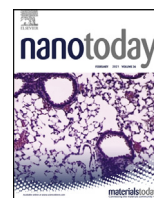




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Nanomedicine strategies to target coronavirus

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ABSTRACT

With the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, the middle east respiratory syndrome CoV (MERS-CoV) in 2012 and the recently discovered SARS-CoV-2 in December 2019, the 21st first century has so far faced the outbreak of three major coronaviruses (CoVs). In particular, SARS-CoV-2 spread rapidly over the globe affecting nearly 25,000,000 people up to date. Recent evidences pointing towards mutations within the viral spike proteins of SARS-CoV-2 that are considered the cause for this rapid spread and currently around 300 clinical trials are running to find a treatment for SARS-CoV-2 infections. Nanomedicine, the application of nanocarriers to deliver drugs specifically to a target sites, has been applied for different diseases, such as cancer but also in viral infections. Nanocarriers can be designed to encapsulate vaccines and deliver them towards antigen presenting cells or function as antigen-presenting carriers themselves. Furthermore, drugs can be encapsulated into such carriers to directly target them to infected cells. In particular, virus-mimicking nanoparticles (NPs) such as self-assembled viral proteins, virus-like particles or liposomes, are able to replicate the infection mechanism and can not only be used as delivery system but also to study viral infections and related mechanisms. This review will provide a detailed description of the composition and replication strategy of CoVs, an overview of the therapeutics currently evaluated in clinical trials against SARS-CoV-2 and will discuss the potential of NP-based vaccines, targeted delivery of therapeutics using nanocarriers as well as using NPs to further investigate underlying biological processes in greater detail.

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Introduction

The 21st century has so far experienced the emergence and epidemic of three major coronaviruses (CoVs): i) the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, ii) the middle east respiratory syndrome coronavirus (MERS-CoV) in 2012, and iii) the recently discovered SARS-CoV-2 in December 2019, which was first reported in the city of Wuhan, Hubei Province, China [1–4]. After their discovery in 1960 up until 2002, human CoVs (hCoVs) were mostly treated as a minor rather than a serious threat due to the fact that these viruses only caused minor respiratory symptoms. However, starting with SARS-CoV in 2002, different CoVs emerged that were associated with serious respiratory diseases including MERS-CoV and the recently discovered SARS-CoV-2. Whereas the incidence of SARS-CoV and MERS-CoV were comparably low with 8,096 and 2,494 reported cases, respectively, the incidence of SARS-CoV-2 is significantly higher [5,6]. Up to September 1st, 2020, around 25,251,334 cases have been reported worldwide with a total of 846,841 related deaths [7]. In particular, the rapid increase in cases over the last months causes immense challenges to health care systems and societies, resulting in more than 50 countries worldwide announcing a 'lockdown' period to limit social contact and decelerate the spreading of the virus. The high incidence and the drastic measures taken by the governments underline the need for therapeutic interventions against SARS-CoV-2. Different therapeutics and vaccines are being developed and tested at rapid speed to treat or contained SARS-CoV-2, of which some are already reaching clinical trials, stoking the hope for a fast solution [8]. This includes repurposing the use of existing therapeutics developed for the treatment of other diseases such as Remdesivir, Ivermectin, and others [8,9]. However, different promising therapeutics, such as hydroxychloroquine failed as potential treatment for SARS-CoV-2 in the clinic due to severe side effects or lack of efficacy [10], whereas other therapeutics such as Remdesivir still need more evaluation before being considered as successful treatment [11].

In the recent years, nanomedicine offered promising strategies to overcome limitations of current therapeutics by providing a platform that increases treatment efficacy, reduced side effects and enabling specific targeting to achieve the desired responses on cellular level [12–18]. On the one hand, nanocarriers can hereby be used to function as either delivery vehicles of a specific vaccine to achieve immunization of the host [12–14], or as nanocarriers to deliver different therapeutics to the target site prolonging their circulation time while protecting the therapeutics from degradation or by reducing side effects of non-encapsulated drugs [15,13–18]. In particular the use of liposomes as nanocarriers forms a promising approach that mimic the infection profile of viruses due to their high resemblance in structure, presenting a lipid bilayer surface and in high similarities in cellular uptake [19–21]. Another strategy can be the use of empty virus particles themselves to function as nanocarriers for a different loading such as the use of gene therapy [22,23]. The combination of aforementioned drugs or newly developed therapeutics with nanocarrier systems could form a promising strategy to deliver therapeutics or vaccine anti-

gens to target cells and thereby stop the life-cycle of CoVs or prevent disease.

In this review, we will critically discuss how nanomedicine can play a crucial role to develop effective treatments for CoVs. First, we will provide a description and classification of CoVs and their specific replication strategy within the host. We then focus on therapeutics that are currently tested to treat CoVs and their specific mechanism of action within the cell as well as describing the potential combination of these therapeutics with nanomedicine to increase their efficacy, reduce side effects and enable directed treatment of SARS-CoV-2 target sites and cells (Fig. 1).

Viral biology and clinical features

CoVs were first discovered in the 1960s and were classified under the subfamily of *Orthocoronavirinae*, within the family of *Coronaviridae*, which themselves form the largest family within the order *Nidovirales* [4,24,25]. SARS-CoV, MERS-CoV and SARS-CoV-2 and the lesser known HCoV-HKU1 and HCoV-OC43, all belong to the so-called β -CoVs, having a high potential to infect humans. CoVs are enveloped viruses containing a single-stranded positive-sense ribonucleic acid ((+)ssRNA) of 27 –

32 kb [24–26]. The genome is protected within the nucleocapsid and encodes for four to five structural proteins, depending on the type of CoV: spike (S), membrane (M), envelope (E), hemagglutinin esterase (HE) and nucleocapsid (N) proteins (Fig. 2). Where only the β -CoVs HCoV-HKU1 and HCoV-OC43 encode for the additional HE protein. It is speculated that differences in the S protein and in particular differences in the cleavage sites, including an additional furin cleavage site, causes the rapid spread of the novel SARS-CoV-2 when compared to other types of CoVs [25,27–30].

Replication strategy

The replication process of CoVs within target cells is a crucial step in the infection and disease progression and one of the major targets for possible therapeutic intervention. As the exact mechanism of CoV infection and replication is already discussed elsewhere, this section will focus on key aspects and targets in this process that form the basis for therapeutic interventions that will be described in the next section of this review [1,25,28,31–33].

The life cycle of CoVs in host cells is similar (Fig. 3), although certain aspects between SARS-CoV, MERS-CoV and SARS-CoV-2 are different resulting in slightly different uptake mechanisms and processing within the host cells. The initial attachment of the virion with the host cell is driven by interaction between the S protein and the specific receptor [28]. SARS-CoV and SARS-CoV-2 bind to the cellular receptor angiotensin-converting enzyme 2 (ACE2) present primarily in the lung, but also in endothelial cells of arteries and veins, the intestine mucosal cells, the tubular epithelial cells of the kidney and the renal tubules as well as cerebral neurons and immune cells [28,33–40]. MERS-CoV binds to the cellular receptor dipeptidyl peptidase 4 (DPP4), also known as cluster of differentiation 26 (CD26), present on epithelial cells in the kidney, alveoli, small intestine, liver, prostate and on activated leukocytes as well as human dendritic cells, T-cells and macrophages, demonstrat-

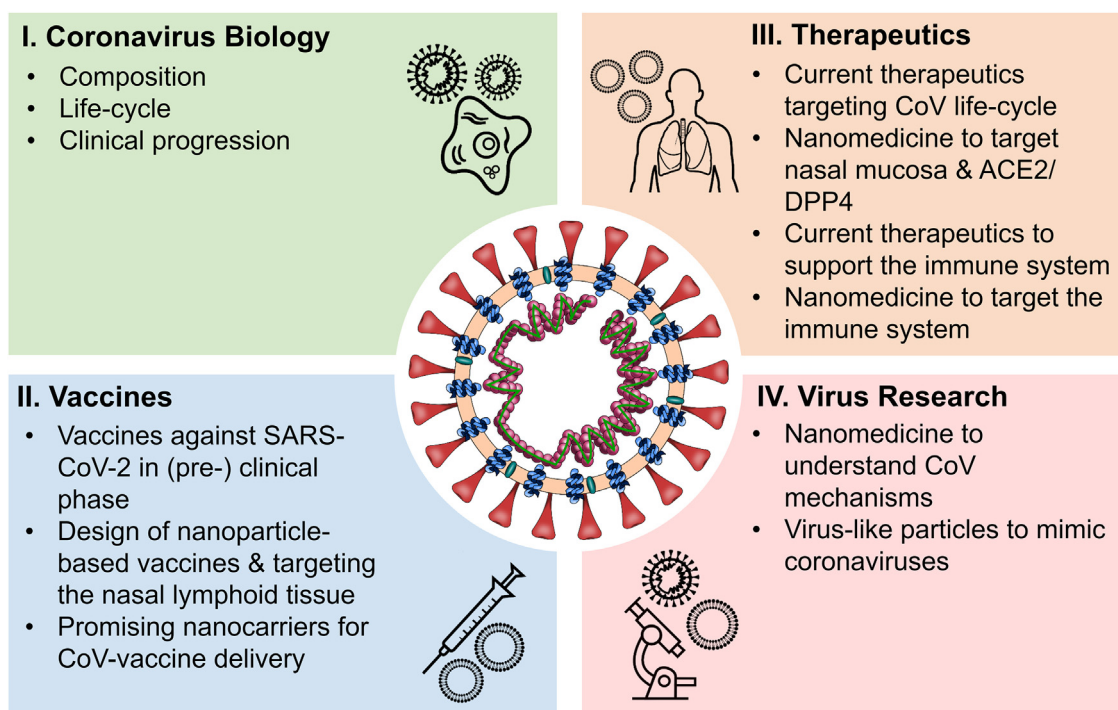


Fig. 1. Schematic overview of the different topics covered in this review.

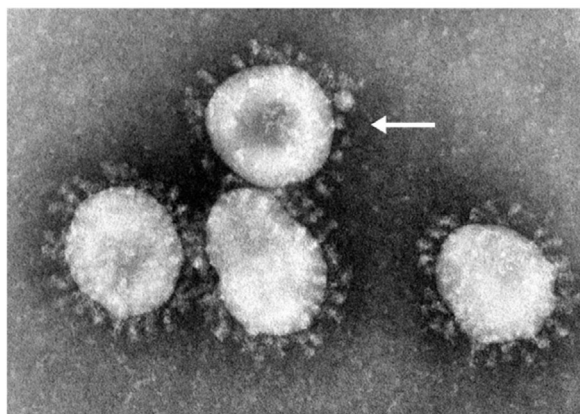
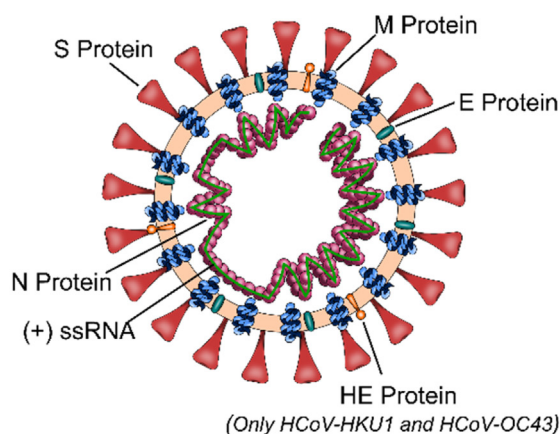


Fig. 2. **Structure of coronaviruses.** Schematic representation of a coronavirus describing structural proteins and the viral genome and representative electron microscopy image of SARS-CoV displaying the characteristic crown-like surface (arrow indicating single virion). Photo credit to Dr. Fred Murphy, reproduced under Creative Common Attribution License CC-BY 4.0 from D.N. Valencia ³⁰.

ing how this virus might be able to affect the immune system and facilitate immune evasion [28,41–49].

After successful fusion of the viral envelope with the host cell membrane, the viral genome is released into the cytoplasm of the host, where the viral RNA is translated resulting in the production of the RNA replicase-transcriptase complex also referred to as RNA-dependent RNA polymerase (RdRP) [28]. This complex generates intermediate full-length negative-sense (–) RNA copies, which are later used as templates for full-length positive sense (+) RNA genomes. Further translation leads to the production of viral proteins. Next, viral nucleocapsids are formed from viral genomic RNA and N-proteins in the cytoplasm followed by budding of the nucleocapsid and structural proteins in the lumen of the endoplasmic reticulum – golgi intermediate compartment (ERGIC) to form novel virions, which are then released (egressed) by exocytosis. This process is reported to be similar for all types of CoVs, including the novel SARS-CoV-2.

