Localized delivery of nanomedicine and antibodies for combating COVID-19

Bin Tu a,b,†, Yanrong Gao a,b,†, Xinran An a,c, Huiyuan Wang a, Yongzhuo Huang a,b,d,e,f,*

a State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
b University of Chinese Academy of Sciences, Beijing 100049, China
c University of Michigan College of Pharmacy, Ann Arbor, MI 48109, USA
d Zhongshan Institute for Drug Discovery, SIMM, CAS, Zhongshan 528437, China
e NMPA Key Laboratory for Quality Research and Evaluation of Pharmaceutical Excipients, Shanghai 201203, China
f Taizhou University, School of Advanced Study, Institute of Natural Medicine and Health Product, Taizhou 318000, China

Received 25 April 2022; received in revised form 1 July 2022; accepted 22 July 2022

Abstract The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has been a major health burden in the world. So far, many strategies have been investigated to control the spread of COVID-19, including social distancing, disinfection protocols, vaccines, and antiviral treatments. Despite the significant achievement, due to the constantly emerging new variants, COVID-19 is still a great challenge to the global healthcare system. It is an urgent demand for the development of new therapeutics and technologies for containing the wild spread of SARS-CoV-2. Inhaled administration is useful for the treatment of lung and respiratory diseases, and enables the drugs to reach the site of action directly with benefits of decreased dose, improved safety, and enhanced patient compliance. Nanotechnology has been extensively applied in the prevention and treatment of COVID-19. In this review, the inhaled nanomedicines and antibodies, as well as intranasal nanodrugs, for the prevention and treatment of COVID-19 are summarized.
1. Introduction

Coronavirus disease 2019 (COVID-19) is a highly contagious respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 has caused an unprecedented public health crisis. As of June 29, 2022, COVID-19 has caused 543,352,927 confirmed cases and 6,331,059 deaths globally. Vaccination is the most effective means of COVID-19 prevention. A variety of vaccines have been marketed in various forms including mRNA (e.g., the Pfizer-BioNTech COVID-19 vaccine); inactivated virus (e.g., Covaxin); recombinant subunit (e.g., Covovax); and adenovirus vector vaccines (e.g., Janssen COVID-19 vaccine). However, the prevalent mutated strains have caused vaccine breakthrough infection and a high transmission rate, thereby aggravating the global pandemic. Normally, COVID-19 vaccines are administrated by intramuscular injection. However, SARS-CoV-2 enters the human body mainly through the nose and mouth to the lung. Intramuscular vaccination cannot efficiently induce mucosal immunity. It was reported that inhaled vaccination, via mimicking the SARS-CoV-2 infection route, can induce potent mucosal immunity, and a clinical trial showed that atomized COVID-19 vaccine can induce both humoral and mucosal antibody responses. Moreover, a variety of specific drugs for COVID-19 treatment are administered orally and intravenously. Yet, the lung is the main lesion of COVID-19 and inhalation could achieve maximum pharmacological targeting with minimal systemic exposure and avoid first-pass metabolism in the liver. Thus, inhalation could be an appropriate drug delivery route for COVID-19 prevention and treatment. In early September, 2022, China approved the world’s first inhaled COVID-19 vaccine (Convidecia Air), which has been granted emergency use as a booster.

Infection cases and deaths of COVID-19 are continuing to rise as the mutant strains emerge and spread. It is of great significance to develop new medications against the infection. Nanotechnology has been extensively studied in the medical field due to its unique properties, e.g., small size, large surface area, multifunctionality, surface adaptability, and enhanced drug solubility. Nanotechnology can improve the detection performance and the therapeutic effect compared with traditional diagnosis and treatment techniques. For example, the nanotechnology-based SARS-CoV-2 detection methods may provide a viable platform for improving the diagnosis efficiency of SARS-CoV-2. Moreover, long-term antiviral therapy usually leads to gradually weakened effectiveness and side effects. Nanomedicines can improve the treatments by increasing the solubility of hydrophobic drugs, prolonging the blood circulation time, delivering to the target tissues, and releasing drugs in a controlled manner. Therefore, nanotechnology-based antiviral strategies may optimize pharmacokinetics and pharmacodynamics, reduce toxic and side effects, and improve therapeutic efficacy.

In this review, we summarized the inhaled delivery strategies including pulmonary and intranasal administration for anti-COVID-19 and discussed the benefits of inhaled nanomedicines for the prevention and treatment of COVID-19. Meanwhile, neutralizing antibodies also play an important role in blocking the infection of SARS-CoV-2. The benefits of inhaled antibodies for the treatment of COVID-19 have also been discussed.

2. The process of the COVID-19 infection

In the past two decades, there have been three coronavirus epidemics: SARS-CoV, the Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2. There are four subgroups of the coronavirus family (i.e., alpha, beta, gamma, and delta coronaviruses). SARS-CoV-2, with a small diameter ranging from 65 to 125 nm, belongs to the beta subgroup. SARS-CoV-2 shares a certain similar sequence and structural homology with SARS-CoV and MERS-CoV; these three belong to coronaviridae with positive-sense single-stranded RNA genomes that are highly conserved. Phylogenetic analysis clustered SARS-CoV-2 in the same group of SARS-CoV and MERS-CoV with similarity scores of 79% and 50%, respectively. The gene sequences of SARS-CoV-2 are as follows: open reading frame 1 (ORF1ab), spike (S), ORF3a, envelope (E), membrane (M), ORF6, ORF7a, ORF7b, ORF8, nucleocapsid (N), and ORF10.

Inhalation of viral particles from respiratory droplets expelled by sneezing, coughing, or talking is the most important transmission route of SARS-CoV-2. Furthermore, SARS-CoV-2 infection can occur via hand-to-eye, hand-to-nose, or hand-tomouth routes after touching virus-laden surfaces, e.g., metal, paper, plastic, and cloth, where viruses could be viable for hours depending on temperature, humidity, and chemical and topological properties of the solid surface. The possibility of fecal-oral transmission of SARS-CoV-2 was also reported.

SARS-CoV-2 infects the host cells relying on the S protein on the surface of the virus. The S protein is the main immunodominant antigen of SARS-CoV-2 and consists of three heterodimers of S1 and S2 subunits. S1 is responsible for the recognition and binding of the host cell surface receptors and S2 mediates the fusion of the viral envelope with the host cell membrane. The S1 subunit can bind to heparan sulfate (HS) on the cell surface, and open the RBD of the S protein, thus facilitating the binding of the S protein to angiotensin-converting enzyme 2 (ACE2) on the host cell surface. ACE2 is a transmembrane protein and is highly expressed on the epithelial cells of the heart, stomach, nasal mucosa, lungs, bladder, intestine, and kidneys, which indicates that these tissues are susceptible to SARS-CoV-2 infection. During infection, the S protein is cleaved at two sites: the cleavage at S1/2 by furin induces a conformational change and facilitates recognition by ACE2, and the S2 cleavage by transmembrane protease, serine 2 (TMPRSS2) initiates virus/cell membrane fusion and intracellular entry. These cleavage steps, called priming, are critical for the virus to bind to the host cell membrane and enter the cell. Subsequently, SARS-CoV-2 hijacks the host cell transcription and initiates replication inside the cells. Finally, the mature viruses are released from the infected cells and infect others. Besides HS and ACE2, other host cell receptors have also been found to be involved in facilitating SARS-CoV-2 entry.
e.g., the neuropilin-1 (NRP1) receptor\textsuperscript{37} and the tyrosine-protein kinase receptor UFO (AXL)\textsuperscript{38}.

During the early stages of infection, SARS-CoV-2 enters the respiratory tract and infects the goblet cells and ciliated cells by depositing on the airway epithelium\textsuperscript{39}. At this stage, SARS-CoV-2 amplifies in the cells, and patients could develop mild symptoms. With the rapid reproduction of the virus, the goblet and ciliated cells die off and release a large number of viruses that cause strong immune responses and severe symptoms like cough, fever, difficult breathing, and coagulation\textsuperscript{40}. Meanwhile, hyper inflammation would occur because the overactivated immune system leads to cytokine storms and progressive damage to the tissues and organs\textsuperscript{41}. A detailed description of this process can be referred to a recent review\textsuperscript{42}. Infectiousness and disease severity are changed among different strains of SARS-CoV-2 and have been summarized in Table 1\textsuperscript{43–67}.

Therefore, through the infection process of SARS-CoV-2, the following therapeutic targets have been investigated: the S protein of SARS-CoV-2, the targets on the host cells (e.g., ACE2 and heparan sulfate proteoglycans, HSPGs), the essential enzymes for virus replication (e.g., RNA dependent RNA polymerase, RdRp), the targets of the virus-associated inflammation (e.g., IL-6 blockage). Moreover, given the transmission nature and the primary target organ (i.e., the lung) of SARS-CoV-2, inhalation would be an ideal drug delivery route for both the prevention and treatment of COVID-19. The International Society for Aerosol Medicine (ISAM) has called for the development of inhaled therapies for COVID-19\textsuperscript{68}. Moreover, a variety of drugs for the prevention and treatment of COVID-19 have been developed in inhalations, e.g., Ad5-nCoV inhaled vaccine\textsuperscript{69}, ivermectin inhalation\textsuperscript{70,71}, ciclesonide inhalation\textsuperscript{2}, and inhaled antibody therapy\textsuperscript{75}.

3. Inhalations for lung or respiratory tract delivery

Inhalation is a traditional method and an important drug delivery strategy for the treatment of lung and respiratory diseases, e.g., asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pneumonia, and pulmonary hypertension\textsuperscript{73,74}. In addition to small molecules, inhalation is also suitable for the delivery of biologics, e.g., peptides, proteins, nucleic acids, and exosomes\textsuperscript{75–78}. Recently, vaccination through inhalation has received great attention. Nebulized measles vaccine eliminated morbidity and mortality in young children and reduced the severity of associated pneumonia, and pulmonary delivery of measles vaccines has shown the promise (detailed information can be referred to a review\textsuperscript{79}). Furthermore, inhalation is an effective and non-invasive strategy, which is patient-friendly, and self-administered inhalation can remove the need for healthcare workers and facilities.

