP) by pattern recognition receptors (PRRs) followed by the activation of innate immunity system and recruitment of innate immune cells, which can clear the pathogen via phagocytosis processes. Further, the activated innate immune system helps in the development of adaptive immune responses (both effector and memory) against a pathogen [32].

Box 2: Key observations that need to be considered for designing the COVID-19 therapeutics

- The RNA of the virus is protected by a lipid layer/envelope that allows the virus to penetrate the cells of the ocular, nasal, and buccal mucosa.
- Spike proteins are responsible for the entry of the virus.
- Unlike other CoVs, SARS-CoV-2 has a slight difference in their temperature withstanding capacity, humidity and type of material where it lies.
- Observational studies of ABO blood groups on large population confirmed that Rh (called Rhesus factor) positive and Rh/ABO blood group is positively and significantly correlating to intubation and death [196]. Likely, the O blood group has a lower risk of infection compared to other blood groups and the B blood group has a higher risk of infection [196,197]. However, earlier studies on SARS-CoV-1 has shown the contrary results [198].
- Although reasons are not known, children are likely to be resistant to SARS-CoV-2 infection and developing severe disease [199,200]. One of the plausible reasons is the existence of rapid producing, broad reactive and variable affinity natural antibodies [201].
- Pre-existing cross reactive T and B cells (including antibodies) against SARS-CoV-2 have been observed in unexposed healthy donors [202-208]. Probably, this contributes a clue to develop tailor-made, cross-protective vaccines against SARS-CoVs.
- Age, gender and underlying chronic diseases have played a significant role in mortality by COVID-19.
- Although the S protein is the best target for vaccine development, nucleic acid-based vaccines should not be undermined.
- M protein in all CoV exhibits N-terminal ectodomain glycosylation site. However, once glycosylated, M protein becomes inactive. Therefore, enhancing the glycosylation is a possible intervention strategy [209].
- It is still premature to confirm the effect of COVID-19 on diseases such as diabetes, hypertension, cardiovascular disease, cancer, inflammatory bowel disease, and others.

- Substantial data are yet be obtained on the effects of COVID-19 in the patients who are under immunosuppressive and/or anti-inflammatory/disease-modifying antirheumatic drugs.



Nanomedicine Therapeutic Strategies for COVID-19 Like Infections