Clinical features of coronavirus infections

General symptoms of an infection with CoVs, and in particular with the novel SARS-CoV-2 (related disease names ‘coronavirus disease’ or COVID-19) include fever, cough, shortness of breath and fatigue, which are similar to SARS and MERS, but can also include headache, haemoptysis or diarrhea [50–52]. So far an exceedingly high number of patients experience (severe) pneumonia up to acute respiratory distress (ARDS), severe sepsis or multiple organ dysfunction [11,50,52]. Moreover new evidences are found that COVID-19 is also related to gastrointestinal symptoms, which might directly be related to viral infections of the intestine due to the high expression of ACE2 receptor within gastrointestinal epithelial cells forming a direct target for the virus [53,54]. Recent studies furthermore displayed the potential of CoVs to infect the central nervous system, explaining symptoms such as headache, nausea and vomiting in some patients [55,56]. Remark-

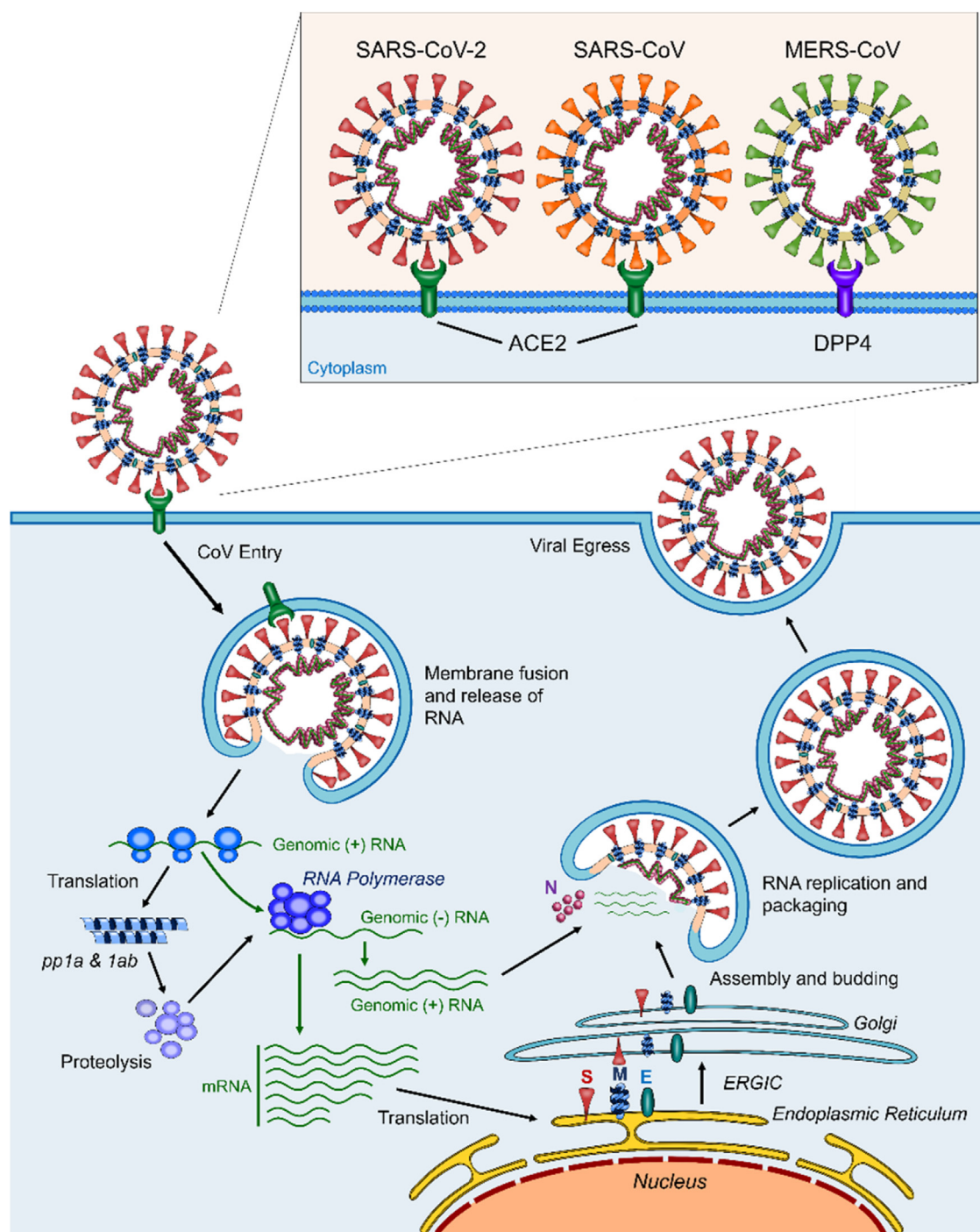


Fig. 3. Replication strategy of SARS-CoV, MERS-CoV and SARS-CoV-2. Schematic representation of (i) attachment of the virus to the specific receptor (ACE2 for SARS-CoV and SARS-CoV-2 and DPP4 for MERS-CoV), (ii) virus cell entry via endocytosis, (iii) fusion of the viral envelop with the endosomal membrane and release of viral RNA, (iv) translation of non-structural proteins including the RdRP and RNA replication, (v) translation of structural viral proteins at the endoplasmic reticulum and the assembly of a new virion in the ERGIC, (vi) packaging of replicated RNA into the novel virions and finally (vii) viral egress.

able for COVID-19 patients is also the high amount of inflammatory cytokines and chemokines, which eventually leads to the cytokine release syndrome (CRS) [57–59]. Severe forms of CRS, in particular in combination with ARDS, can lead to severe multi-organ failure and eventually the patient's death. Although CRS is a life-threatening and severe diagnosis for the patient, the underlying mechanisms and cytokines and chemokines involved, such as interleukins 1 or 6 (IL-1, IL6) or tumor necrosis factor α (TNF α) are well understood, facilitating the use of already known drugs in the treatment of this syndrome.

Therapeutic interventions and nanomedicine strategies

To date, there are no existing antiviral drugs that are known to efficiently treat COVID-19 progression, although, there are several therapeutics in clinical trials that show the potential to be effective against the virus progression. Most of these drugs were originally designed for the treatment of other infections and are now evaluated for their potential to be used for COVID-19 (re-purposing). Currently, around 300 clinical trials falling under the European Union Drug Regulating Authorities Clinical Trials

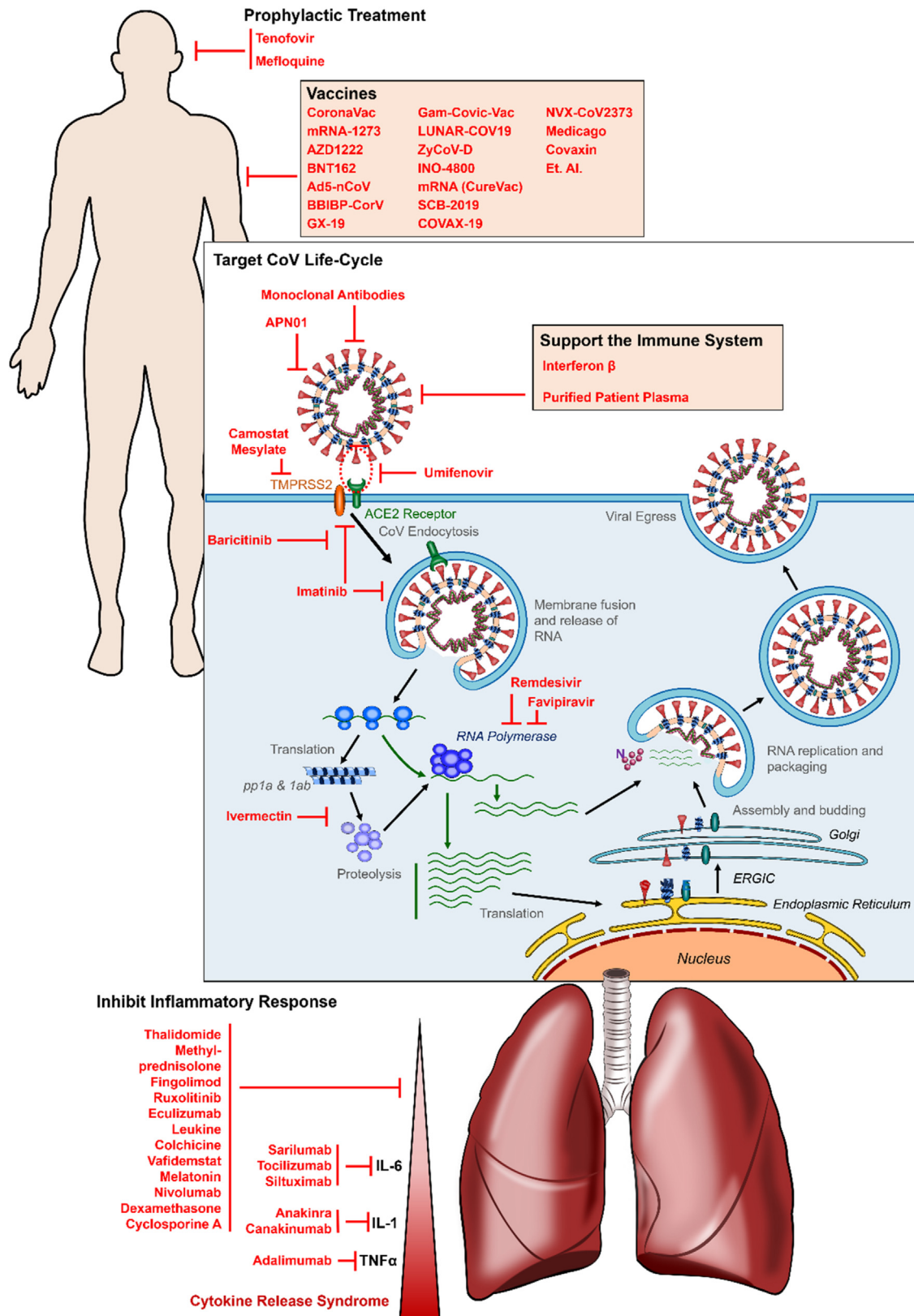


Fig. 4. Schematic representation of potential target sites for SARS-CoV-2 therapeutics currently evaluated in ongoing clinical trials. Starting with (i) prophylactic treatments, (ii) vaccines, (iii) therapies that target the life cycle of SARS-CoV-2 before and after entering the host cells, (iv) treatment to support the immune system and (v) therapeutics to reduce COVID-19 symptoms including anti-inflammatory therapeutics to inhibit cytokine release syndrome as well as therapeutics to avert long-term tissue damage.

Database (EudraCT) are listed in the EU Clinical Trials Register focusing on the treatment or prevention of COVID-19 [8]. In general, these treatments can be divided into four categories: (i) vaccines against SARS-CoV-2, (ii) therapies targeting the CoV's life-cycle, either blocking cell entry or inhibiting the viral cycle within the host cell, (iii) therapies to target the immune response and (iv) other treatments, including prophylactic treatments or preventing long-term lung damage (Fig. 4).

The re-purposing of such drugs does not only display a major reduction in the necessary time to develop a treatment against COVID-19 as these drugs have often been tested extensively before, but scientists can also benefit from the already existing knowledge of how these drugs function in patients and the risk of potential side-effects. For instance, hydroxychloroquine, which was considered one of the most promising therapeutics against SARS-CoV-2 infections, failed in recent clinical trials due to severe side-effects, which have been known and reported for years [60–62]. Nonetheless, the mechanism of function of this drug makes it highly interesting for the use in CoV infections. Whereas, the drug alone did not succeed, the combination of this drug with nanomedicine strategies might be able to reduce known side-effects and overcome limitations in efficacy to make this drug interesting again for the treatment of SARS-CoV-2 [63].

Over the last decade, nanomedicine, describing the use of colloidal carriers, also called nanocarrier systems, has found broad application in pharmacology and aimed to improve the treatment of several diseases [64]. In particular cancer research has rapidly facilitated the use of such nanocarriers to render drug delivery more efficient and specific [15]. There are several advantages in the use of nanocarriers for the delivery drugs, including (i) enhancing the solubility of certain drugs, (ii) the controlled sustained-release of drugs providing a long-term treatment or high drug exposure, (iii) the suitability to deliver macromolecules, which are protected from degradation within the body or protected from clearance by the immune system, (iv) a general decrease in side-effects, (v) the potential increase drug internalization by cells as well as (vi) the capability to target specific cells [65]. Furthermore, nanocarriers can be altered in their size, shape, charge or surface chemistry facilitating the fabrication of tailorable biological properties. Moreover, nanocarrier systems can be administered via different routes, such as sub-cutaneous or muscular injections, via oral or intranasal administration and are capable of penetrating capillaries and mucosal surfaces [66]. These characteristics make nanocarriers also highly interesting for the treatment of CoVs, either by delivering vaccines and stimulate the immune response, or by delivering drugs to infected cells to improve their efficiency and specificity.