3.1. Orally inhaled delivery

The drugs can be directly delivered through the mouth to the lower respiratory tract by oral inhalation. Moreover, the complex physiological structure in the nose and the mechanical obstruction are also part of the reasons for less pulmonary drug deposition\textsuperscript{80}. Therefore, orally-inhaled delivery is preferable for inhalations. An aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) has passed phase I clinical trial and the result shows good safety, tolerability, and immunogenicity\textsuperscript{11}. It is inspired that only 1/5 of the intramuscular injection dose by inhalation can reach the same level of immune response\textsuperscript{11}. Moreover, the latest clinical data show that orally administered aerosolized heterologous vaccination, serving as the 2nd booster, has good safety, and its immunogenicity to the wild-type and delta strains is significantly higher than that of homologous inactivated vaccine\textsuperscript{81}.

Normally, orally-inhaled drugs could directly pass through the throat and enter the lower respiratory tract with the help of a device. However, a part of inhaled drugs will be trapped in the larynx due to the limitations of airway anatomy\textsuperscript{82}. The drugs deposited in the lung may undergo pulmonary absorption and metabolism, which is dependent on the physiological characteristics of the biological membranes and the properties of the drugs\textsuperscript{83}.

Stabilizers are commonly used to maintain particle size and shape, and prevent particle aggregation in a given aqueous suspension medium\textsuperscript{84}. However, the stabilizers can be a source of cytotoxicity. For example, Solutol HS 15 can contribute to the cytotoxicity of nanoparticles measured in a cell-based assay\textsuperscript{85}. In this regard, aerosol is an effective method to prevent particle agglomeration. Metered-dose inhalers (MDI), dry powder inhalers (DPI), and nebulizers are commonly used to generate aerosols for enhanced pulmonary delivery and reduced side effects\textsuperscript{86}. The
MDI, also named pressurized metered-dose inhalers (pMDI), can atomize drug solutions and the DPI atomizes drug powders; both require patients to cooperate with inhalation or actuation. The power-driven nebulizers can convert a drug solution or suspension into an aerosol, which is friendly to the elderly, children, and unconscious patients. Therefore, it is important to select an appropriate inhaler according to the nature of the drugs, the age of the patients, and the disease stages.

### Table 1: The basic information of the SARS-CoV-2 (wild-type and variants of concern, as of April 2022).

| Variant | Pangolin name | Number of mutations | Infectiousness | Disease severity | Therapeutic effectiveness | Vaccine effectiveness | Ref. |
|---------|---------------|---------------------|----------------|------------------|--------------------------|----------------------|------|
| Wild-type | − | − | High transmissibility | Pneumonia, supplemental oxygen requirement, and ICU admission | Be effectively neutralized by monoclonal antibodies (mAbs) for clinical use | High antibody neutralizing titers elicited by mRNA vaccine (mRNA-1273 and BNT162b2) | 43,44 |
| Alpha B.1.1.7 | Spike: 10 Overall: 24 | 43%—90% more transmissible than the previous lineages | Cough, sore throat, fatigue, and myalgia occur more frequently and anosmia is less common; higher risk of hospitalization and increased mortality than in previous variants | Just be resistant to a few mAbs | Only a modest reduction in neutralizing titers elicited by mRNA-1273 or BNT162b2 (less than 3-fold) | 43,45–50 |
| Beta B.1.351 | Spike: 10 Overall: 20 | 1.5 times more transmissible than the previous lineages | More frequent symptomatic cases than delta; higher risk of hospitalization and ICU admission than delta and non-VOC cases | Be resistant to most mAbs | 4.9-fold reduction in neutralizing titers elicited by 2-dose BNT162b2 | 43,49–53 |
| Gamma P.1 | Spike: 12 Overall: 24 | 1.4—2.2 times more transmissible than the previous lineages | Higher risk of hospitalization and ICU admission than delta and non-VOC cases and higher mortality than the previous lineages | Be similar to beta | Significant reduction in neutralizing titers elicited by mRNA-1273 and BNT162b2 (4.5- and 6.7-fold) | 43,52,54–56 |
| Delta B.1.617.2 | Spike: 9 Overall: 22 | Higher transmissibility than the previous lineages | Higher risk of pneumonia than the wild-type; higher rates of ICU admission and death than other variants. | Be highly resistant to bamlanivimab but retain susceptibility to many mAbs | 5.8-fold reduction in neutralizing titers elicited by 2-dose BNT162b2 | 43,53,57–60 |
| Omicron B.1.1.529 | Spike: more than 30 Overall: more than 60 | Higher transmissibility than delta | Low risk of pneumonia and symptoms are mostly upper respiratory tract infection; lower rates of hospitalization, ICU admission, and death than delta | Be resistant to many commercial mAbs in various degree | 22-fold reduction in neutralizing titers elicited by 2-dose BNT162b2 | 43,61–67 |

−, not applicable.

3.2. Intranasal delivery

The intranasal route is another inhalation option. Notably, the intranasal vaccination can simulate a respiratory infection of a virus and elicit potent mucosal immunity, which has been clinically used for influenza vaccination\(^{87}\). The dNS1-RBD, based on a live attenuated influenza virus vector, is the first reported intranasal vaccine entering clinical trials to prevent COVID-19\(^{88}\). The
The lung and systemic delivery of salbutamol was compared in a clinical investigation following inhalation from an MDI, an MDI attached to a spacer, and a nebulizer. It was found that using MDI attached to a spacer resulted in more pulmonary drug deposition and less drug in the systemic circulation than the other two devices, indicating the importance of spacers. Therefore, the abovementioned factors should be taken into consideration in the design of inhaled products.

It is generally believed that the types and activities of metabolic enzymes in the lung are few and low, and the interaction between pulmonary drugs and metabolic enzymes is often ignored. However, for the inhalations with a long retention time in the lung, it is essential to investigate the interaction between pulmonary inhalations and metabolic enzymes. Detailed information on the phase I and phase II metabolic enzymes in the human lung is summarized in a review.

The common cytochrome P450 (CYP) isoforms in the human lung are CYP2B6, CYP1B1, CYP2E1, CYP3A5, CYP2J2, and CYP1A1 (in smokers). Besides the CYP family, other biotransformation enzymes (e.g., uridine diphosphate glucuronosyltransferase, glutathione-S-transferases, flavin monoxygenase, peptidase, esterases, cyclooxygenase, and sulfotransferase) are also expressed in the lung. It should be noted that the metabolic enzymes in the lung differ from those in the liver. For example, CYP3A4 is the most abundant metabolic enzyme in the liver, but its activity in the lung is about 20% compared to that in the liver. In fact, the isof orm of CYP3A5 plays an important role in lung metabolism. Meanwhile, species differences should be taken into consideration in the study of pulmonary drug metabolism. Furthermore, more metabolism models should be established for the evaluation of the enzyme metabolism process of pulmonary inhalations, which currently are largely dependent on the human lung microsomes or rat lung homogenates.

Macroscopic pharmacokinetic studies cannot reflect the drug concentration in the target cells, which may lead to a weak correlation between blood concentration and efficacy. Therefore, a cell pharmacokinetic study is helpful for inhaled drug development; for instance, revealing the way and extent of inhalations to enter the target cells in the lung will be beneficial. Some cell lines (e.g., A549) are often used as a model for cellular pharmacokinetic study of inhalations. However, if the cells cannot form the tight junctions, it may reduce their utility in drug transport research. Meanwhile, the excessive proliferation and the mucous absence of cells may also affect the results. The appropriate cell lines should be selected according to the experimental purposes, and primary cells may be applied if necessary. In addition, the inhaled drugs in the lung could re-distribute to the systemic circulation, and the permeability of drugs should be also evaluated. Often, the Calu-3 cell line is utilized as a model to examine the permeability of drugs in the lung. Moreover, inhaled drugs deposited in the lung could be cleared by alveolar macrophages, thus resulting in off-target delivery.

In addition, in vitro simulation methods are often applied to predict the in vivo fate of the inhaled drugs. However, the gap between in vitro and in vivo studies is still difficult to bridge. An in-depth understanding of the whole lung architectures and a clear illustration of the pulmonary distribution patterns and deposition mechanisms of the inhaled drugs at 3D, in situ, and single-particle levels is important. An attractive method was reported for acquiring high-precision cross-scale visualization of the entire lung anatomy by using the advanced Micro-Optical Sectioning Tomography (MOST) system coupled with whole lung Nissl-
staining, while the inhaled material was labeled with Alex Fluor-488. The cross-scale lung architectures were reconstructed by overlapping about 80,000 coronal images for qualitative analysis of the lung structure (airways, arteries, veins, and alveoli) and quantitative computing of the inhaled material deposition in different areas (Fig. 2A).

Particle size is a critical parameter affecting the pharmacokinetics of inhalations. The wind tunnel studies showed that particles smaller than 5 μm can be inhaled completely and the efficacy of inhalations would decrease with the increasing size of particles. Meanwhile, particles of different sizes would be delivered to different regions in the lung with different mechanisms. For example, particles (5–9 μm, slow inhalation; 3–6 μm fast inhalation) deposited in large airways through impaction, particles (1–5 μm) deposited in smaller airways through gravitational sedimentation, particles (1–3 μm) deposited in respiratory bronchioles through gravitational sedimentation, and particles (≤0.5 μm) deposited in alveoli through Brownian diffusion; detailed information can be found in a review. A recent report revealed the influence of particle size on the inhaled drug distribution in the lung after intratracheal administration in a mouse model. In that study, the particles with different sizes and similar release rates were used, and the in vivo fate of inhaled drugs was vastly different in lung retention, lung tissue absorption, and lung targeting efficiency (Fig. 2B), compared to the previous reports; it suggests that the effect of drug release rate matters.