Over the last years several different materials have been extensively investigated for the use as drug delivery systems including polymeric nanoparticles (NPs), facilitating the use of polymers such as poly(lactic-co-glycolic acid) (PLGA), poly(γ -glutamic acid)(γ -PGA), polystyrene or poly-alkyl acrylate; inorganic NPs, based on gold, silica or carbon; liposomes and lipid-based NPs or virus-like particles (VLPs), which is based on the implementation of viral proteins to form carriers (Fig. 5). The advantages and disadvantages of the different systems are extensively discussed elsewhere [13,22,66]. This review will mainly focus on the potential of lipid-based NPs as well as virus-like NPs for the use in CoV-targeted NP-based vaccine (NPb-V) due to the high similarity of such systems with natural viruses. Such virus-mimicking NPs are able to display similar characteristics as viruses in term of size and performance within the body, promoting their capability to follow the same mechanism of entering and spreading throughout the body as viruses. In this section, we will summarize different drugs and therapeutics that are currently evaluated for the treatment of SARS-CoV-2 infections and take a detailed look on different nanocarrier

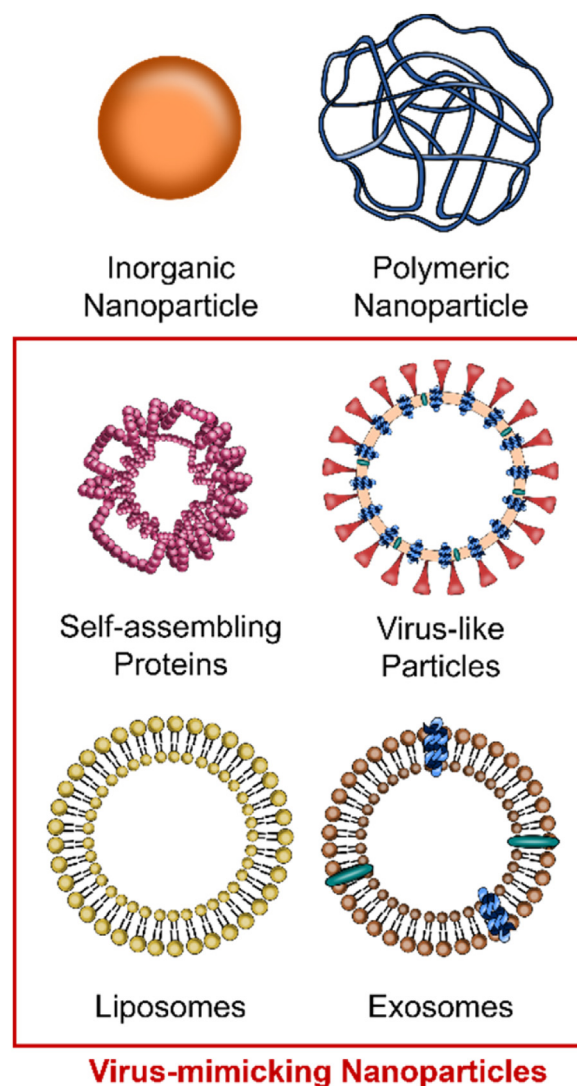


Fig. 5. Different nanoparticles for vaccine/ drug delivery. Schematic representation of different nanoparticles able to deliver vaccines or therapeutics towards target cells, highlighting nanoparticles that display virus-mimicking properties.

systems with the potential to render these therapeutics more efficient and safer.

Vaccines against CoVs and the role of nanomedicine

Arguably the earliest point of therapeutic intervention is the clearance of the virus before it can even infect target cells or spread throughout the body. Vaccines allow for such a type of intervention with the potential to provide a long-lasting effect against CoVs, however, the development of vaccines can take several months up to years to reach the market, leaving vaccines as a strategy to prevent outbreak of SARS, MERS or COVID-19 in the future. Nonetheless, due to the rapid sequencing of the SARS-CoV-2 genome, different vaccine candidates have already been developed that are evaluated in clinical trials.

Vaccines basically introduce specific viral antigens to the body, which are also produced in patients undergoing the disease, however, in a safe fashion for the patient [67]. Such antigens are often presented on the cell surface of so called antigen presenting cells (APCs), in particular dendritic cells, embodied in the major histocompatibility complex (MHC) I and II [68]. These presented antigens are crucial for the adaptive immune system, which rec-

ognized such antigens as hostile invaders and further produces antibodies or trigger T cells to kill the invader. Memory B cells furthermore develop virus specific antibodies on its cell surface, which upon recognition of the virus, trigger a fast immune response to clear the viral infection. Several different types of vaccines are currently in use depending on the type of infection, such as live attenuated vaccines, inactivated vaccines, conjugated vaccines, or more recently DNA or RNA vaccines [26]. Despite its presence for nearly 20 years, so far no effective vaccine against human CoVs could be developed due to inefficiency of the vaccine or induced side-effects upon treatment, demonstrating the need of a novel strategy to target CoVs [69].

In particular the importance of the S protein in CoVs, made it a promising target for vaccines [70]. Currently different clinical trials are running to investigate the efficacy of mRNA, DNA and non-replicating adenovirus vector-based vaccines. Moderna's mRNA-1273, BioNTech's BNT162a1, b1, b2 and c2, Arcturus Therapeutics' LUNAR-COV19 and an unnamed vaccine candidate by CureVac are mRNA vaccines that target the S protein of CoVs or specific regions within. Similarly, Inovio Pharmaceuticals' INO-4800, Genexine's GX-19 and Zydus Cadila's ZyCoV-D are DNA vaccines targeting the S protein of CoVs [8]. Both, RNA and DNA vaccines have several advantages compared to conventional vaccines, in particular lower productions costs and simple purification, however, the delivery of such vaccine to the target cells is a challenge due to stability and specificity, which makes them suitable for nanomedicine-based delivery systems which will be discussed at a later stage in this review. The University of Oxford in collaboration with AstraZeneca recently presented a different vaccine to tackle CoVs composed on a non-replicating adenovirus vector able to replicate the S protein of SARS-CoV-2 called AZD1222 (formerly ChAdOX1) demonstrating a different way for vaccine development. Similarly Ad5-nCoV from CanSino Biologics and Gam-COVID-Vac from the Gamaleya Research Institute facilitate the same strategy to find a vaccine candidate against SARS-CoV-2. Recently, the use of inactivated CoVs has also shown efficacy to induce immunity and are currently in phase III clinical trials including CoronaVac from Sinovac or two vaccine candidates developed by Sinopharm in collaboration with the Wuhan Institute of Biological Products or the Beijing Institute of Biological Products. Furthermore, protein-based vaccines such as NVX-CoV2373 from Novavax or COVAX-19 from Vaxine PTY Ltd. are currently in evaluation. In addition, several other candidates are currently developed that have not yet reach clinical phases [71].

Nanomedicines to deliver vaccines against CoVs

With the increasing interest in RNA and DNA-based vaccines, the combination of such vaccines with nanocarriers has become an interesting strategy to overcome limitations of the current delivery of these vaccines. The combination of RNA with nanocarriers has been an effective approach to deliver small interfering RNA (siRNA) for the treatment of several diseases such as malignancies, infections, autoimmune disease and neurological diseases [72]. For instance, the aforementioned RNA-based vaccine mRNA-1273 from Moderna is based on the combination of the RNA drug substance with a nanocarrier system, which will be discussed later in this section in greater detail. Similarly, nanocarriers can be used for the delivery of antigens avoiding premature degradation of such molecules in the body, as well as assist in the translation of these molecules into functional immunogens avoiding potential side-effects caused by the treatment [12].

In general, the delivery of vaccines using NPs systems (NPb-Vs) can be divided into two main strategies: (i) NPb-Vs where the antigen or RNA/DNA is encapsulated within the nanocarrier and (ii) attaching antigens on the nanocarrier surface exposing it to the surrounding (Fig. 6) [13,66]. The encapsulation of antigens or RNA/DNA

vaccine within nanocarriers mainly aims on protecting the antigens from proteolytic degradation and to allow directed targeting of the vaccine towards APCs [13]. These APCs take up the NPb-V and either process the induced antigens towards the cell surface, or translate the infused RNA or DNA to the respective antigen before implementing it into the surface [73]. Hereby, RNA might be preferable when comparing to DNA as RNA can be directly translated in the cell cytoplasm while DNA must reach the nucleus of the target cell first [73]. Encapsulating RNA or DNA into nanocarriers can be a promising approach to create NPb-V against CoVs. As aforementioned, different RNA or DNA vaccines are currently evaluated in clinical trials, which makes the combination of these ribonucleases with nanocarriers a highly promising strategy. Furthermore, NPb-V based on antigen encapsulation also show the potential to exert a local depot effect, which prolongs the exposure of antigens towards the immune cells [74]. The second strategy to create NPb-Vs is to directly attach or conjugate antigens onto the nanocarrier surface [66]. In such way the NPb-V is not directly aimed to bring a cargo to APCs but to mimic the virus itself. For instance, presenting S protein specific antigens on top of a nanocarrier could trigger a specific immune response towards these antigens, forming a promising strategy against CoVs. In particular, the purification of immunoglobulins from patient plasma, as aforementioned, in combination with a nanocarrier could create a promising NPb-V against CoVs.

Design considerations of NP-based vaccines. CoVs are thought to be spread from human host to host by respiratory droplets, mainly produced when an infected person sneezes or coughs. Recently it has been shown that the major route for SARS-CoV-2 to enter the body is through the nasal cavity, in particular through mucosal epithelial cells including mucus-producing goblet cells and ciliated cells, forming the main target site of corona infections [75,76]. The present lymphoid tissue, called nasal-associated lymphoid tissue (NALT), comprises of lymphoid follicles (B-cell areas), interfollicular areas (T-cell areas), macrophages and dendritic cells, which when activated or triggered support the clearance of infectious agent either by activated killer cells or by the production of antigen-specific antibodies presented in the mucus layer, which forms a liquid layer on top of the epithelial cells of the mucosa [66,77]. As a result of such response, the NALT is a promising target for vaccines against respiratory virus and more interestingly for CoVs. Ideally, NPb-Vs against CoVs should follow the same path as CoVs to reach the NALT and trigger a specific mucosal immune response. To achieve this, NPb-Vs have to be designed presenting similar kinetics as viruses within the host, which can be dependent on the size, shape, charge and general surface properties. The size of CoVs has been measured to be between 50–150 nm using electron cryomicroscopy, with the average diameter size of 82–94 nm (excluding the spikes) [78]. Most nanocarrier systems are aimed to achieve a size between 20–200 nm, making them suitable to mimic the size of CoVs as well to follow the same mechanism of infection to the NALT [14,15].

Another crucial aspect when targeting the NALT, besides the size, is the route of NPb-V administration. It has been shown that intravenous or intramuscular injections of vaccines induce a systemic immunity, however, only a weak mucosal response [66]. The application of vaccines via the nasal route, similar to the infection mechanism of CoVs, is therefore more promising to trigger a systemic immunity on the one hand, while also inducing immunity on the mucosal surfaces. It has been shown that the nasal route of vaccine administration resulted in increased proliferation of antigen-specific lymphocytes, increased cytokine production as well as induction of antigen-specific antibodies when compared to subcutaneously or systematic administration [79–81]. A promising strategy to administer NPb-Vs is the use of a nasal spray that deliv-

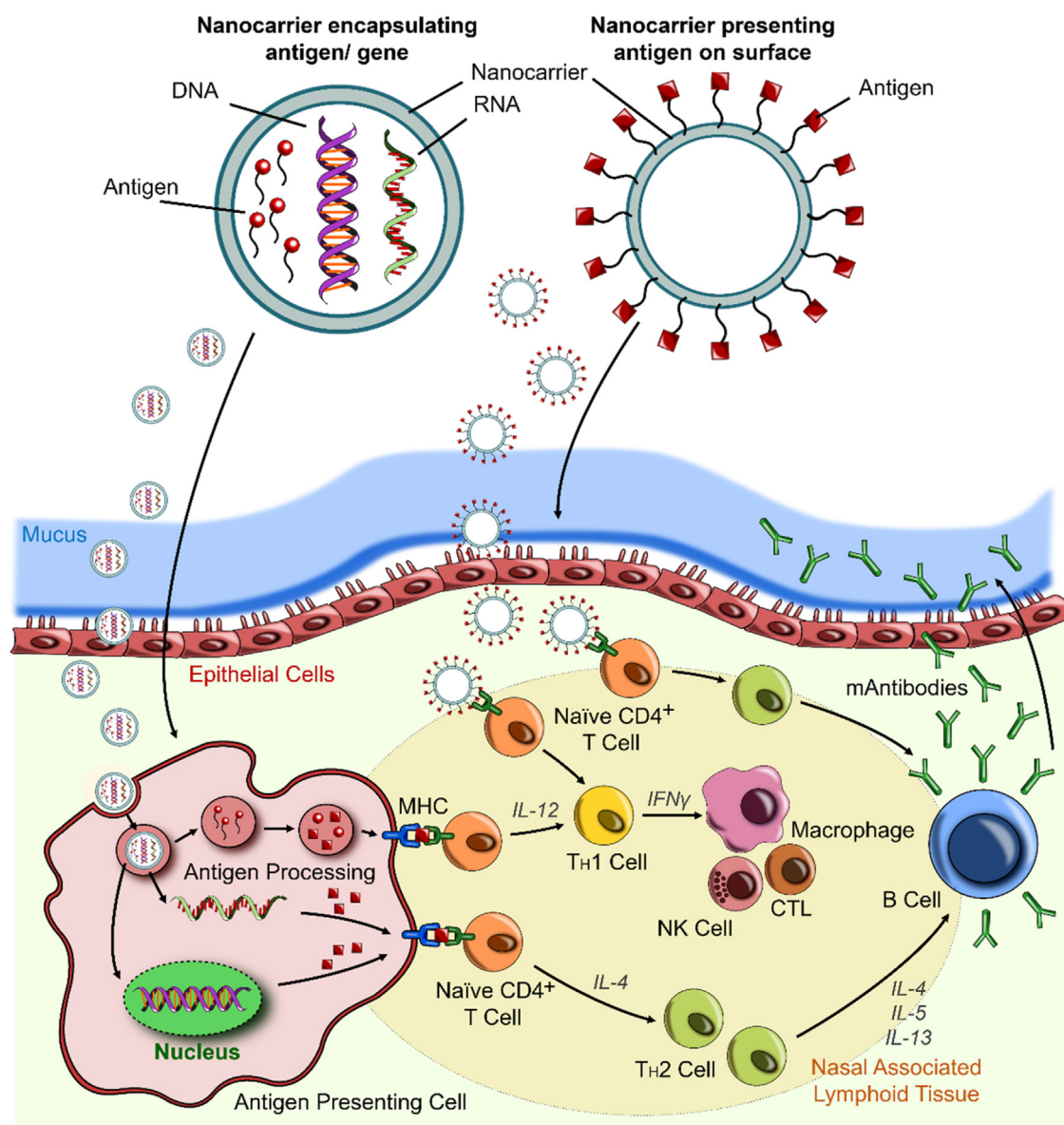


Fig. 6. Strategies to deliver vaccines against CoVs using nanoparticles. Schematic representation of (i) nanoparticle-based vaccines encapsulating a gene or antigen for the delivery towards antigen-presenting cells which causes an immune reaction or (ii) nanoparticle-based vaccines with an antigen conjugated to the nanocarrier surface to function as antigen-presenting carriers themselves.

ers the vaccine directly to the target site, however, such systems currently display several limitations including insufficient antigen uptake in the mucosa, rapid mucociliary clearance or toxicity of the administered therapeutics as well as challenges in stable fabrication of NPb-Vs for nasal delivery [82]. Nonetheless, different strategies have emerged over the last decades facilitating the use of a nasal spray for vaccine delivery. One of the first nasal vaccines that reached clinical trials was developed in 2010, describing the use of dried live attenuated measles vaccines [83,84]. Although this vaccine did not involve nanocarriers system, this study demonstrated the potential use nasal spray for application. Besides sprays, nasal application of therapeutics can also involve droppers or needleless syringes, which are less complicated in the fabrication process [85].

Remarkable for the clinical symptoms of the recent SARS-CoV-2 outbreak is also the high infection potential of the intestine, central nervous system as well as indications for infections of endothelial cells in blood vessels and capillaries as aforementioned. Although the nasal route of access is considered the primary infection site of SARS-CoV-2 and potentially the most promising route to design

a vaccine, so far it is unknown if other potential infection routes are possible. In such way, besides targeting the NATL, NPb-Vs might also be designed to target lymphoid systems in the intestine or to reach immune cells in the brain to block viral infections of the central nervous system, of which the nasal administration could be a promising strategy as different drug delivery approaches use nasal administration to reach the brain environment [86]. To potentially treat all target sites, a combination of intranasal and subcutaneously or intravenously injected NPb-Vs could form a promising strategy for SARS-CoV-2. For instance, a recent study demonstrated that intranasal combined with intravenous injection of the antigen keyhole limpet hemocyanin in mice resulted in the increased production antibodies against this antigen when compared to either injection alone [87]. However, subcutaneous injection did not demonstrate similar beneficial effects. A similar study to investigated the combined injection of intranasal and subcutaneous vaccine injection against influenza also did not show a significant additional value of subcutaneous injection compared to the nasal administration alone [88], demonstrating in particular the

combination with an intravenous injection forms a promising strategy. Such combinational strategies should be taken into account in SARS-CoV-2 due to the rapid spread of the virus away from the main target site towards other secondary sites.

Promising nanocarriers for efficient vaccine delivery. Since the outbreak of SARS-CoV in 2002, several different vaccine candidates in combination of nanocarriers have been developed (Table 1), however, only with the recent outbreak of SARS-CoV-2, such vaccines are now reaching clinical phases due to high urgency to develop a novel vaccine. Arguably, the most straight-forward strategy to fabricate a NPb-V to mimic an actual virus' mechanism of body entry, is the use of viral components as basis for the nanocarrier. A NPb-V approach that has recently been used to target respiratory viruses, in particular human respiratory syncytial virus (RSV), is the use of self-assembling protein NPs (SAPNs) which are 20–100 nm sized nanocarriers that are based on the oligomerization of monomeric proteins [89]. For instance, the N protein of RSV has been used to create self-assembled sub-nucleocapsid ring structures that provoked an antigen-specific T-cell response towards the protein. *In vivo* examination in a RSV BALB/c mouse model displayed enhanced immunity towards RSV after treatment [90]. In a later stage, these N protein structures were combined with palivizumab, a FcII-protein antagonist, to further enhance immunity against RSV [91]. In a similar fashion, the N protein of RSV was combined with the ectodomain of the influenza virus A matrix protein 2 (M2e) to induce immunity towards the influenza (H1N1) virus [92]. Furthermore, this study demonstrated the advantage of nasal administration when compared to subcutaneous injection. The use of SAPNs has also shown application in CoVs, demonstrating the potential of such strategies to be used in the novel SARS-CoV-2 as well. For example, the purified S protein of CoVs self-assembling into micellular NPs in combination with a Matrix M1 adjuvant was used to induce immunity towards CoVs in BALB/c mice. It was found that the mice displayed enhanced presence of neutralizing antibodies after vaccination [93]. In a recent approach, a similar strategy of using purified S protein-based NPs formulated with an adjuvant (Aluminum) displayed a similar response in MERS-CoV [94]. Currently the use micellular NPs based on the S protein in combination with a Matrix M1 adjuvants, in a study led by Novavax (NVX-CoV2373), is evaluated as potential vaccine for SARS-CoV-2 demonstrating the potential use of S protein-based vaccines for CoVs [8,95].

Similar to SAPNs, VLPs are based on viral proteins, in particular viral capsid proteins, that self-assemble into spherical nanocarriers of 20–200 nm. Such VLPs have successfully been tested for their vaccination capability in influenza as well as RSV. For instance, VLPs comprised of A/PR8/34 (H1N1) hemagglutinin and matrix (M1) were investigated for their immunogenic potential in influenza after nasal administration in mice [96]. Similarly, VLPs comprising of multiple ectodomains of matrix protein 2 (M2e5x VLPs) have been evaluated for induction of influenza immunity using nasal administration [97]. Both studies displayed enhanced immunity towards influenza after treatment, which showed the potential of VLPs in vaccine applications. Chimeric VLPs, comprising of proteins from different types of viruses, have also shown the potential to induce immunity. A VLP comprised of the F protein or G protein of RSV and M1 protein of influenza displayed enhanced immunogenic potential against RSV in mice [98]. Although not demonstrated in SARS-CoV, VLPs based on MERS-CoV viral proteins have been demonstrated to induce immunity in mice. In this study, MERS-CoV VLPs were generated by using a chimeric approach of combining canine parvovirus VP2 structural gene with the receptor binding domain (RBD) of MERS-CoV to mimic the S protein binding site of this virus [99]. Intramuscular injection of these chimeric VLPs displayed increased immunity against MERS-CoV, demonstrating the

potential of this VLP against MERS-CoV infections. In a more recent study, MERS-CoV VLPs were prepared including the full S protein of MERS-CoV to induce immunity by generating VLPs using insect cells [100]. Although this approach lacks the evaluation *in vivo* so far, VLPs expressing the full S protein of CoVs, might demonstrate increased immunity compared to specific sequences such as the RBD. Currently Medicago, a company from Quebec City, Canada, is evaluating a VLP-based vaccine candidate against SARS-CoV-2 aiming to start a phase I clinical trial including 180 patients soon [101].

Lipid-based NPs, such as liposomes or solid-lipid NPs (SLNs), display high similarities to virus particles resembling a similar surface structure as viruses, which can additionally be chemically modified. Although the use of liposomes in NPb-V against respiratory viruses is limited, the use of these systems is highly favorable in vaccine development because the carriers can be altered to achieve similar characteristics as viruses without the use of virus-specific peptides. 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) liposomes containing either the adjuvants monophosphoryl lipid A or trehalose 6,6' dimycolate or short synthetic peptides which have been derived from conserved regions of pathogen-derived proteins have been shown to induce immunity against influenza in mice when nasally administered, demonstrating the use of liposomes as NPb-V for nasal administration [102]. The same strategy has been applied to induce immunity against SARS-CoV by coupling synthetic peptides mimicking the N protein of SARS-CoV that were based on HLA-A*0201 transgenic mice and recombinant adenovirus for expressing these peptides onto the surface of liposomes [103]. They observed induced CoV-specific T lymphocyte activity as well as enhanced viral clearance in HLA-A*0201 transgenic mice after treatment, demonstrating a potential strategy to use liposomes combined with virus-specific antigens as NPb-V. In particular antigens purified from patient plasma combined with lipid-based NPs forms a promising strategy to create immunity towards CoVs in the near future. A different advantage of using these NPs for the development of NPb-Vs, is their unmet capability to encapsulate RNA and DNA, one of the major investigated type of vaccines at the moment. The use of liposomes has been widely applied for the delivery of different RNA cargos, for example of siRNA as well as messenger RNA (mRNA), demonstrating how these system are able to project the cargo encapsulated while being rapidly taken up by target cells [72,73]. It has been proven that mRNA liposome complexes were able to infect the lung tissue and translate the induced mRNA into functional proteins, demonstrating the use of liposomes for efficient gene delivery [104]. Currently one of the most promising strategies to develop a vaccine against SARS-CoV-2 is based on nanocarrier systems encapsulating RNA to enhance their stability and performance such as Moderna's mRNA-1273. This therapeutic is based on the encapsulation of the RNA drug substance within a SLN. Although their therapeutic is administered intramuscularly and not via nasal administration, it demonstrates the potential of combining RNA-based vaccines with nanocarrier systems to enhance their performance. Furthermore, a current clinical trial led by Arcturus Therapeutics evaluates the combination of self-replicating RNA with lipid NPs as potential vaccine candidate for SARS-CoV-2 following a similar strategy as Moderna further demonstrating the high potential of lipid-based systems for vaccine delivery [105]. Two additional studies led by the University of Washington (HDT-301) and CanSino Biologicals (NP-based Ad5-nCoV), respectively, facilitate the same strategy to combine RNA vaccine with lipid-based nanocarriers to effectively deliver the vaccine candidates towards the target site [106].

A different nanocarrier, which has recently been investigated for the use as NPb-V, are extracellular vesicles or exosomes [107]. Exosomes are vesicles that are released from cells upon fusion of multivesicular bodies with the plasma membrane, liberating intra-

Table 1
Overview of different nanoparticle-based vaccines for coronaviruses and the advantages and disadvantages of the respective nanoparticle platform.