5. Inhaled administration of nano-formulations for prevention and treatment of COVID-19

Various nanotechnology strategies for the prevention, diagnosis, and treatment of COVID-19 have been widely explored. The
Extracellular vesicles (EVs) are heterogeneous membrane structures, which are secreted from almost all human cells. They are categorized into various subgroups including microvesicles, exosomes, and apoptotic bodies. As an important pathway for intercellular communication, EVs can transfer various cargos, such as proteins, lipids, and nucleic acids, to the adjacent or distant cells and exert pathological or physiological effects. EVs can carry both endogenous and exogenous compounds for therapeutic purposes. For example, EVs can be engineered to deliver exogenous proteins or nucleic acids, with the benefits of stability, safety, and biomimetic nature. Exosomes carrying the S protein of SARS-CoV-2 have been investigated as a vaccine candidate.

To prevent viral entry into the host cell, the blockade of the ACE2 receptor could be an effective strategy. A study shows that the COVID-19 patient-derived EVs expressing ACE2 can neutralize SARS-CoV-2 by competing with the ACE2-bearing cells. Notably, the neutralization strategy using the EVs displaying ACE2 protein of SARS-CoV-2 have been investigated as a vaccine candidate.

Infection of SARS-CoV-2 could result in hyperinflammation. EVs were also investigated to relieve lung inflammation. It has been reported that the mesenchymal stem cells (MSCs)-derived EVs can mitigate acute lung injury by transferring proteins, lipids, and RNA from MSCs to the injured cells. Meanwhile, MSC-EVs were also investigated to relieve lung inflammation. It has been reported that the mesenchymal stem cells (MSCs)-derived EVs can mitigate acute lung injury by transferring proteins, lipids, and RNA from MSCs to the injured cells.

Table 2: The inhaled nanomedicines and antibodies for COVID-19 in the clinical trials (https://clinicaltrials.gov).

| Name                      | Drug types or delivery system | Administration | Clinical stage   | Clinical status | NCT number     |
|---------------------------|-------------------------------|----------------|------------------|-----------------|----------------|
| CSTC-Exo                  | T cell-derived exosomes       | Oral inhalation | Phase 1          | Unknown         | NCT04389385    |
| MSCs-derived exosomes     | Allogenic adipose mesenchymal stem cells | Oral inhalation | Phase 1          | Completed       | NCT04276987    |
| COVID-19EXO2              | Mesenchymal stem cell exosomes | Oral inhalation | Phase 2          | Enrolling by invitation | NCT04602442    |
| TLC19                     | Hydroxychloroquine liposome   | Oral inhalation | Phase 1          | Completed       | NCT04697654    |
| Remdesivir (GS-5734) and NA-831 (NEUROSIVIR) | Liposome | Intranasal inhalation | Phase 2/3        | Completed       | NCT04475120    |
| Liposomal lactoferrin     | Nanoparticle                  | Oral inhalation | Phase 1          | Recruiting      | NCT04480333    |
| CT-P63 and CT-P66         | Monoclonal antibodies         | Oral inhalation | Phase 3          | Not yet recruiting | NCT05224856    |
| combination therapy       |                               |                |                  |                 |                |
| DZIF-10c                  | Monoclonal antibody           | Oral inhalation | Phase 1/2a       | Completed       | NCT04631705    |
| STI-2099 (COVID-DRPSTM)   | Monoclonal antibody           | Oral inhalation | Phase 2          | Completed       | NCT04906694    |
| STI-9199                  | Monoclonal antibody           | Intranasal inhalation | Phase 2         | Not yet recruiting | NCT05372783    |
| IGM-6268                  | Immunoglobulin M antibody     | Intranasal inhalation | Phase 1         | Recruiting      | NCT05184218    |

inhaled nanomedicines for the prevention and treatment of COVID-19 in the clinical trials were summarized in Table 2. The major roles of nanotechnology-based inhaled delivery in the prevention and treatment of COVID-19 in the preclinical phase are reviewed in this article from five aspects: extracellular vesicles, cell membrane vesicles, liposomal formulations, polymeric nanoparticles, and inorganic nanoparticles.

5.1. Extracellular vesicles for prevention and treatment of COVID-19

The EVs administered intravascularly could relieve pro-inflammatory cytokine secretion and respiratory dysfunction in vivo, which indicated the implication of MSC-EVs for relieving the lung inflammation of severe patients infected by SARS-CoV-2. Clinical trials of MSC-EVs for the treatment of COVID-19 have been described by Yan et al. Furthermore, the effectiveness of MSCs exosomes by inhalation was examined in a small sample size of COVID-19 patients, and the results showed nebulization of MSCs exosomes reduced the hospitalization time for COVID-19 patients, without inducing toxic and side effects.
5.2. Cell membrane-modified vesicles for prevention and treatment of COVID-19

The cell membrane-based systems have great promise as biomimetic carriers. Derived from various cells, the membrane can be extruded into small vesicles or encapsulated on the surface of the synthetic nanoparticles. Various kinds of cell membranes have been used for therapeutic or drug delivery purposes, including red blood cells, platelets, cancer cells, immune cells, and bacterial membranes. In the case of COVID-19, the specific ligands can be displayed on the cell membranes for neutralizing the virus. A cell membrane-based nanosystem for COVID-19 treatment was developed by using the cell membrane of the human lung epithelial type II cells or human macrophages that express ACE2 to coat the poly (lactic-co-glycolic acid) (PLGA) nanoparticles. Such a system served as the nanosponges (NS) for neutralizing SARS-CoV-2 and displayed a concentration-dependent manner in vitro. In another report, the hACE2-containing nanocatchers (NCs) with a mucoadhesive excipient hyaluronic acid were developed into an inhalable dry formulation via lyophilization for a prolonged retention effect in the lung via intratracheal inhalation. The nanocatchers were prepared by extruding the cell membranes derived from the engineered 293 T cells with stable hACE2 expression, and the neutralization potency against the pseudoviruses of wild-type SARS-CoV-2 and D614G variants was demonstrated. The in vivo investigations showed that the intratracheally administered nanocatchers efficiently inhibited the infection of pseudoviruses.
In addition to blocking the virus infection, the cell membrane-based nanomedicine can also be used to treat COVID-19 complications. Cytokine storm is a potentially severe consequence of COVID-19. To address this problem, the two-step neutralizable cell-membrane-based nanovesicles were designed by Rao et al. Two types of cells were used in this study, i.e., ACE2-engineered 293 T cells and precursor human myeloid mononuclear THP-1 cells. The two types of cell membranes were extracted and fused into a final nanodecoy (Fig. 4E). The results showed that the nanodecoy not only neutralized the pseudovirus and authentic SARS-CoV-2 but also neutralized the inflammatory cytokines interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in the lungs, because of the inherent expression of IL-6 receptor on the THP-1 cell membrane and GM-CSF receptor on both types of cell membranes. With intratracheal administration of the nanodecoy to the mice, the immune disorder and lung injury were effectively improved in an acute lung inflammation mouse model. To further improve the treatment, Wang, et al. developed a microfluidic microsphere-based inhaled aerosol (termed iAE-PMS) in which two kinds of cell membrane nanovesicles (one from the ACE2-expressed HEK293 cells, another from the pro-inflammatory M1 macrophages that expressed inflammatory cytokine receptors) were fused. The hybrid nanovesicles were loaded into the pores of the negatively charged methacrylate hyaluronic acid hydrogel microspheres via electrostatic interaction to neutralize the complex immunoregulatory molecules (Fig. 4F). The mice inhaled the iAE-PMS through a mask of the atomizer and the iAE-PMS was...
distributed throughout the whole respiratory system, including the nasopharynx, trachea, and alveolus, where the iAE-PMS competitively bound with SARS-CoV-2 via ACE2/S protein interaction, thus protecting the body against infection. Meanwhile, the inflammatory cytokines were neutralized via binding with the iAE-PMS and the hyperinflammatory state was alleviated.

Apart from treatment, the cell-membrane vesicles can also be applied for vaccination against COVID-19. The outer membrane vesicles (OMVs), naturally released by gram-negative bacteria, are mainly comprised of lipids, lipopolysaccharide (LPS), integral membrane proteins, and lipoproteins\(^{140}\). OMVs have been explored as a vaccination platform\(^{41}\). For instance, *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) were genetically engineered to produce the detoxified OMVs displaying a receptor-binding domain (RBD) of the S protein\(^{142}\). Intranasal immunization with the RBD-OMVs induced a robust S protein-specific immune response, with a high antibody titer against the S protein\(^{142}\).

The aforementioned results demonstrate that the cell membrane-based formulations via intranasal or orally inhaled administration may be the potential approaches for the prevention and treatment of COVID-19.

5.3. Liposomal formulations for prevention and treatment of COVID-19

In the development of COVID-19 mRNA vaccines, lipid nanoparticles (LNPs) play an essential role in the delivery ability of mRNA. Two LNP vaccines of mRNA have been approved by FDA to prevent the infection of SARS-CoV-2, e.g., Pfizer BioNTech and Moderna\(^{43}\). A liposome (LPX)-based mRNA vaccine was developed by Huang et al.\(^{144}\), which had good immunogenicity with multiple routes of administration including intravenous (i.v.), intramuscular (i.m.), hypodermic (i.h.), intradermal (i.d.), or intraperitoneal (i.p.) injection, which thus provides other options. However, there is no report on inhaled LNP/mRNA COVID-19 vaccine. The low transfection efficacy in the lung seriously hindered the development of inhaled LNP/mRNA vaccine\(^{45}\). A recent article illustrates some critical issues of inhaled LNP/mRNA design. Lokugamage et al.\(^{146}\) reported a cluster-based iterative screening approach for identifying and optimizing different chemical components in lipid nanoparticles for the lung delivery of LNP/mRNA vaccines. In that work, the authors found that the polyethylene glycol (PEG) molarity and the structure and charge of the helper lipid influence the mRNA delivery efficacy in the lung, and showed that LNPs with high PEG density and cationic lipids were more efficient than those with low PEG density because of the PEG steric effect and electronic expelling, which could resist the aggregation of LNPs during nebulization. It was also pointed out that the lung biology issues (e.g., mucus and physiological barriers in the lung) predominate and contributes simultaneously to lung delivery efficiency along with the factors of nebulization\(^{46}\). Similarly, Suberi, et al.\(^{147}\) found that different components and PEG density in the nanoparticles influence the transfection efficacy of the inhaled mRNA vaccine in vivo and pointed out the importance of formulation optimization for the inhaled mRNA vaccine delivery.