Nanocarrier	Characteristics & Pros/ Cons	Target	Formulation	Vaccine	Stage	Ref
Self-assembling protein NPs (SAPNs)	(Purified) proteins that self-assemble into NPs (e.g. micelles). Pro: Simple preparation, surface charge and size prevent clearance, kinetically stable. Con: High costs prevent industrial upscaling, short-term stability in vivo.	SARS-CoV & MERS-CoV	Protein-protein micellular nanoparticles based on CoV S protein, administered with Matrix M1 adjuvant	S protein	Early Stage	[93]
		MERS-CoV	Protein-protein micellular nanoparticles based on CoV S protein, formulated with aluminum adjuvant	S protein	Early Stage	[94]
		SARS-CoV-2	Protein-protein micellular nanoparticles based on CoV S protein	S protein	Phase I C.T.	Novavax, [95]
Virus-like particles (VLPs)	Based on structural viral proteins (e.g. capsid proteins) that form spherical NPs. Often obtained from plants, bacteria or inactive human viruses. Pro: High biocompatibility and biodegradability, mimics virus's distribution in vivo due to similar characteristics. Con: Difficult to scale up industrially, depending on source can cause side-effects or unwanted immune reactions.	MERS-CoV	VP2 structural protein of canine parvovirus with receptor binding domain (RBD) of MERS-CoV	RBD (S protein)	Early Stage	[99]
		MERS-CoV	Nanovesicles based on structural E, M and S proteins obtained from expressing Bm5 cells.	S protein	Early Stage	[100]
		SARS-CoV-2	Plant-based	Not clarified	Phase I C.T.	Medicago, [101]
Lipid-based NPs	NPs based on a lipid bilayer with a hydrophilic compartment inside or as solid-lipid NPs. Pro: Simple preparation, long physical stability, sustained release, high control on surface properties and size, high feasibility for industrial upscaling, highly suitable for RNA/DNA delivery. Con: Limited loading capacity, burst release of load.	SARS-CoV	Liposomes composed of dioleoyl phosphatidyl choline, dioleoyl phosphatidyl ethanolamine, dioleoyl phosphatidyl glycerol acid and cholesterol with synthetic peptide conjugated to surface	N protein	Early Stage	[103]
		SARS-CoV, MERS-CoV & SARS-CoV-2	Solid-lipid NPs composed of ionizable lipid, SM-102, with cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000)	RNA (encodes for S protein)	Phase III C.T. (for SARS-CoV-2)	Moderna
		SARS-CoV-2	Solid-lipid NPs composed of ionizable lipid, ATX, cholesterol, DSPC, DMG-PEG2000	RNA (not clarified)	Phase I/II C.T.	Arcturus Therap.
		SARS-CoV-2	Lipid-inorganic NPs composed of superparamagnetic iron oxide NPs within a hydrophobic squalene core	RNA (encodes S protein)	Pre-clinical	Uni. Of Wash., [106]
		SARS-CoV-2	Unspecified lipid NP	Non-replicating adenovirus type 5 (S protein)	Early Stage	CanSino
Exosomes	Vesicles released from cells upon fusion of multivesicular bodies with the plasma membrane. Pro: High biological relevance and compatibility, can be modified to express specific protein in membrane, highly suitable for RNA/DNA delivery. Con: Complex fabrication process, not suitable (yet) for industrial upscaling, high costs and labor intensive.	SARS-CoV	Exosomes obtained from 293 T cells transfected with CoV S protein-expressing plasmid.	S protein	Early Stage	[110]

luminal vesicles into the extracellular milieu which are further called exosomes [108]. As the compositions of exosomes are similar to the body's own cells, they are widely considered non-immunogenic, which makes them highly promising for gene or drug delivery [109]. Furthermore, exosomes have recently been demonstrating their potential for the delivery of RNA and DNA for vaccination purposes [107]. Furthermore, similar to liposomes, exosomes can be modified to express viral antigens. In 2007, it was demonstrated that transfecting 293 T cells with S protein-expressing plasmids resulted in exosomes that included the S protein into their compositions [110]. Animal experiments using BL57/6 mice showed induced neutralizing antibody levels upon treatment, clearly showing how exosomes can be used as NPb-V. However, as exosomes are produced by cells, the amounts that can be fabricated are currently limited.

Targeting CoV's life-cycle and nanomedicine strategies

Therapies that directly target the CoVs life-cycle can have different targets or sites of action, including blocking the CoV-specific receptor to prevent CoV binding, the fusion of the viral envelop with the cell membrane, the viral proteases which among others facilitate the generation of the RdRP, or directly inhibit the RdRP. In this subsection, we will describe the different therapeutics categorized based on their specific site of action, before discussing the potential of nanocarrier systems to make the delivery of these therapeutics more specific and efficient.

Blocking receptor-mediated cell entry (extracellular)

Arguably the earliest strategy of intervention besides governmental lockdown or a vaccine, is avoiding the cell entry of the virus by blocking the CoV-specific receptors. Two drug candidates are currently evaluated that can block the entry of CoVs into the host cells – Umifenovir (brand name: Arbidol) and Camostat mesylate. Umifenovir, which so far is only licensed as a broad-spectrum antiviral drug in Russia and China, has shown to block different life-cycle steps of the influenza virus including blocking the virion attachment to the cell by interacting with the viral S protein. This makes Umifenovir particularly interesting for blocking the cell entry for SARS-CoV, MERS-CoV and SARS-CoV-2 [111]. Camostat mesylate is another therapeutic that shows promising application for the treatment for SARS-CoV, MERS-CoV and SARS-CoV-2 by inhibiting TMPRSS2, eventually blocking the S protein cleavage and therefore the fusion with the cell membrane [112]. Furthermore, Camostat mesylate has also shown suitability to be combined with nanocarriers for drug delivery, making it a promising candidate for future targeted delivery applications [113]. Similarly the inhibition of furin could also block the cleavage of the S protein and the mediated cell entry, however such approaches are so far not investigated in clinical trials. Nonetheless, in particular due to the additional furin-dependent cleavage site in the novel SARS-CoV-2, the inhibition of furin could form a promising approach to block SARS-CoV-2 cell entry. Starting in 1994, different studies have investigated the potential of furin inhibition in viral infections, however, such approaches have not reached clinical trials [114]. Also a study which successfully blocked furin in Ebola infections, did not show a reduction in the viral replication *in vitro*, demonstrating that Ebola might have different mechanisms of cell entry besides furin-mediated S protein cleavage [115]. However, as differences between Ebola and SARS-CoV-2 might alter the efficiency of therapeutics, the inhibition of furin might form a functional approach in CoVs, which needs further investigation. For instance, Remdesivir, one of the most promising therapeutics against SARS-CoV-2, which will be discussed in more detail in a later section of this review, did not show efficacy in Ebola but promising results in SARS-CoV-2 clinical trials.

Besides drug candidates, neutralizing monoclonal antibodies (mAbs) form another promising therapeutic to prevent cellular entry of the virus. Since the outbreak of SARS-CoV and MERS-CoV several different antibody candidates have been developed that show the capability to block cell entry by binding to different sites of the S protein and attenuate binding of the virion to the specific ACE2 or DPP4 receptor for SARS-CoV / SARS-CoV-2 or MERS-CoV respectively. Such candidates for SARS-CoV and MERS-CoV have been discussed in detail elsewhere [24]. Due to the high similarities in cell entry and binding site, mAbs that show promising application for SARS-CoV might also form a promising strategy to target SARS-CoV-2 [116]. In particular, the mAbs 80R, CR3014, 201 and 68 have shown effective blocking of SARS-CoV to the ACE2 receptor in small animal models [117–123]. Promising mAbs for MERS-CoV, which have proven their performance *in vivo*, include 4C2, m336, MERS-GD27, MCA1, CDC2-C2 and 7D10 [124–134]. Recently, the human monoclonal 47D11 antibody has been identified to bind to the SARS2-S-S1_B domain of the S protein in SARS-CoV and SARS-CoV-2, inhibiting the binding to the cell receptor ACE2 [135]. *In vitro* studies have shown that this monoclonal antibody can inhibit viral uptake in Vero cells transfected with SARS-CoV and SARS-CoV-2, however, did not affect the uptake of MERS-CoV. Clinical trials involving such neutralizing antibodies are currently performed to ensure patient's safety and efficacy. In particular the combination of antibodies with nanocarriers might form a promising strategy to enhance the stability of these antibodies *in vivo* as well as allow for a targeting approach to the site of interest. Such combinations have already shown beneficial characteristics for the treatment of different diseases compared to antibodies alone [136].

Another approach, that is currently evaluated in clinical trials, is the use of soluble human ACE2 called APN01. Recent studies have shown that human recombinant ACE2 can block the uptake of SARS-CoV-2 in human blood vessel organoids and human kidney organoids as well as reduce the recovery of SARS-CoV-2 from Vero cells by a factor of 1.000–5.000 displaying promising strategies to block early stages of SARS-CoV-2 infections [137]. Furthermore, as SARS-CoV is employing the ACE2 receptor, the overall ACE/ACE2 balance is disrupted leading to higher levels of angiotensin II (Ang II) in COVID-19 patients, which goes along with severe lung injury and ARDS [138]. APN01 has shown the potential to reduce Ang II levels in the blood by covering the virus and thereby reconstituting the ACE/ACE2 balance [139]. Due to the combined working of APN01 it might form a promising therapeutic for use in SARS-CoV-2 applications.

Blocking cell entry and fusion of the virus envelop with the cell membrane (intracellular)

Chloroquine (CQ) and its more soluble and less toxic metabolite hydroxychloroquine (HCQ) were first used as prophylactic treatment for malaria and were long considered a promising treatment for SARS-CoV-2 due to its broad spectrum of functions [30,140–146]. However due to severe side effects of CQ/HCQ several clinical trials failed and eventually studies were discontinued by the WHO [147–149]. Despite this fact, the combination of HCQ and nanocarriers, might reduce the found side effects and facilitate the use of HCQ in the treatment of SARS-CoV-2. The combination of CQ with SLNs for example has shown promising performance in the treatment of CQ-resistant malaria in the past [150]. Mefloquine, a drug which is considered to be similar to CQ is currently evaluated as prophylactic treatment for SARS-CoV-2 infections, however, the exact mechanism of action is not completely understood which limits its direct use [140].

Other potential drugs that are able to inhibit virus endocytosis are Imatinib and Baricitinib. Imatinib on the one hand is protein-kinase inhibitor that inhibits the bcr-abl tyrosine kinase, which has been shown to be involved in the cell entry and fusion of the viral

envelope and the cell membrane in hepatitis C and ebola, and might be similarly involved in the life-cycle of CoVs [151–153]. Baricitinib, on the other hand, is a Janus kinase inhibitor, which was previously used in the treatment of rheumatoid arthritis. It might also be used for blocking CoV cytosol due to its affiliation with the AP2-associated protein AAK1 [154,155].

Blocking viral proteases

Viral proteases are crucial for the generation of non-structural viral proteins, such as the RdRP as well as transport of viral proteins to the nucleus [9]. Ivermectin is an anti-parasitic agent, which has previously been used for human immunodeficiency virus (HIV) and dengue virus [156]. It dissociates the IMP α / β 1 homodimer related with the nuclear transport of viral proteins. Ivermectin additionally showed to reduce viral RNA in Vero cells up to 5000 fold demonstrating its potential for the use in CoVs [157]. Another therapeutic that was mentioned in relation with the treatment of SARS-CoV-2 is the HIV therapeutic Darunavir, which, however, is not confirmed as potential treatment for CoVs and is still in premature trials to show its potential as stated by the manufacturer Janssen [158].

Inhibiting the RNA-dependent RNA polymerase

The RdRP is a crucial protein in the life-cycle of CoVs and thereby blocking the RdRP is a promising strategy to stop the viral spreading within the body and treat CoVs. One of the most promising treatments against SARS-CoV-2 and other CoVs is Remdesivir, a therapeutic developed by Gilead Sciences (USA) under the brand name GS-5734 [159]. Although it did not show efficacy against its original target Ebola, it was proven to be safe for patient use, which facilitated rapid clinical testing against CoV [160]. Furthermore, studies have proven its efficacy to inhibit the replication SARS-CoV and MERS-CoV in primary human airway epithelial cell cultures as well as being effective against bat CoVs and circulating contemporary human CoVs. Its efficacy has also been demonstrated *in vivo* in a SARS-CoV mouse model [161]. Remdesivir has been shown to be effective *in vitro* using SARS-CoV-2 infected Vero cells. A recent study performed in the US was able to show a recovery time of patients from 15 days to 11 days in the treatment groups as well as a drop in patient mortality from 11.6 % in the placebo to 8 % in the treatment group, demonstrating its potential to also treat the novel SARS-CoV-2 [162].

Favipiravir, a therapeutic designed for treating influenza, displays a similar mechanism of action as Remdesivir by inhibiting the RdRP, however, it is less experimentally supported compared to Remdesivir [163]. Nonetheless, Favipiravir has been recently approved by the National Medical Products Administration of China for the treatment of COVID-19 patients after a clinical trial where Favipiravir was combined with interferon- α showed clinical benefits of the drug combination [164,165]. Furthermore, Tenofovir, a different RNA transcriptase inhibitor, is currently in evaluation for the use a prophylactic treatment [166].

Nanomedicine strategy to target pathological cells

As aforementioned, nanocarriers are promising for the stable delivery of drug candidates towards target cells avoiding rapid clearance by the immune system while preventing side-effects [18,64]. Due to variety of different nanocarriers available and the possibility to tune these to fit the drug load as well as the target, most of the aforementioned drug candidates against CoV can be included into nanocarriers to target the lung, prevent early degradation or clearance as well as avert side effects [167]. In fact, different studies of drug-loaded nanocarriers have been reported in the past, involving CQ-loaded SLNs to treat CQ-resistance malaria [150], Tenofovir-loaded PLGA NPs to induce long-acting prevention of HIV vaginal transmission [168] or Ivermectin-loaded SLNs to facilitate transdermal delivery for the treatment of sca-

bies [169]. Similarly different nanocarriers have been evaluated for the delivery of antibodies [170,171]. These studies demonstrate that the combination of currently evaluated drug candidates with nanocarrier systems is feasible and capable to improve the drug characteristics and properties. Due to the big variety of possible candidates and drug-nanocarrier combinations, this review will focus on possible delivery strategies that are capable to deliver drugs towards CoV infected cells as well as cells involved in the COVID-19 disease progression.

Targeting the nasal mucosa. As aforementioned, the main target cells of CoVs are mucus-producing goblet cells and ciliated cells located in the nasal mucosa. Targeting these cells with nanocarriers is a promising strategy to deliver drug candidates to the infected cells (Fig. 7). A particular barrier found in the nasal administration of therapeutics is the mucus. The mucus forms an aqueous layer on top of the mucosal endothelial cells, which based on the secretion of mucin, cilia action and cough is kept in constant flow facilitating rapid clearance of agents present in the mucus film [172,173]. Additionally the mucus contains several secretory antibodies forming a first line of defense against pathogens, which, as aforementioned, makes the nasal mucosa a promising target for vaccines aimed against respiratory viruses [174]. Although the rapid clearance from the mucosa is favorable against pathogens, it creates a hard to penetrate barrier for nanocarriers to reach underlying epithelial cells. In the nose, the mucus is reported to have a thickness of around 15 μ m, including a rapidly flowing loosely adherent mucus layer on top of a firmly adherent slowly flowing layer covering the epithelial cells [175]. Clearance of agents from the mucus can occur within 30 min, giving applied nanocarriers a limited time to overcome the barrier and being taken up by or pass the epithelial lining [175]. Nonetheless several mucus-penetrating NPs (MPPs) have been developed over the last years, as extensively discussed elsewhere [173,175,176]. A general inspiration for the design of such MPPs are viruses, such as CoVs, that are able to penetrate the mucus with similar diffusion rates compared diffusion in water. Different studies suggested that the surface charge and related hydrophilicity as well as the size play a crucial role in the penetration of viruses through the mucus [175]. Furthermore, glycolized viruses or particles display enhanced penetrating properties, indicating the PEGylation (the conjugation of polyethylene glycol onto a nanocarrier surface) can increase the efficacy of MPPs and explains how CoVs, with their heavily glycolized S proteins can penetrate the mucus as well [177]. Generating nanocarriers for the delivery of therapeutics should therefore be greatly inspired by such virus-like properties. As described aforementioned, viral particles or liposomes can be tuned in terms of charge, size and surface chemistry (conjugation of PEG or other peptides to create a muco-inert surface) to reach these characteristics facilitating delivery of therapeutics towards goblet cells or ciliated cells in the mucosa. For instance, nanocarriers modified with the goblet cell-targeted peptide (CSKSSDYQC (CSK)), have demonstrated enhanced mucus penetrating characteristics, reduced mucus clearance as well as specific uptake into goblet cells. Studies demonstrate the CSK peptide enhanced nanocarrier uptake in the oral mucosa for the delivery of insulin or exenatide for the treatment of diabetes [178–181], as well as enhanced uptake of gemcitabine in intestinal goblet cells for the treatment of breast cancer after oral administration [182]. The combination of nanocarriers loaded with drug candidates focusing on the treatment of CoVs combined with such surface peptides could form a promising strategy for the nasal delivery towards CoV-infected epithelial cells.

An additional strategy to rapidly allow targeting of the mucosal epithelial cells is disrupting the mucus to enhance drug delivery towards underlying cells. Such adjuvant therapies can be particularly advantageous in diseases including a change in the mucus,

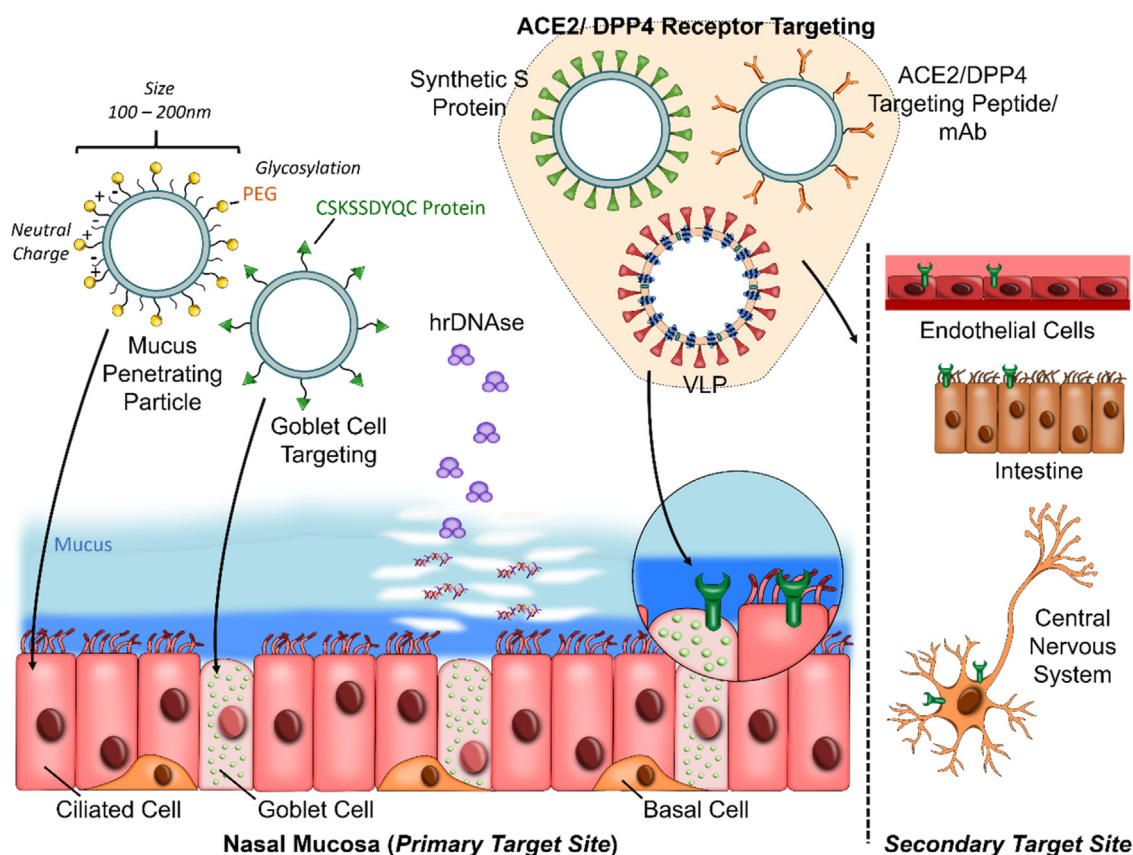


Fig. 7. Strategies to deliver therapeutics to target cells using nanoparticles. Schematic representation of different nanocarriers delivering therapeutics towards nasal mucosa (primary target site) as well as secondary target sites including the central nervous system, intestine or blood vessel endothelium. Nanocarriers include mucus penetrating particles and their characteristics, specific goblet cell targeted nanoparticles, DNase strategies to increase mucus penetration of nanoparticles and different strategies to directly target the ACE2/DPP4 receptor including virus-like particles based on coronaviruses or targeting ligands involving synthetic S proteins, targeting peptides and antibodies.

rendering it highly viscous, such as cystic fibrosis or chronic obstructive pulmonary disease (COPD). Given the symptoms of cough and sometimes bloody mucus in COVID-19 patients [183], it is likely that the mucus of these patients present abnormal mechanical properties which can directly affect the penetration of nanocarriers towards mucosal epithelial cells. Dornase alpha, a recombinant human DNase (rhDNase), has shown to hydrolyze DNA involved in the dense crosslinks of glycoproteins within the mucus [184]. It is also currently evaluated in COVID-19 patients related to reduction of ARDS [185]. Dornase alpha is an adjuvant, which can be administered in form of an aerosol, enabling it to be combined with anti-CoV therapeutics and being administered using the nasal delivery route in form of a spray or syringe [186].

Nanocarrier systems specifically targeting the ACE2/ DPP4 receptor. Although the nasal mucosa is the primary target site of CoVs, several other sites and tissues affected by CoVs have been announced recently with the potential that other tissues can also be affected, which are yet to be discovered. Targeting the virus-specific receptors, ACE2 and DPP4 is a promising strategy to deliver therapeutics to all CoV-infected cells throughout the host's body. Efficient targeting of these receptors can be achieved by different means including the conjugation of a targeting agent (peptide or antibody) onto a nanocarrier surface or by the use of a VLPs comprised of CoV-based proteins. Using the virus' own characteristics and behavior to target ACE2 expressing has been demonstrated recently, using structural proteins (M, E, S) from HCoV-NL63 to generate VLPs that could effectively transfect ciliated cells of the nasal mucosa [187]. Furthermore, these VLPs could be loaded with a fluorescent cargo

demonstrating the capability of these VLPs for efficient protein delivery. Although this is so far the only attempt to use a CoV-based VLP for the delivery of therapeutics towards infected cells, the use of VLP might form one of the most promising strategies for efficient drug delivery, as these VLPs, similar to the virus, are able to escape immune clearance, penetrate the mucus and might display similar characteristics in infecting not only the main target site of the lung but also secondary target sites, that are also affected by SARS-CoV-2.

A different strategy to target the ACE2 or DPP4 receptor is using a targeting ligand, such as peptides or mAbs, and conjugate these onto the surface of nanocarriers, enabling the active targeting of these receptors as frequently applied in recent nanomedicine applications [188,189]. These peptides are specifically designed to bind to CoV-specific receptors similar to the actual SARS-CoV, MERS-CoV or SARS-CoV-2. In recent studies, such peptides or mAbs were mostly applied to work as ACE2 or DPP4 antagonist and block the function of the receptor to prevent cell entry of CoVs during their life-cycle [190,191]. However, these inhibitors also show the potential to be used as targeting ligand combined with nanocarriers to direct therapeutics towards infected cells. In such way, these nanocarrier might exert a double-sided effect by delivering therapeutics towards infect cells, while blocking the virus entry on non-infected cells. Nonetheless, as these and potential novel inhibitors are not directly designed as targeting ligands and have been extensively described elsewhere for ACE2 [190–193] and DPP4 [194–197], respectively, this review will not focus on such inhibitors in greater detail. So far, no studies describe the use ACE2 or DPP4 antagonistic peptides or mAbs in the context of targeted

delivery of drugs towards SARS-CoV, MERS-CoV or SARS-CoV-2. However, a recent study describes the targeting of the angiotensin II receptor type 1 to specifically deliver PLA/PLGA-PEG NPs towards mesangial cells for the potential treatment or detection of diabetic nephropathy. Hereby, angiotensin-I was covalently bound onto the surface of the NPs to allow binding to ACE receptors of the first target cell. The binding to ACE allowed for enzymatic processing of angiotensin-I towards angiotensin-II, which further allowed uptake of these NPs through AT1R mediated endocytosis by a second target cell. This strategy was mainly inspired by the cellular uptake of influenza A viruses, which first undergo ectoenzymatic activation of hemagglutinin by one cell which facilitates the uptake of the virus by a secondary target cell. Although their study did not aim for ACE-mediated cellular uptake, the strategy of using angiotensin-coated nanocarriers is a promising strategy to specifically target ACE2 expressing cells. A different strategy is based on the recent sequencing of the S protein, enabling deeper understanding of the protein structure of the S protein, which further allows to generate a synthetic peptide mimicking this structure. The synthetic S protein can potentially be conjugated onto the surface of nanocarriers functioning as targeting ligand. Studies have reported the potential of such synthetic S proteins for their antiviral capability in SARS-CoV-2 by blocking the ACE2 receptor similar to the actual virus [198]. Such synthetic proteins would allow for the targeting of the ACE2 receptor without the need for purification of the S protein from actual CoVs.