For blocking the binding between SARS-CoV-2 and ACE2, a liposomal nanotrapping platform was designed and functionalized with either recombinant ACE2 protein or neutralizing antibodies of SARS-CoV-2 and phagocytosis-specific phosphodiesterases on the surfaces (Fig. 5A)\(^{48}\). The inhaled nanotrapping could capture SARS-CoV-2 via binding with ACE2 or antibodies and then be cleared by macrophages mediated by phosphodiesterases. Notably, this nanotrapping exhibited good safety and inhibited pseudotyped SARS-CoV-2 infection in the dissected human lungs by intratracheal administration.

5.4. Polymeric nanoparticles for prevention and treatment of COVID-19

Compared with the cell membrane vesicles like exosomes, the non-cell-derived nanomaterials can be easier to prepare on a large scale in a controllable and simple manner. The inhaled polymeric nanoparticles developed against COVID-19 can be divided into various applications, including vaccination, blockage of the binding between SARS-CoV-2 and the S protein, and inhibition of SARS-CoV-2 replication.

Inhaled vaccines have the advantages in the prevention of SARS-CoV-2 infections as discussed above. However, biopharmaceutical obstacles and nasal clearing will largely limit the effectiveness of inhaled vaccines, but these limitations could be overcome by adjuvants and delivery systems\(^{49}\). Nanoparticle-based vaccines have become an innovative strategy for inhalation delivery of antigens\(^{50}\). For instance, an intranasally inhaled nanoparticle vaccine (RBD-TMC NPs) was developed, in which the antigenic spike RBD of SARS-CoV-2 was loaded into the N,N,N-trimethyl chitosan nanoparticles\(^{51}\). The RBD-TMC NPs induced a more robust local mucosal immunity, systemic antibody responses, and systemic immune responses compared with the free form of spike RBD antigen\(^{51}\). Another work is about the biodegradable poly (amine-co-ester) (PACE) polyplexes for an inhalable spike (S) protein mRNA vaccine for SARS-CoV-2\(^{47}\). The inhaled PACE nanoparticles of mRNA vaccine could systemically and locally induce immune responses in vivo; for example, the draining lymph node germinal center was activated, and S-specific memory B cells, antibody-secreting cells, circulating S-specific CD8\(^+\) T cells, and the lung-resident S specific tissue memory CD8\(^+\) T cells were induced. The PACE-mRNA vaccination protected K18-hACE2 mice from lethal viral challenges. Notably, PEG density in the PACE nanoparticles influences the inhaled mRNA vaccine efficacy, and the optimal PACE-PEG concentration (10%) for the inhaled mRNA vaccine delivery achieved high transfection targeted to the lung\(^{45}\).

In addition, blocking the binding between the S protein of SARS-CoV-2 and heparan sulfate on the cell surface is another promising strategy for interfering interaction between the S and ACE2 protein\(^{53}\). Heparin is an analog of heparan sulfate and the most commonly used anticoagulant drug, which may mimic heparan sulfate to interact with the S protein and inhibit the cell entry of SARS-CoV-2. A recent research exhibited that heparin could block SARS-CoV-2 infection by allosterically hindering S protein to bind with the host cell receptor, directly competing with heparan sulfate proteoglycan co-receptors to bind with the S protein, and preventing the S protein from cleavage by furin\(^{52}\). The in vitro data also revealed that heparin could inhibit the cell entry of SARS-CoV-2 and infection\(^{4}\). Meanwhile, it has been reported that heparin could be modified on the cell membrane and suppress the cell entry of SARS-CoV-2\(^{154}\). While these studies indicate that heparin has great potential against SARS-CoV-2 infection, there are only heparin injections clinically available. Pulmonary heparin delivery would be superior for the
early treatment of COVID-19, because of its self-administrability and reduced systemic exposure and bleeding risk. Our group developed an inhaled heparin nanodecoy to competitively bind the S protein, thus inhibiting the interaction between the virus and the cell surface heparan sulfate (Fig. 5B)\textsuperscript{154}. This nanodecoy significantly inhibited the infection of pseudovirus of SARS-CoV-2 and variants by intratracheal administration, and the neutralized pseudovirus as a complex with heparin nanodecoy was quickly phagocytosed and cleared by the macrophages in the lung\textsuperscript{154}.

Furthermore, small-molecule drugs with anti-SARS-CoV-2 effect can be loaded into the inhaled nanoparticles for improving targeting delivery and reducing side effects. Nanostructure aggregates and liposomes have been applied for preparing the inhaled nanomedicines of Remdesivir (an inhibitor of RNA polymerase)\textsuperscript{155,156}.

5.5. Inorganic nanoparticles for prevention and treatment of COVID-19

Inorganic nanoparticles (e.g., silver and gold nanoparticles) have been explored for the prevention and treatment of COVID-19. Silver nanoparticles have been widely applied in air, water, and surface disinfection because of their broad spectrum of antibacterial, anti-fungal, and anti-viral activity\textsuperscript{157}. Interestingly, the dynamics of silver nanoparticles by inhalation showed that only a small percentage of silver nanoparticles reached the lungs and most of them are more likely to remain in the upper respiratory tract\textsuperscript{158}. Inhaled silver nanoparticles have been investigated \textit{in vivo} to inhibit the infection of viruses, e.g., H3N2 influenza virus\textsuperscript{159} and respiratory syncytial virus\textsuperscript{160}. The application of silver nanoparticles against SARS-CoV-2 infection is potential through various anti-virus mechanisms (Fig. 5C)\textsuperscript{161}. Inhaled silver
nanoparticles for the treatment of COVID-19 were revealed to be feasible by a computation method\textsuperscript{162}, but the practicability of inhalation of silver nanoparticles for inhibiting SARS-CoV-2 infection needs to be further explored \textit{in vivo}. Another study reported an ultrathin two-dimensional CuInP\textsubscript{2}S\textsubscript{6} (CIPS) nanosheet could selectively bind with the S protein of wild-type SARS-CoV-2 and its variants (e.g., Delta and Omicron) against infection in the human ACE2-transgenic mice via intranasal instillation\textsuperscript{163}.

As for gold nanoparticles, an inhaled COVID-19 DNA vaccine carried by the chitosan-modified gold nano-star, triggered a strong immune response and induced the formation of memory T cells with good biosafety in the lung\textsuperscript{164}. In addition, gold nanoparticles...
have been studied to enable rapid point-of-care diagnosis and infection monitoring of SARS-CoV-2. However, due to the non-degradability, the safety concern of inorganic nanoparticles still is a formidable barrier against clinical translation, and further strict safety evaluation using a standard protocol as drug development must be carried out.

6. Inhaled antibodies and nanobodies for prevention and treatment of COVID-19

Antibody therapy (e.g., bamlanivimab/etesevimab and BRII-196/198) has been approved for clinical use to treat COVID-19, and monoclonal antibody-based treatments have shown therapeutic effects in patients with mild symptoms of COVID-19. However, monoclonal antibodies require very large doses (usually a few grams) for intravenous injection. Moreover, the concentrations of the i.v.-injected antibodies in the lung are hundreds of times lower than in the serum. Inhaled antibodies can partially overcome this problem. Furthermore, multivalent antibodies have shown enhanced potent efficacy in the treatment of COVID-19. Data from Wang et al. exhibited that the anti-SARS-CoV-2 efficacy of IgA monomers was two-fold less than that of IgG, but the anti-SARS-CoV-2 efficacy of IgA dimers was 15 times that of monomers IgA. IgM, another kind of mucosal antibody, is pentamers and has huge potential for the treatment of COVID-19. A study has shown that inhaled IgM antibodies have long-term retention in the nasal cavity and lung, and thus could protect and treat the infection of SARS-CoV-2. Meanwhile, the IgM antibodies also showed the inhibition of SARS-CoV-2 variants in vitro.

The production of monoclonal antibodies is both time-consuming and pricey. To reduce the cost of the antibody preparation, egg yolk immunoglobulin (IgY) which is the major serum antibody in avians and a counterpart to mammalian IgG, was developed for the neutralization of SARS-CoV-2 and variants. The IgY is thermostable and can be stored at 25 °C for three months. Meanwhile, nasal delivery of the IgY exhibited significant inhibitions of the infection of SARS-CoV-2 in the trachea and lung. Furthermore, the animals infected by SARS-CoV-2 treated with IgY could reduce the risk of cross infections between animals and humans at a low cost. The inhaled antibodies for the prevention and treatment of COVID-19 in the clinical trials are summarized in Table 2.

Nanobodies are a practical alternative to overcome the difficulties of antibody application in the treatment of COVID-19. Conventional antibodies produced by mammals have a heterotetrameric structure and are normally composed of two heavy chains and two light chains. Camels (e.g., alpacas, llamas, and dromedaries) and sharks can produce a heavy chain-only antibody, from which nanobody is derived as a single variable domain (VHH) (Fig. 6A). Although the lack of the variable domain of the light chain might be unfavorable in terms of antigen binding, nanobodies have various advantages such as small size, good stability, cost-effective production, high specificity, low immunogenicity, and identification of variable epitopes. Notably, due to the much smaller size than the full-length antibodies, nanobodies have the advantage of better tissue penetration and extravasation.