The surface properties of nanocarriers have also shown to facilitate binding to CoV-related receptors. It has been described recently that cationic NPs, in particular polyamidoamines (PAMAMs), bind to ACE2 receptor, subsequently blocking the cleavage of angiotensin, which can cause ARDS [199]. Interestingly, anionic nanocarriers did not display such binding properties, demonstrating how the surface charge of nanocarriers can also facilitates specific receptor binding. However, it is unclear whether such characteristics would allow for the targeting of ACE2-expressing ciliated or goblet cells in the nasal mucosa, as cationic nanocarriers might not be able to successfully penetrate mucus layer.

This study also demonstrates a particular challenge when targeting the ACE2 receptor. The blocking of this receptor has shown to reduce the enzymatic cleavage of angiotensin-II, disrupting the renin-angiotensin system (RAS) and eventually increasing its blood levels, which subsequently can promote ARDS [38,200,201]. Additional targeting of this receptor might further block ACE2 receptor levels which can enhance this cascade. Nonetheless by applying a multilayered therapy, such as for instance a treatment using ACE2-targeted delivery of therapeutics followed by the administration of the previously described APN01, a soluble form of ACE2, could avert such side effects, however, these hypothetical strategies remain to be proven in experimental settings. Another potential challenge in the targeting of ACE2 or DPP4 receptors for the directed delivery of therapeutics to infected cells, is the reduced presence and expression of these receptor after infections with SARS-CoV, MERS-CoV and SARS-CoV-2, which eventually might reduce the overall amount of therapeutics that can be delivered towards target cells [38,200]. Similarly, despite that DPP4 targeting might allow for the targeted delivery of MERS treatment towards infected cells, DPP4 blocking or inhibition has shown profound influence on T cell-specific immunity, downregulating T cell activity and maturation. Although the exact consequences of DPP4 blocking on the immune system are so far not well understood, the probable side-effects of DPP4 targeted peptides or mAbs must be taken into consideration when designing targeted systems for delivery towards ACE2 or DPP4 expressing cells.

Despite these different challenges, direct targeting of ACE2 or DPP4 might form a promising strategy to not only deliver therapeutics to the major target site but also specifically reach secondary

sites of infections. The proper *in vivo* evaluation of such strategies is crucial to avert potential side effects and ensure the targeted delivery of treatments. A different strategy to avert side effects might be the search for different shared surface receptors except ACE2 or DPP4, however, due to the variety of affected cells, a common receptor that does not exert side effects upon targeting might be challenging to find.

Targeting the immune system and nanomedicine strategies

As described before, SARS-CoV-2 is characterized by a rapid progression of the disease where an exceedingly high number of patients experiences severe pneumonia or ARDS. In particular patients with a weak immune system based on age or other pre-existing conditions are more prone to a lethal outcome of the disease compared to younger healthier patients. Currently different strategies are developed to inhibit the progression of COVID-19 and reduce patient mortality. Such strategies mainly focus on supporting the immune system to enhance the clearance of the virus from the body or to inhibit the inflammatory response caused by the viral infection and related to ARDS and severe lung damage.

Supporting the immune system

A strategy that is currently evaluated for the use in COVID-19 patients is the administration of interferon beta 1a (IFN β), a body-own cytokine with extensive antiviral functions [202,203]. The administration of interferon has been investigated to treat SARS-CoV and MERS-CoV demonstrating promising results *in vitro* and *in vivo* which, however, could not be translated to clinical settings in the past [203–205]. Also the exact role of interferons in viral infections can be highly dependent on the infection type and progress as interferons are also reported to promote inflammatory responses and increase patient mortality [202]. Nonetheless, administration of IFN β might form a promising strategy for certain COVID-19 patients.

Another strategy to facilitate antibody therapy in the targeting of CoVs, is the use of purified plasma for patients that are already recovered from CoVs [206]. The plasma of such patients, when proven safe to have no traces of the specific pathogen left, might include sufficient antibodies/ immunoglobulins to support the body's own immune system against the CoVs. As the plasma from SARS, MERS or COVID-19 patients would include specific Abs against these viruses, it might form a promising strategy to support viral clearance before entering the host cells and when given intravenously (referred to as intravenous immunoglobulins (IVIGs)) in combination with antiviral drugs could form a promising treatment against CoVs.

Inhibiting the inflammatory response

As mentioned before, COVID-19 patients often experience increased levels of inflammatory cytokines and chemokines (CRS) related to ARDS, which forms one of the main reasons for the high mortality of patients [57,58]. In particular, ARDS might also cause severe long-lasting damage to the lungs involving scarring of the lung tissue and substantial reduction of the patient's quality of life, which is why a treatment to attenuate the inflammatory response leading to ARDS is a promising strategy to treat the consequences of a CoV infection [207]. Such treatments involve cell-based treatments, treatments with intravenous immunoglobulins, general immunomodulatory drugs or drugs targeting specific pathways involved in the CRS.

Besides the use of antibodies or immunoglobulins to target and neutralize CoVs, IVIGs, isolated from healthy donors, have also shown the potential to exert anti-inflammatory properties in patients with severe pneumonia by suppressing

inflammatory cells, inhibit phagocytosis and interfering with antibody-dependent cytotoxicity [206,208,209].

A common strategy to attenuate the inflammatory response is the use of already known therapeutics that function as immunomodulators. Hereby, it can be differentiated between drugs that in general show immunomodulatory properties or drugs that target a specific cytokine that is related to CRS. Therapeutics that show immunomodulatory properties currently evaluated comprise Thalidomide [210], Methylprednisolone [209], Fingolimod [211], Ruxolitinib [212], Eculizumab [213], Leukine [214], Colchicine [215], Vafidemstat [216], Melatonin [217], Nivolumab [218], Dexamethasone [219] or Cyclosporine A [220,221].

Targeting specific pathways and cytokines forms another promising, more focused, strategy to treat the inflammatory responses in patients. In particular, IL-6, IL-1 and TNF α have been identified as the key players in CRS and form the most pro-inflammatory cytokines in the human body [57–59]. Recently it has been also shown that IL-6 is directly involved in the need for mechanical ventilation of patients and poor survival prognosis [222]. Sarilumab is a mAb functioning as a IL-6 antagonist that is currently evaluated in clinical trials, which binds to soluble as well as membrane bound IL-6 receptors (sIL-6R and mIL-6R), blocking the related receptor mediated IL-6 signaling [223,224]. Originally applied in rheumatoid arthritis, Sarilumab is now a promising agent for the treatment of COVID-19 patients. Similarly, the IL-6 antagonists Tocilizumab, a therapeutic also used in rheumatoid arthritis, and Siltuximab, originally applied in multicentric Castleman's disease, are currently evaluated for their efficacy in COVID-19 [225]. Besides IL-6, IL-1 is also directly related to CRS and shown to be highly expressed in COVID-19 patients [226]. Anakinra and Canakinumab are both IL-1 antagonists, which are currently investigated for their efficacy and their potential use for the treatment of COVID-19 [227,228]. Lastly, targeting TNF α , a cytokine well-known for its pro-inflammatory potential, is also currently evaluated in clinical trials for the treatment of COVID-19 using Adalimumab, a TNF α antagonist, which has been used to treat rheumatoid arthritis or Morbus Crohn in the past [229].

Nanomedicine strategies to target immune system

Besides facilitating targeting of the infected cells in CoV to prevent the virus from spreading throughout the body, nanocarriers also show promising capabilities to deliver anti-inflammatory drugs towards immune cells to avert CRS. Different from the nanocarriers discussed before that aimed to reach the NALT and provoke an immune response towards presented antigens causing immunity, such nanocarriers are aimed to deliver anti-inflammatory agents towards inflammatory macrophages and T cells, which are mainly involved in CRS and block the production of IL-6, IL-1, TNF α and other cytokines. In particular the targeting of macrophages has been recently in focus of countless studies, due to the involvement of macrophages in inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, multiple sclerosis or diabetes, cardiovascular diseases, cardiac diseases as well as cancer as summarized elsewhere [230]. Similar to therapeutics aimed for the treatment of CoV- infected cells, several studies have been described in the past that combined currently evaluated anti-inflammatory therapeutics with nanocarriers to enhance their stability and resistance to degradation, prolong drug exposure or allow targeted delivery of these therapeutics. For instance, Tocilizumab, an IL-6 antagonist, was combined with hyaluronate-gold NPs for the treatment of rheumatoid arthritis [231], dexamethasone acetate was loaded into SLNs for the delivery to the lung [232], or colchicine-loaded lipid bilayer-coated mesoporous NPs for the treatment of cancer [233].

Although different strategies to target macrophages have been emerged over the last years, directed targeting of pro-inflammatory macrophages remains challenging and studies that target macrophages with context of the CRS are rare [230]. Nonetheless, a few approaches have demonstrated the use of nanocarriers designed to target pro-inflammatory immune cells. A promising target for specific delivery towards macrophages is the presence of mannose receptor (also known as CD206) on the surface of such macrophages. For instance, NPs were generated comprised of mannosylated bioreducible cationic polymers facilitating the presence of mannose receptor for cellular uptake [234]. These nanocarriers were designed to deliver siRNA against TNF α expression in macrophages, which eventually reduced the pro-inflammatory response in inflammatory bowel disease. However, mannose receptor is not only expressed on pro-inflammatory macrophages, and in fact, displays overexpression in anti-inflammatory macrophages as well. This highlights a challenge in the specific targeted delivery of therapeutics towards macrophages – macrophage specific markers might be shared by both, pro- and anti-inflammatory markers, making the design of specifically targeted nanocarriers particularly challenging [230]. Different potential surface markers for the targeting of inflammatory macrophages and averting CRS are CD80 or CD86, also being reported as surface markers for inflammatory macrophages [235].

In general, nanocarriers that are aimed for the delivery of anti-inflammatory therapeutics for preventing CRS face similar barriers as NPb-Vs and other lung-aimed nanocarriers described in detail before including the mucus layer of top of the pulmonary lining as well as the need to cross the pulmonary barrier to reach the capillaries and eventually inhibit the secretion of pro-inflammatory cytokines from macrophages and T cells.

Nanomedicines to understand CoV's mechanisms

Remarkable for all the efforts of finding an efficient treatment or vaccine against the novel SARS-CoV-2 or the related disease COVID-19 is the fact that most drugs currently in clinical trials are repurposed from other disease targets including other viral infections, such Ebola or HIV, but also cancer or rheumatoid arthritis. With the increasing number of cases and related deaths, a fast treatment was crucial and therapeutics already effective against known pathways or already evaluated for safety in clinical trials are the most rapid strategy to find a treatment against SARS-CoV-2 infections. Nonetheless, SARS-CoV and MERS-CoV have been known for nearly 20 years, and so far, no efficient treatment against these diseases is available and remarkable for the current outbreak and the related clinical trials is that no drug is currently evaluated that is directly aimed to treat SARS or MERS. As SARS-CoV and MERS-CoV only had limited case numbers, which led to the outbreaks being controlled comparably fast, funding for finding a vaccine or treatment against these viruses was limited and efforts to find a vaccine were shelved after governmental funding was stopped [236,237]. Nonetheless, the fact that after nearly 2 decades no efficient treatment is available, also demonstrates that it is also crucial to further investigate and understand the virus, and the related processes involving cellular uptake and replication, to design an efficient treatment directly targeted to SARS-CoV-2, and potentially other CoVs. Recently, a wide range study analyzing protein interactions involved in host cell-virus responses has identified 332 possible protein-protein interactions of which 66 demonstrated a drugable profile [238]. These interactions can be targeted by 69 known drugs which are either already FDA-approved for different diseases or in clinical trials. In particular, therapeutics targeting mRNA translation and predicted regulators of the Sigma1 and Sigma2 receptors demonstrated promising profiles for the treatment of SARS-CoV-2

infections. The detailed analysis of the protein interactions of SARS-CoV-2 and host cells is crucial steps towards a more specialized and directed treatment of SARS-CoV-2 and highlights promising candidates that might find their application in the treatment of these viral infections soon.

As aforementioned, several different nanocarriers can be used as NPb-Vs or drug delivery systems, whereas viral proteins, VLPs and liposomes are exclusively able to mimic the infection pattern and mechanics similar to actual viruses such as CoVs. These nanocarriers have been demonstrated for the use in the vaccine or therapeutic delivery towards CoV-infected cells presenting tunable characteristics and properties such as size, surface charge and surface modifications. However, compared to liposomes, viral proteins and VLPs on the one hand display more limitations regarding their composition and architecture due to the pre-defined proteins from the viral source. Liposomes, on the other hand, can be generated using a variety of lipids where the exact composition allowed for the specific tuning of liposome characteristics and behavior as well as the encapsulation of various agents ranging from RNA/ DNA towards therapeutics and others [239,240]. Furthermore, liposomes can easily be modified by surface conjugation of peptide sequences such as PEG or targeting ligands to allow immune escape, mucus penetration or drug delivery to specific target cells [239,240].

The characteristics of liposomes also facilitate a unique use of these systems in investigating viral infections. Liposomes can be designed to mimic the viral composition, eventually rendering their characteristics and behavior (Fig. 8) [19,241,242]. The use of liposomes as model virus in CoV is an interesting approach in particular due to higher safety in handling compared to live viruses allowing laboratories that do not own facilities to handle SARS-CoV, MERS-CoV or SARS-CoV-2 to investigate potential treatment in a realistic fashion as well as due to the high tunability of the model properties and characteristics. In general, a liposome mimicking a CoV comprises of (i) a liposomal bilayer that mimics the viral envelope, (ii) peptides or purified protein conjugated onto the liposome surface mimicking the viral S protein and (iii) an encapsulated agent such as a drug or gene mimicking the viral genome.

Such virus mimics have been generated in the past to investigate the process of binding and fusion of the viral envelopes with the endosomal membranes as well as potential pH triggered release mechanisms [241]. Furthermore, the conjugation of viral antigens (hen egg lysozyme) onto the liposome and encapsulation of nucleic acids, mimicking the viral genome, was used to study the specific response of B cells towards these antigens, demonstrating the use of such systems to investigate underlying mechanisms in a controlled fashion [242]. Such systems can be similarly used to investigate the behavior of CoVs in a realistic and well-controlled environment. In particular the combination of such systems with purified or synthetic CoV S proteins would allow for the detailed investigation of the cellular entry of these viruses and the exact mechanism of how the virus is able to fuse with endosomal membrane. Furthermore, *in vivo* studies involving such CoV models could help in understanding the mechanism of viral spread throughout the body as well as help in identifying potential secondary target sites which so far have not been found or taken into consideration. By, for example, using a fluorescent cargo within the liposomal CoV model, novel secondary target can easily be identified *in vivo*. Moreover, the simple modification of the CoV model composition might also facilitate the research of a universal corona vaccine by, for instance, altering sites within a synthetic S protein to mimic a random mutation within. In such way a potential vaccine could be investigated in more detail for its potential use in future CoV outbreaks.

In summary, mimicking CoVs with liposomal formulations forms a promising strategy to further investigate underlying biological mechanisms as well as to evaluate current and future

treatments and vaccine for CoVs in a realistic biologically relevant environment. Using such CoV models *in vivo* could help to identify secondary CoV target sites and due to the safety of working with a CoV model instead of the actual virus enables research groups with limited virus-related facilities to research the virus in greater detail, allowing for the application of multidisciplinary strategies for understanding and treating SARS-CoV, MERS-CoV or SARS-CoV-2.

Conclusions and future outlook

The rapid spread and high incidence of the novel SARS-CoV-2 has sparked unprecedented joint scientific efforts to develop novel vaccines or therapeutic agents for the prevention of SARS-CoV-2 infections or treatment of COVID-19, respectively. However, several challenges and hurdles remain in the development of an effective treatment against SARS-CoV-2 as well as in the prevention of potential future outbreaks of novel CoVs.

Recently, the development of an effective vaccine has been in particular focus of different studies and several companies currently work and evaluate different types of vaccines, some in combination with nanocarriers, for the treatment of SARS-CoV-2. One of the critical hurdles in the development of such a vaccine is the time that is necessary to fulfil all criteria that allows for the approval of these vaccines for treating patients. Although the first vaccine candidates were already designed a few weeks after the composition and genetic sequence of SARS-CoV-2 was known, it might take several months up to more than a year before a potential candidates can reach the market. Although the development and approval time, which can usually take several years, is drastically reduced in the current situation due to the desperate need of an effective treatment or prevention of SARS-CoV-2 infections, this time period will lead to recurrent virus outbreaks and hotspots and eventually increase the number of patient deaths. One of the major hurdles in the development is also the lack of suitable *in vitro* and *in vivo* models that precisely predict the immunization capabilities of novel vaccine candidates. In particular, a solid *in vivo* model that presents clinically relevant characteristics of CoV infection could limit the time necessary to develop and evaluate vaccine candidates for their efficacy in safety. Recently, a mouse model using BALB/c mice showed similar clinical features found in COVID-19 patients after infection with an isolated SARS-CoV-2 [243]. This model was also evaluated for the protective efficacy of a recombinant RBD virus demonstrating the potential of such a clinically relevant *in vivo* model in the development and evaluation of vaccine candidates before entering clinical phases.

A different challenge in vaccine development is the prevention of potential future outbreak of CoVs. Despite the tremendous effort to develop a vaccine against SARS-CoV-2, it is rather certain that there will be another outbreak of a different type of CoV in the future. As shown in Table 1, the S protein of CoVs is currently in major focus for its use as antigen, however, other structural proteins of viruses might have the potential to be used an antigen as well and might provide a more solid vaccine compared to the S protein. One of the major antigens of influenza vaccines is the hemagglutinin protein, presenting the major surface protein of this virus, similar to the S protein in CoV [244,245]. However, the hemagglutinin protein is often target for mutation resulting in a constant need for an updated vaccine based on this protein as antigen. An often discussed solution for the mutation of this protein is the use of a so-called universal influenza vaccine that tries to present an antigen that does not undergo frequent mutation and does not need to be updated on regular basis such as current influenza vaccines. For instance, the M proteins and N proteins of influenza have been evaluated for immunization in the

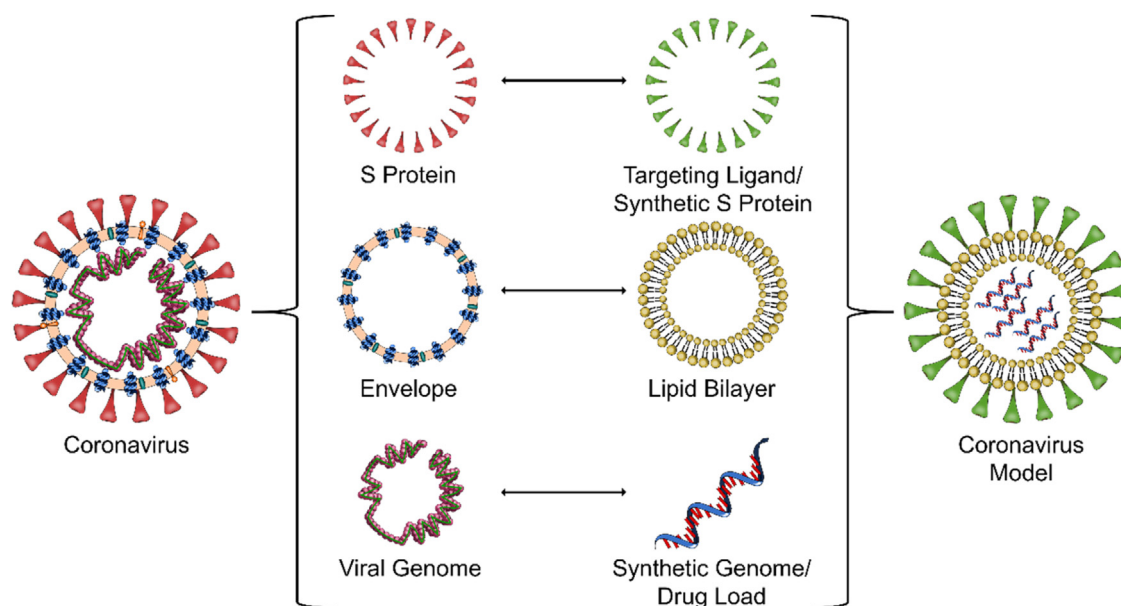


Fig. 8. Design of a virus model using nanoparticles. Strategy to mimic a virus for the investigation of biological processes and virus behavior using nanomedicine strategies displaying the use of targeting ligands or synthetic proteins to mimic the S protein of a coronavirus, a lipid bilayer to mimic the viral envelope and synthetic RNA/ DNA or a drug to mimic the encapsulated viral genome.

past demonstrating their potential as antigen used in an universal influenza vaccine [246]. Similarly the S protein of CoVs, in particular SARS-CoV, MERS-CoV and SARS-CoV-2, displays significant differences leading to the different characteristics and behaviors of these viruses. Also the recent evidence of a specific mutation of the S protein in SARS-CoV-2 compared to SARS-CoV underlines the tendency of mutation within this protein. A universal corona vaccine could be a promising strategy, presenting antigens such as the M protein, onto the surface of a nanocarrier or a gene or direct cargo within a nanocarrier. In such way future outbreaks of CoV could be prevented in a more efficient manner while providing a delivery system in form of a nanocarrier that can enable stable delivery via the intranasal or intravenous route.

A different hurdle in the development of an effective treatment for CoV infections is a lack of understanding of the exact biological mechanism of the infection as well as the related clinical features. Although the primary site of infection has been clearly identified, the understanding of potential secondary sites is still limited. As a result the long-term effects of CoV infections are hard to predict. The repurposing of known drug candidates has presented a highly promising and rapid solution to fulfil the urgent need of a treatment for COVID-19. In particular the use of already approved drug candidates that were originally designed for a different virus were rapidly evaluated for their potential to treat COVID-19 as well. Although such drugs might increase the overall disease progression and survival of patients in the first place, it is unclear whether such therapeutics prevent long-term damage of secondary targets which are yet unknown. Targeted delivery of different components to infected cells using nanocarriers, either by prolonging the circulation time of therapeutics increasing the chance to reach potential secondary site or by active targeting of for instance the ACE2/DPP4 receptors, presents a promising strategy to render treatments more specific. Despite the current efforts of rapidly finding a treatment that increases the survival of current patients, further research is needed into CoV-specific treatment strategies for future outbreaks, in particular as the development of a vaccine might take multiple months. The use nanocarriers can form a crucial aspect hereby to increase the specificity of drug candidates, reducing their side-effects and prolonging the circulation time. In particular a pro-

longed circulation time makes such nanocarrier-based therapeutics also interesting for the use as prophylactic treatment. Especially healthcare workers or other people that are highly exposed to CoVs on daily basis could benefit from a prolonged prophylactic treatment to eventual form a basic line of resistance.

In general, nanomedicine offers an attractive approach to further investigate the virus' behavior and identify potential secondary targets sites, which are yet unknown. Virus mimics, as previously described, can mimic the virus' behavior using nanocarrier-based replicates and study their kinetics and spread through the body. For instance, fluorescently or paramagnetic NPs that display CoV-like kinetics and target binding can be used to study the spread of the virus throughout the body without eventually using the actual virus. This also makes CoV research more available to groups that are not directly focused on the research with viruses or do not have the facilities to work with such biological compounds. In such way understanding the virial behavior can become a multidisciplinary research combining different fields such as immunology, biomaterials, biophysics and others. Such interdisciplinary approaches are needed to eventually find a successful long-term treatment for CoV infections as well as form a strong basis to prevent or handle future CoV outbreaks.

In summary, the urgent efforts made by the scientific and health community, pharmaceutical industries and governing bodies to develop a vaccine against SARS-CoV-2 and find a treatment for COVID-19 demonstrate this infection as an unseen threat to the human race. New insights into the infection pattern, secondary target sites and efficiency of treatments are identified and published on daily basis. Data on SARS-CoV-2 is growing and evolving rapidly and certain descriptions and conclusions in this review might change due to new and more detailed insights into the viral characteristics and properties. Certain ideas and hypothetical descriptions in this review might need to be adapted based on new findings and potential long-term effects of a SARS-CoV-2 infection are yet to be found and explored. Furthermore, nanomedicine-based strategies that already displayed promising application in SARS-CoV and MERS-CoV, might also find application in SARS-CoV-2 and present treatment options that are currently not evaluated. The constant progress and new insights into the mechanism and

related disease patterns of SARS-CoV-2 infections will facilitate the fast development of novel therapeutics, where nanomedicine can provide several promising platforms to render treatments more efficient.

CRedit authorship contribution statement

Marcel Alexander Heinrich: Conceptualization, Visualization, Writing - original draft. **Byron Martina:** Writing - review & editing. **Jai Prakash:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

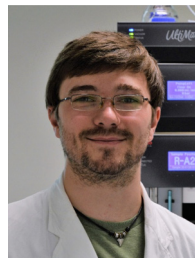
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