Nanobodies have shown great potential for COVID-19 treatment. The S protein, especially the RBD, is the main target for developing therapeutic nanobodies against SARS-CoV-2. Three strategies have been established by distinguishing the interactions between nanobodies and viral S proteins: receptor binding site, non-receptor binding site, and the overlapping cahoots. For receptor binding sites, nanobodies competitively bind to ACE2 receptor-associated epitopes on the RBD, which results in the abrogation of viral entry. Non-receptor binding site means nanobodies interact with a non-RBD part of the S protein, which can also hinder the interaction between ACE2 and the S protein by distorting the conformational freedom of the S protein. For the overlapping cahoots, nanobodies can interact with both the RBD and other parts of the S protein.

The biophysical properties of nanobodies, e.g., good stability and small size, could benefit inhalation administration. Inhaled nanobodies have been explored for COVID-19 treatment. For instance, an inhaled nanobody, Nb11-59, was developed (Fig. 6B), with a strong neutralization activity against SARS-CoV-2 by binding the RBD of the S protein (IC50 0.55 µg/mL). Meanwhile, Nb11-59 showed good stability after nebulization and could be produced on a large scale in Pichia pastoris. In another report, the in vivo efficacy of nanobodies (PiN-21 nanobodies) for the inhibition of the SARS-CoV-2 infection was evaluated. The intranasally inhaled PiN-21 nanobodies effectively achieved lung targeting delivery and exhibited high therapeutic efficacy against SARS-CoV-2 infection in Syrian hamsters. Importantly, intranasal inhalation of nanobodies can reach the lungs directly and retain a longer time compared to intraperitoneal (i.p.) or intravenous (i.v.) routes (Fig. 6C and D). The nanobodies after nebulization could retain a high inhibition efficiency against SARS-CoV-2 (Fig. 6E).

Frequent mutations of SARS-CoV-2 have substantially reduced the prevention effect of the currently used vaccines, and elicited antibodies with the high neutralizing activity to the wild-type SARS-CoV-2 showed a sharply decreased efficacy against the mutated strain of Omicron, thus often resulting in vaccine breakthrough infection. It is of great significance to develop antibodies with broadly neutralizing ability. It was reported that there are six different binding sites (Ia, Ib, Ia, Iib, Iic, and IV) on the RBD of the S protein that can interact with antibodies, identified by using cryo-electron microscopy (Fig. 6F). Both Ia and Ib sites overlap with the receptor-binding motif (RBM) with which ACE2 interacts. Neutralizing antibodies that bind to the Ia and Ib sites can prevent the S protein from entering the host cells by competing with ACE2. Ila, Iib, and Iic are cryptic sites of the RBDs and could bind with the neutralizing antibodies with open conformations of two or three RBDs. Neutralizing antibodies that bind to the Ila, Iib, and Iic sites do not compete with ACE2, but can create steric hindrance against the virus access to ACE2 and thus disabling the RBD binding with ACE2. Site IV, far from the core RBM of RBD, is structurally conserved and could be a target for antibody-drug development, and its affinity to a specific antibody is not affected by the RBD conformation. Antibodies bound to site IV do not compete with ACE2 too, but notably, could induce antibody-dependent cell-mediated cytotoxicity (ADCC). Therefore, the neutralizing antibody to site IV can yield a complementarity with the neutralizing antibody that binds to sites Ia and Ib as a combination therapy for neutralizing SARS-CoV-2 and mutant strains. So far, the ambivirumab (P2C–1F11, BRII-196), binding site Ia, and romisevirumab (P2B-1G5, BRII-198), binding site IV, have been included in the official guidance of COVID-19 treatment in China.

By searching the conserved sequences of the S proteins between the wild-type and mutant strains and through sequence comparison, it was found that the RBD mutation of Omicron mainly occurred in
the RBM region, but the cryptic epitopes hidden or partially hidden inside the trimeric interface and the lateral surface epitopes outside the trimeric interface are relatively conservative, compared with the wild-type S protein \textsuperscript{186}. Based on that, two humanized nanobodies (n3113v and n3130v) have been developed with the two conserved regions above, respectively, and then a small bispecific humanized nanobody (bn03) conjugate (MW 27 kDa) was obtained by connecting n3113v and n3130v with a flexible polypeptide linker composed of glycine and serine \textsuperscript{186}. The developed bn03 can bind and neutralize SARS-CoV-2 and the major mutants \textit{in vitro} and showed a significant neutralizing ability against SARS-CoV-2 \textit{in vivo} by pulmonary administration. Moreover, inhaled bn03 had a higher pulmonary concentration and longer retention than that by intravenous administration.

7. Perspectives

The world has still been struggling with the ongoing pandemic of COVID-19. This global crisis indicates that the development of vaccines and drugs for a quick response to an emerging virus outbreaks is essential for society. The inhaled nanotechnology is a promising measure to combat SARS-CoV-2 infection. On one hand, intranasal or orally inhaled delivery provides the benefit of specific drug distribution to the targeted site (e.g., nasal epithelium and lung) while minimizing systemic exposure; moreover, the easy-handling and self-administrable features offer great convenience for personal care. On the other hand, nanotechnology can improve drug solubility, stability, and drug bioavailability.

Innovative inhaled medicines for lung diseases have been developed and there are a range of opportunities for new inhaled drugs \textsuperscript{187}. Inhalation can be a useful intervention to prevent the spread of COVID-19. It should be noted that inhaled medicine relies on the technical support of inhalation devices, e.g., pressurized metered-dose inhalers (pMDIs), dry powder inhalers (DPIs), and nebulizers for inhalation. In addition, the potential safety concern of inhaled nanoparticles should be addressed, too. The inhaled nanoparticles enter the alveolar cells and lung-resident immune cells and possibly induce lung toxicity including the generation of oxidative stress, DNA damage, and inflammation \textsuperscript{188}.

The intranasal application provides another promising method for the prevention of viral infection. The applied drugs retained in the airway can neutralize the entering virus. The increased viral titer of Omicron in the upper respiratory makes it easy for Omicron to spread from infected patients. The intranasal application could be a promising route for preventative management. The studies have shown that the binding capacity between the S protein and the ACE2 is increased from the wide-type SARS-CoV-2 to Delta and Omicron variants \textsuperscript{189,190}. Therefore, the ACE2 protein can potentially inhibit the spread of SARS-CoV-2, especially variants.

It should be noted that variants of SARS-CoV-2 can cause a change in COVID-19 symptoms. Rates of infections and replications of Omicron are much faster than that of original SARS-CoV-2 and Delta variants in human bronchus tissues, while Omicron infects and replicates in lung tissues much slower than Delta and the original strain do \textsuperscript{191}. As a result, Omicron typically affects the upper respiratory tract but rarely develops into severe symptoms. Compared to the wide-type SARS-CoV-2 and Delta variants, Omicron is milder and less pathogenic. Patients with Omicron infections resulted in a lower rate of hospitalization (1.9% for Omicron vs. 2.6% for Delta), but an increased rate of upper respiratory tract symptoms (e.g., sore throat and hoarseness) \textsuperscript{192}. Therefore, due to the change in Omicron-related pathology, the therapeutic strategy and drug delivery should also be adjusted, \textit{i.e.}, for targeting the upper respiratory tract by using a proper formulation and administration route. Particles with different diameters can deposit in different regions of the respiratory system through oral inhalation \textsuperscript{193}. Therefore, fine-tuning the physical properties of inhaled nanodrugs could be a potential method for better improving the prevention and treatment of Omicron-related infection.

The approved vaccines have shown reduced effectiveness against the variants of SARS-CoV-2, especially Omicron. In addition to developing a new vaccine targeting the Omicron variant, improvement of the current vaccination efficacy could also be an alternative method. Considering the infection route of SRAS-CoV-2, mucosal immunity plays an essential role in vaccination protection. Yet, conventional intramuscular immunization is not an ideal way to elicit mucosal immunity (e.g., IgA secretion). In this sense, developing an inhaled vaccine could be a promising vaccination method for enhancing immunological protection against SRAS-CoV-2, of which there would be several advantages. First, self-administrable vaccination can relieve the burden on the healthcare system during the pandemic. Second, robust mucosal immunity can provide specific protection in the respiratory tract. Third, an inhaled vaccine can serve as a part of “hybrid immunization” along with other conventional i.m. injected vaccines. It has been reported that a hybrid immunization (e.g., i.m. + inhaled administration) can induce higher neutralizing antibody responses than a single route of immunization \textsuperscript{11}.

Additionally, the development of nanomaterials for reducing virus exhalation from patients will play an important role in reducing virus transmission. For example, masks based on nanomaterials can almost completely capture the aerosols containing SARS-CoV-2 \textsuperscript{194}. Such a design can also reduce the risk of virus infection for susceptible populations.

8. Conclusions

In this review, we focus on the role of orally inhaled and intranasal nanoformulations in the prevention and treatment of COVID-19. There is still a lack of understanding of the \textit{in vivo} fate of orally inhaled or intranasal nanomedicines. To address the complex challenges of applying orally inhaled and intranasal nanoformulations to combat COVID-19, it requires collaboration among researchers from different disciplines including pharmaceutical scientists, physicians, and mechanical engineering. The development of formulations in this field will not only combat the current pandemic but also promote better preparation for the future.

Acknowledgments

We are thankful for the support of the National Key Research and Development Program of China 627 (2021YFE0103100, China), National Nature Science Foundation of China (81925035), Shanghai Sci-Tech Innovation Initiative (194319030100 and 18430740800, China), and the Sanofi-SIBS Yong Faculty Award (China), and the Youth Innovation Promotion Association (China). ZIDD has been granted as High-level New R&D Institute.
References

1. Coronaviridae Study Group of the International Committee on Taxonomy of V. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5:536–44.

2. WHO. WHO coronavirus (COVID-19) dashboard. Available from: https://covid19.who.int/ (Accessed June 29).

3. U.S. Food and Drug Administration. FDA approves first COVID-19 vaccine. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine.

4. Food and Drug Administration Philippines. Whole virion, inactivated coronavirus vaccine [Covaxin]. Available from: https://www.fda.gov/whole-virion-inactivated-corona-virus-vaccine-covaxin/.

5. Food and Drug Administration Philippines. SARS-CoV-2 r5 protein nanoparticle vaccine [Covovax]. Available from: https://www.fda.gov/sars-cov-2-rs-protein-nanoparticle-vaccine-covovax/.

6. U.S. Food And Drug Administration. Coronavirus (COVID-19) update: FDA limits use of Janssen COVID-19 vaccine to certain individuals. Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-limits-use-janssen-covid-19-vaccine-certain-individuals.

7. Dai L, Gao GF. Viral targets for vaccines against COVID-19. Nat Rev Immunol 2021;21:73–82.

8. Sa-Nguanmoo N, Namdee K, Khongkhw M, Ruktanonchai U, Zhao Y, Liang XJ. Review: development of SARS-CoV-2 immunoenhanced COVID-19 vaccines with nano-platform. Nano Res 2022;15:2196–225.

9. Tiboni M, Casettari L, Illum L. Nasal vaccination against SARS-CoV-2: synergistic or alternative to intramuscular vaccines?. Int J Pharm 2021;603:120686.

10. Xu F, Wu S, Yi L, Peng S, Wang F, Si W, et al. Safety, mucosal and systemic immunopotency of an aerosolized adenovirus-vectorized vaccine against SARS-CoV-2 in chusus macaques. Emerg Microb Infect 2022;11:438–41.

11. Wu S, Huang J, Zhang Z, Wu J, Zhang J, Hu H, et al. Safety, tolerability, and immunogenicity of an aerosolised adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in adults: preliminary report of an open-label and randomised phase 1 clinical trial. Lancet Infect Dis 2021;21:1654–64.

12. Huang L, Chen Y, Xiao J, Luo W, Li F, Wang Y, et al. Progress in the research and development of anti-COVID-19 drugs. Front Public Health 2020;8:365.

13. Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine 2013;9:1–14.

14. Srivastava M, Srivastava N, Mishra PK, Malhotra BD. Prospects of nanomaterials-enabled biosensors for COVID-19 detection. Sci Total Environ 2021;754:142365.

15. Qiao Q, Liu X, Yang T, Cui K, Kong L, Yang C, et al. Nanomedicine for acute respiratory distress syndrome: the latest application, targeting strategy, and rational design. Acta Pharm Sin B 2021;11:3060–91.

16. Chhatwal J, Wang X, Ayer T, Kabiri M, Chung RT, Hur C, et al. Hepatitis C disease burden in the United States in the era of oral direct-acting antivirals. Hepatology 2016;64:1442–50.

17. Topalis D, Gillemot S, Sneeck R, Andrei G. Distribution and effects of amino acid changes in drug-resistant alpha and beta herpesviruses DNA polymerase. Nucleic Acids Res 2016;44:9530–54.

18. Choi YH, Han HK. Nanomedicines: current status and future perspectives in aspect of drug delivery and pharmacokinetics. J Pharm Invest 2018;48:43–60.

19. Abd Elkodous M, Olejodo SO, Morsi M, El-Sayyad GS. Nanomaterial-based drug delivery systems as promising carriers for patients with COVID-19. ACS Adv 2021;11:26463–80.

20. Stadler K, Masignani V, Eickmann M, Becker S, Abrigani S, Klenk HD, et al. SARS—beginning to understand a new virus. Nat Rev Microbiol 2003;1:29–18.

21. Petherick A. MERS-CoV: in search of answers. Lancet 2013;381:2069.

22. El-Atab N, Quiser N, Badghaish H, Shaikh SF, Hussain MM. Flexible nanoporous template for the design and development of reusable Anti-COVID-19 hydrophobic face masks. ACS Nano 2020;14:7659–65.

23. Hartog N, Faber W, Frisch A, Baus J, Bupp CP, Rajasekaran S, et al. SARS-CoV-2 infection: molecular mechanisms of severe outcomes to suggest therapeutics. Expert Rev Proteomics 2021;18:105–18.

24. Yang X, Wu C, Li X, Song Y, Yao X, Wu X, et al. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev 2020;7:1012–23.

25. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265–9.

26. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020;382:1199–207.

27. Kampf G. Potential role of inanimate surfaces for the spread of coronaviruses and their inactivation with disinfectant agents. Infect Prev Pract 2020;2:100044.

28. Zhang Y, Chen C, Zhu S, Shu C, Wang D, Song J, et al. Isolation of 2019-nCoV from a stool specimen of a laboratory-confirmed case of the coronavirus disease 2019 (COVID-19). China CDC Wkly 2020;2:123–4.

29. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 2020;158:1831–1833 e3.

30. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. The receptor-binding domain of SARS-CoV-2. Cell 2020;181:281–292 e6.

31. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260–3.

32. Guo M, Song W, Zhou H, Xu J, Chen S, Xiang Y, et al. Cryo-electron microscopy structures of the SARS-CoV-2 spike glycoprotein reveal a prerequisite conformational state for receptor binding. Cell Res 2017;27:119–29.

33. Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD, et al. SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. Cell 2020;183:1041–1057 e15.

34. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ Res 2000;87:E1–9.

35. Essalmani R, Jain J, Susan-Resiga D, Andreu O, Evagelidis A, Derbali RM, et al. Distinctive roles of furin and TMPRSS2 in SARS-CoV-2 infectivity. J Virol 2022;96:e0012822.

36. Zhang Q, Xiang R, Hua S, Zhou Y, Jiang S, Wang Q, et al. Molecular mechanism of interaction between SARS-CoV-2 and host cells and interventional therapy. Signal Transduct Target Ther 2021;6:233.
Localized delivery of nanomedicine and antibodies for combating COVID-19

37. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djanatian M, Franz J, Kuivinen S, et al. Neutrophil-1 facilitates SARS-CoV-2 cell entry and infectivity. Science 2020;370:856–60.

38. Wang S, Qiu Z, Hou Y, Deng X, Xu W, Zheng T, et al. AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. Cell Res 2021;31:126–40.

39. Adivitiya, Kaushik MS, Chakraborty S, Veleri S, Kateriya S. Mucociliary respiratory epithelium in molecular defense and susceptibility to pulmonary viral infections. Biology 2021;10:95.

40. Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kuijper EJ, et al. Amsterdam elite COVID-19 ICU patients with COVID-19. Thromb Res 2020;191:145–7.

41. Lotfi R, Kalmarzi RN, Roghani SA. A review on the immune responses against novel emerging coronavirus (SARS-CoV-2). Immunol Lett 2021;269:213–24.

42. Wadam Y, Cousin-frankel J, Kaiser J, Mataric C. How does coronavirus kill? Clinicians trace a ferocious rampage through the body, from brain to toes. Available from: https://www.science.org/content/article/how-does-coronavirus-kill-clinicians-trace-ferocious-rampage-through-body-brain-toes.

43. GISAID. Overview of variants/mutations. Available from: https://covar.sails.org/var/variants.

44. Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature 2021;592:616–22.

45. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Abbott S, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372.eaag3055.

46. Coronavirus (COVID-19) Infection Survey: characteristics of people testing positive for COVID-19, countries of the UK, 9 February 2021. Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/articles/coronavirus/covid19infectionsinthecommunityinengland/characteristicsofpeopleestimatingpositivcoronovc79inEngland9February2021 (Accessed 22 April).

47. Bager P, Wohlfahrt J, Fonager J, Rasmussen M, Albertsen M, Michaelsen TY, et al. Risk of hospitalisation associated with infection with SARS-CoV-2 lineage B.1.1.7 in England: an observational cohort study. Lancet Infect Dis 2021;21:1507–17.

48. Davies NG, Jarvis CI, Group CCW, Edmunds WJ, Jewell NP, Diaz-Ordaz K, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature 2021;593:270–4.

49. Wang P, Nair MS, Liu L, Betani S, Luo Y, Guo Y, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021;593:130–5.

50. Collier DA, De Marco A, Ferreira IATM, Meng B, Datir RP, Walls AC, et al. Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. Lancet Infect Dis 2021;21:856–80.

51. Pearson CAB, Russell TW, Davies NG, Kucharski AJ, Edmunds WJ, Eggo RM. Estimates of severity and transmissibility of novel South Africa SARS-CoV-2 variant 501Y.V2. 2021. Available from: https://cmmid.github.io/topics/covid19/sa-novel-variant.html.

52. Funk T, Pharris A, Spiteri G, Bundle N, Melidou A, Carr M, et al. Characteristics of SARS-CoV-2 variants of concern B.1.1.7, B.1.351 and P.1: data from seven EU/EEA countries, weeks 38/2020 to 10/2021. Euro Surveill 2021;26:2100348.

53. Wall EC, Wu M, Harvey R, Kelly G, Warchal S, Sawyer C, et al. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. Lancet 2021;397:2331–3.

54. Faria NR, Mellan TA, Whitaker C, Claro IM, Candido DDS, Mishra S, et al. Genomics and epidemiology of the P1 SARS-CoV-2 variant lineage in Manaus, Brazil. Science 2021;372:825–51.

55. Dejnirattisai W, Huo J, Zhou D, Zahradnık J, Supasa P, Liu C, et al. Antibody evasion by the P.1 strain of SARS-CoV-2. Cell 2021;184:2939–54.

56. Garcia-Beltran WF, Lam EC, St Denis K, Nitido AD, Garcia ZH, Hauser BM, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. Cell 2021;184:2372–83.

57. Dougherty K, Mannell M, Naqui O, Matson D, Stone J. SARS-CoV-2 B.1.617.2 (Delta) Variant COVID-19 outbreak associated with a gymnastics facility—Oklahoma, April-May 2021. MMWR Morb Mortal Wky Rep 2021;70:1004–7.

58. Ong SWX, Chiew CJ, Ang LW, Mak TM, Cui L, Toh M, et al. Clinical and virological features of SARS-CoV-2 variant Delta to antibody neutralization. Nature 2021;596:276–80.

59. Fisman DN, Tuite AR. Progressive increase in virulence of novel SARS-CoV-2 variants in Ontario, Canada. medRxiv 2021. Available from: https://doi.org/10.1101/2021.07.05.21260050.

60. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 2021;596:276–80.

61. Ranjan R. Omicron Impact in India: analysis of the ongoing COVID-19 third wave based on global data. medRxiv 2022. Available from: https://doi.org/10.1101/2022.01.09.22268969.

62. Nishiura H, Ito K, Anzai A, Kobayashi T, Piantanham C, Rodriguez-Morales AJ. Relative reproduction number of SARS-CoV-2 Omicron (B.1.1.529) compared with Delta variant in South Africa. J Clin Med 2021;11:30.

63. Wolter N, Jassat W, Walaza S, Welch R, Moutrie H, Groome M, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Lancet 2022;399:437–46.

64. Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. Comparison of outcomes from COVID infection in pediatric and adult patients before and after the emergence of Omicron. medRxiv 2022. Available from: https://doi.org/10.1101/2021.12.30.21268495.

65. Jassat W, Karim SA, Mudura C, Welch R, Oozugwu L, Groome MJ, et al. Clinical severity of COVID-19 in patients admitted to hospital during the omicron wave in South Africa: a retrospective observational study. Lancet Glob Health. 2022;10:e961–9.

66. Dejinrattisai W, Hoo J, Zhou D, Zahradnık J, Supasa P, Liu C, et al. SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. Cell 2022;185:467–84.

67. Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature 2022;602:654–6.

68. Mitchell JP, Berlinski A, Canisius S, Cipolla D, Dolovich MB, Gonda I, et al. Urgent appeal from international society for aerosols in medicine (ISAM) during COVID-19: clinical decision makers and governmental agencies should consider the inhaled route of administration: a statement from the ISAM regulatory and standardization issues networking group. J Aerosol Med Pulm Drug Deliv 2020;33:235–8.

69. Phase I/II clinical trial of recombinant novel coronavirus (COVID-19) vaccine (Adenovirus Type 5 Vector) for inhalation. Available from: https://clinicaltrials.gov/ct2/show/NCT04510233.

70. Ivermectin nasal spray for CV2019 patients. Available from: https://clinicaltrials.gov/ct2/show/NCT04510233.

71. Albarigi AH, Wang Y, Chang RYK, Quan DH, Wang X, Kalfas S, et al. Pharmacokinetics and safety of inhaled ivermectin in mice as a potential COVID-19 treatment. Int J Pharm 2022;619:121688.

72. Iwabuchi K, Yoshie K, Kurakami Y, Takahashi K, Kato Y, Morishima T. Therapeutic potential of ciclesonide inhaled for COVID-19 pneumonia: report of three cases. Int J Clin Pract 2018;72:2732017.

73. Guo Y, Bera H, Shi C, Zhang L, Cun D, Yang M. Pharmaceutical strategies to extend pulmonary exposure of inhaled medicines. Acta Pharm Sin B 2021;11:2565–84.
Zoulikha M, Xiao Q, Boafo GF, Sallam MA, Chen Z, He W. Pulmonary delivery of siRNA against acute lung injury/acute respiratory distress syndrome. Acta Pharm Sin B 2022; 12:600–20.

Griffin DE. Current progress in pulmonary delivery of measles virus vaccines. Expert Rev Vaccines 2014; 13:751–9.

Lane AP. Nasal anatomy and physiology. Clin Physiol Funct Imag 2000; 19:507–70.

Heyder J, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W. Deposition of particles in the human respiratory tract in the size range 0.005–15 μm. J Aerosol Sci 1986; 17:811–25.

Munkholm M, Mortensen J. Mucociliary clearance: pathophysiological aspects. Clin Physiol Funct Imag 2014; 34:171–7.

Nassini M, Schleif C, Lauenstein HD, Hussein R, Hoymann HG, Koch W, et al. A toxicological evaluation of inhaled solid lipid nanoparticles used as a potential drug delivery system for the lung. Eur J Pharm Biopharm 2010; 75:107–16.

Jones MC, Kumar A, Spina D, Forbes B, Page C, Dailey LA. In vivo safety and particoxinetics of inhaled nanomedicines. J Drug Deliv Sci Technol 2011; 21:339–46.

Singh S, Kanbar-Agha F, Sharafkhaneh A. Novel aerosol delivery devices. Semin Respir Crit Care Med 2015; 36:543–51.

Rose MA, Zielen S, Baumann U. Mucosal immunity and nasal influenza vaccination. Expert Rev Vaccines 2012; 11:595–607.

Zhu F, Zhang C, Chu K, Zhang L, Zhao H, Huang S, et al. Safety and immunogenicity of a live-attenuated influenza virus vector-based intranasal SARS-CoV-2 vaccine in adults: randomised, double-blind, placebo-controlled, phase 1 and 2 trials. Lancet Respir Med 2022; 10:739–48.

Keller LA, Merkel O, Popp A. Intranasal drug delivery: opportunities and toxicologic challenges during drug development. Drug Deliv Transl Res 2022; 12:735–57.

Grassin-Delhy S, Buenestado A, Naline E, Faisy C, Bouquet-Layez S, Couderc LJ, et al. Intranasal drug delivery: an efficient and non-invasive route for systemic administration: focus on opioids. Pharmacol Ther 2012; 143:366–79.

Jiao J, Zhang L. Influence of intranasal drugs on human nasal mucociliary clearance and ciliary beat frequency. Allergy Asthma Immunol Res 2019; 11:306–19.

Quadir M, Zia H, Needham TE. Toxicological implications of nasal formulations. Drug Deliv 1999; 6:227–42.

Choi HY, Lee YH, Lim CH, Kim YS, Lee IS, Jo JM, et al. Assessment of respiratory and systemic toxicity of Benzalkonium chloride following a 14-day inhalation study in rats. Part Fibre Toxicol 2020; 17:5.

Stanaland BE. Once-daily budesonide aqueous nasal spray for allergic rhinitis: a review. Clin Therapeut 2004; 26:473–92.

Newman B, Witzmann K. Addressing the regulatory and scientific challenges with generic orally inhaled drug products. Pharmaceut Med 2020; 34:93–102.

Sadik MW, Holz O, Ellinghusen BD, Faulenbach C, Muller M, Badorrek P, et al. Lung pharmacokinetics of inhaled and systemic drugs: a clinical evaluation. Br J Pharmacol 2021; 178:4400–51.

Wu X, Cheng L, Fu M, Huang B, Zhu L, Xu S, et al. A potent bispecific nanobody protects hACE2 mice against SARS-CoV-2 infection via intranasal administration. Cell Rep 2021; 37:109869.

Okuda T, Morishita M, Mizutani K, Shibayama A, Okazaki M, Okamoto H. Development of spray-freeze-dried siRNA/PEI powder for inhalation with high aerosol performance and strong pulmonary gene silencing activity. J Control Release 2018; 279:99–113.

FDA. Draft guidance for industry: bioavailability and bioequivalence studies for nasal aerosols and nasal sprays for local action. 2003. Available from: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070111.pdf (Accessed April 22).

Aljorga J, Andrade L, Medina M, Kirkov V, Arsova S, Li F, et al. Pharmacokinetic bioequivalence of two inhaled tiotropium bromide formulations in healthy volunteers. Clin Drug Invest 2016; 36:753–62.

Silkstone VL, Corlett SA, Chrystyn H. Relative lung and total systemic bioavailability following inhalation from a metered dose inhaler compared with a metered dose inhaler attached to a large volume plastic spacer and a jet nebuliser. Eur J Clin Pharmacol 2002; 57:781–6.

Somers GI, Lindsay N, Lowdon BM, Jones AE, Freathy C, Ho S, et al. A comparison of the expression and metabolizing activities of phase I and II enzymes in freshly isolated human lung parenchymal cells and cryopreserved human hepatocytes. Metab Dispos 2007; 35:1797–805.

Enlo-Scott Z, Backstrom E, Mudway I, Forbes B. Drug metabolism in the lungs: opportunities for optimising inhaled medicines. Expert Opin Drug Metabol Toxicol 2021; 17:611–25.

Hukkanen J, Pelkonen O, Hakkola J, Raunio H. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. Crit Rev Toxicol 2002; 32:391–411.

Anttila S, Hukkanen J, Hakkola J, Stjernwall T, Beane P, Edwards RJ, et al. Expression and localization of CYP3A4 and CYP3A5 in human lung. Am J Respir Cell Mol Biol 1997; 16:242–9.

Hukkanen J, Vaisanen T, Lassila A, Piiipari R, Anttila S, Pelkonen O, et al. Regulation of CYP3A5 by glucocorticoids and cigarette smoke in human lung-derived cells. J Pharmacol Exp Ther 2003; 304:745–52.

Oesch F, Fabian E, Landsiedel R. Xenobiotica-metabolizing enzymes in the lung of experimental animals, man and in lung human models. Arch Toxicol 2019; 93:3419–89.

Patel B, Rashid J, Alsan F. Aerosolizable modified-release particles of montelukast improve retention and availability of the drug in the lungs. Eur J Pharm Sci 2017; 96:560–70.

Zhu X, Kong Y, Liu Q, Lu Y, Xing H, Lu X, et al. Inhalable dry powder prepared from folic acid-conjugated docetaxel liposomes alters pharmacodynamic and pharmacokinetic properties relevant to lung cancer chemotherapy. Palm Pharmaco Ther 2019; 55:50–61.

Luo Y, Liu C, Qu Y, Fang N. Towards single-cell analysis for pharmacokinetics. Bioanalysis 2012; 4:453–63.

Forbes B, Erhardt C. Human respiratory epithelial cell culture for drug delivery applications. Eur J Pharm Biopharm 2005; 60:193–205.

Foster KA, Oster CG, Mayer MM, Avery ML, Audus KL. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. Exp Cell Res 1998; 243:359–66.

Erhardt C, Kneuer C, Fiegel J, Hanes J, Schaefer UF, Kim KJ, et al. Influence of apical fluid volume on the development of functional intercellular junctions in the human epithelial cell line 16HBE14o-l for implications of the use of this cell line as an in vitro model for bronchial drug absorption studies. Cell Tissue Res 2002; 308:391–400.

Gruenert DC, Finkbeiner WE, Widdicombe JH. Culture and transformation of human airway epithelial cells. Am J Physiol 1995; 268: L347–60.

Foster KA, Avery ML, Yazdanian M, Audus KL. Characterization of the Calu-3 cell line as a tool to screen pulmonary drug delivery. Int J Pharm 2000; 208:1–11.
116. Grainger CI, Greenwell LJ, Lockley DJ, Martin GP, Forbes B. Culture of Calu-3 cells at the air interface provides a representative model of the airway epithelial barrier. *Pharm Res (N Y)* 2006;23:1482–90.

117. Lombray C, Edwards DA, Preat V, Vanbever R. Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L1002–8.

118. Patel B, Gupta N, Ahsan F. Particle engineering to enhance or lessen particle uptake by alveolar macrophages and to influence the therapeutic outcome. *Eur J Pharm Biopharm* 2015;89:163–74.

119. Sun X, Zhang X, Ren X, Sun H, Wu L, Wang C, et al. Multiscale Co-reconstruction of lung architectures and inhalable materials spatial distribution. *Adv Sci* 2021;8:2003941.

120. Phalen RF, Hinds WC, John W, Lioy PJ, Lippmann M, Sun X, Zhang X, Ren X, Sun H, Wu L, Wang C, et al. Multiscale Co-reconstruction of lung architectures and inhalable materials spatial distribution. *Adv Sci* 2021;8:2003941.

121. Yan YY, Zhou WM, Wang YQ, Guo QR, Zhao FX, Zhu ZY, et al. Oxygen therapy to enhance or lessen particle uptake by alveolar macrophages and to influence the therapeutic outcome. *Eur J Pharm Biopharm* 2015;89:163–74.

122. El-Shennawy L, Hoffmann AD, Dashzeveg NK, McAndrews KM, Grainger CI, Greenwell LJ, Lockley DJ, Martin GP, Forbes B. Culture of Calu-3 cells at the air interface provides a representative model of the airway epithelial barrier. *Pharm Res (N Y)* 2006;23:1482–90.

123. Liu Q, Zhang X, Xue J, Chai J, Qin L, Guan J, et al. Exploring the intrinsic micro-nanoparticle size on their in vivo fate after lung delivery. *J Control Release* 2022;347:435–48.

124. van Niel G, D’Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol* 2018;19:213–28.

125. El-Shennawy L, Hoffmann AD, Dashzeveg NK, McAndrews KM, Mehli PJ, Cornish D, et al. Circulating ACE2-expressing extracellular vesicles block broad strains of SARS-CoV-2. *Nat Commun* 2022;13:405.

126. Grainger CI, Greenwell LJ, Lockley DJ, Martin GP, Forbes B. Culture of Calu-3 cells at the air interface provides a representative model of the airway epithelial barrier. *Pharm Res (N Y)* 2006;23:1482–90.

127. Kim HK, Cho J, Kim E, Kim J, Yang JS, Kim KC, et al. Engineered extracellular vesicles enriched with palmitoylated ACE2 as COVID-19 therapy. *Adv Mater* 2021;33:e2010347.

128. Kim HK, Cho J, Kim E, Kim J, Yang JS, Kim KC, et al. Engineered small extracellular vesicles displaying ACE2 variants on the surface protect against SARS-CoV-2 infection. *J Extracellular Vesicles* 2022;11:e12179.

129. Wu C, Xu Q, Wang H, Tu B, Zeng J, Zhao P, et al. Neutralization of SARS-CoV-2 pseudovirus using ACE2-engineered extracellular vesicles. *Acta Pharm Sin B* 2022;12:1523–33.

130. Wang J, Huang R, Xu Q, Zheng G, Qiu G, Ge M, et al. Mesenchymal stem cell-derived extracellular vesicles alleviate acute lung injury via transfer of miR-146a. *Crit Care Med* 2020;48:926–36.

131. Cloer C, Roussari L, Rochelle L, Petrie T, Welch M, Charest J, et al. Mesenchymal stromal cell-derived extracellular vesicles reduce lung inflammation and damage in nonclinical acute lung injury: implications for COVID-19. *PLoS One* 2016;11:e0259732.

132. Yan YY, Zhou WM, Wang YQ, Guo QR, Zhao FX, Zhu ZY, et al. Potential role of extracellular vesicles in COVID-19 treatment: opportunity and challenge. *Front Mol Biosci* 2021;8:699929.

133. Chu M, Wang H, Bian L, Huang J, Wu D, Fei F, et al. Nebulization therapy for COVID-19 pneumonia with embryonic mesenchymal stem cells-derived exosomes. Available from: https://doi.org/10.2139/ssrn.3678558.

134. Fang RH, Kroll AV, Gao W, Zhang L. Cell membrane coating nanotechnology. *Adv Mater* 2018;30:e1706759.

135. Liu Y, Luo J, Chen X, Liu W, Chen T. Cell membrane coating technology: a promising strategy for biomedical applications. *Nano-Micro Lett* 2019;11:100.

136. Zhang Q, Honko A, Zhou J, Gong H, Downs SN, Vasquez JH, et al. Cellular nanoparticles inhibit SARS-CoV-2 infectivity. *Nano Lett* 2020;20:5570–4.

137. Zhang H, Zhu W, Jin Q, Pan F, Zhu J, Liu Y, et al. Inhalable nanocatchers for SARS-CoV-2 infection. *Proc Natl Acad Sci U S A* 2021;118:e2102957118.
156. Sahakjipijarn S, Moon C, Koleng JJ, Christensen DJ, Williams Li RO. Development of remdesivir as a dry powder for inhalation by thin film freezing. *Pharmaceutics* 2020;12:1002.

157. Tran QH, Nguyen VQ, Le AT. Silver nanoparticles: synthesis, properties, toxicityology, applications and perspectives. *Adv Nat Sci* 2013;4:03001.

158. Andriamasinoro SN, Dieme D, Marie-Desvergne C, Serventi AM, Debia M, Haddad S, et al. Kinetic time courses of inhaled silver nanoparticles in rats. *Arch Toxicol* 2022;96:487–98.

159. Xiang D, Zheng Y, Duan W, Li X, Yin J, Shigdar S, et al. Inhibition of A/Human/Hubei/3/2005 (H3N2) influenza virus infection by silver nanoparticles in vitro and in vivo. *Int J Nanomedicine* 2013;8:4103–13.

160. Morris D, Ansar M, Speshock J, Ivanciuc T, Qu Y, Casola A, et al. Pilaquinga F, Morey J, Torres M, Seqqat R, Pina MLN. Silver nanoparticles as a potential treatment against SARS-CoV-2: a review. vol. 13. Wiley Interdiscip Rev Nanomed Nanobiotechnol; 2021, e1707.

161. Zachar O. Formulations for COVID-19 early stage treatment via silver nanoparticles inhalation delivery at home and hospital. *ScienceOpen Preprints* 2020. Available from: https://doi.org/10.14293/S2199-1006.1.SOR-.PPHBJEO.v1.

162. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-CoV, a neutralizing antibody cocktail, in outpatients with COVID-19. *N Engl J Med* 2021;384:238–51.

163. DeFrancesco L. COVID-19 antibodies on trial. *Nat Biotechnol* 2020;38:1242–52.

164. Wang Z, Lorenzi ICC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. *Sci Transl Med* 2021;13:eabh0315.

165. Liu Z, Xie X, Hinton PR, Liu X, Ye X, Muruato AE, et al. Nasal delivery of an IgM offers broad protection from SARS-CoV-2 variants. *Nature* 2021;595:718–23.

166. Fan W, Sun S, Zhang N, Zhang Y, Jiao P, Wang J, et al. Nasal delivery of thermostable and broadly neutralizing antibodies protects mice against SARS-CoV-2 infection. *Signal Transduct Target Ther* 2022;7:55.

167. Konning D, Zielonka S, Grzeschik J, Empting M, Valldorf B, Krah S, et al. Camelid and shark single domain antibodies: structural features and therapeutic potential. *Curr Opin Struct Biol* 2017;45:10–6.

168. van Hecke E, Allorys K, De Brabandere V, De Smedt T, Detalle L, de Fougherolles A. Nanobodies(R) as inhaled biotherapeutics for lung diseases. *Pharmaco Ther* 2017;169:47–56.

169. Asaadi Y, Joungheghi FF, Janani S, Rahbarizadeh F. A comprehensive comparison between camilid nanobodies and single chain variable fragments. *Biomark Res* 2021;9:87.

170. Chanter T, Chames P. Nanobody engineering: toward next generation immunotherapies and immunomaging of Cancer. *Antibodies* 2019;8:13.

171. Fan W, Sun S, Zhang N, Zhang Y, Jiao P, Wang J, et al. Nasal delivery of thermostable and broadly neutralizing antibodies protects mice against SARS-CoV-2 infection. *Signal Transduct Target Ther* 2022;7:55.

172. Konning D, Zielonka S, Grzeschik J, Empting M, Valldorf B, Krah S, et al. Camelid and shark single domain antibodies: structural features and therapeutic potential. *Curr Opin Struct Biol* 2017;45:10–6.

173. Van Hecke E, Allorys K, De Brabandere V, De Smedt T, Detalle L, de Fougherolles A. Nanobodies(R) as inhaled biotherapeutics for lung diseases. *Pharmaco Ther* 2017;169:47–56.

174. Asaadi Y, Joungheghi FF, Janani S, Rahbarizadeh F. A comprehensive comparison between camilid nanobodies and single chain variable fragments. *Biomark Res* 2021;9:87.

175. Chanter T, Chames P. Nanobody engineering: toward next generation immunotherapies and immunomaging of Cancer. *Antibodies* 2019;8:13.