New Therapeutical Approaches for COVID-19

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The pandemic of coronavirus disease (COVID-19) stimulated an unprecedented international collaborative effort for rapid diagnosis, epidemiologic surveillance, clinical management, prevention, and treatment. Since the beginning of the COVID-19 pandemic, treatment of the SARS-CoV-2 infection was a real challenge. An overview of the viral structure and life cycle of SARS-CoV-2 is given and future therapeutical options are discussed.

COVID-19 treatment future therapy antiviral drugs antibodies immunomodulators nanobodies interferons

1. Viral Structure, Life Cycle and Therapeutic Targets

SARS-CoV-2 is an enveloped, positive single-stranded RNA virus, member of the *Coronaviridae* family (named after the crown-like shape of the spike glycoproteins projections on the envelope). Phylogenetic studies demonstrated that SARS-CoV-2 belongs to genus *Betacoronaviridae*, subgenus *Sarbecovirus*, lineage 2B, is highly related to bat coronaviruses, and resembles more closely the SARS-CoV than the MERS-CoV, with genomic similarities of 79% and 50%, respectively [1][2]. The viral life cycle is initiated by SARS-CoV-2 attachment to cellular receptors (angiotensin-converting enzyme 2-ACE2), a series of host factors that promote viral penetration by fusion with the cellular or endosomal membrane. The spike (S) glycoprotein is the major antigenic component of the virus and controls viral entry in susceptible human cells, binding to the ACE2 receptor, found on epithelial cells in the respiratory tract, oral cavity (mouth and tongue), lungs, intestine, kidney, and blood vessels [2][3][4][5].

During cell attachment, the S protein is cleaved into two functional subunits: S1, which includes the receptor-binding domain (RBD); and S2, essential for membrane fusion. Activation of viral infectivity is mediated by cellular proteases: TMPRSS2 (type II transmembrane serine protease), furin (highly expressed in the lungs), and furin-like proteases, which recognize an additional polybasic PRRAR site at the S1/S2 cleavage site-specific to SARS-CoV2, thought to have played an important role in the human adaptation of the virus. Structural rearrangements of the S protein trimer are essential for the transition from a closed to an open shape of the RBD, allowing efficient cell binding and the switch from a prefusion to a post-fusion conformation [6].

Following the fusion of the virus envelope and host cell membrane, mediated by the S2 subunit containing the fusion peptide, the RNA genome is released into the cytoplasm of the host cell. The positive-sense single-stranded RNA genome is directly translated into two large polypeptides—pp1a and pp1ab—further cleaved by two important

viral cysteine proteases (papain-like protease-Plpro, and the chymotrypsin-like protease-3Clpro/main protease-Mpro) to form 16 nonstructural proteins, many of which will participate in the replicase–transcriptase complex (RTC). This includes the RNA-dependent RNA polymerase (RdRP), which conducts the synthesis of the new RNA genome, together with viral and host co-factors, and the Nsp14 exonuclease, with RNA proofreading activity, that limits viral variability. A set of sub-genomic RNA (sgRNA) species are formed during the genome replication from the negative-sense RNA intermediate and serve for translation of the structural and accessory proteins [7]. Double-membrane vesicles, convoluted membranes, and open double-membrane spherules produce a protective microenvironment for viral RNA replication and transcription of sgRNA. Translated structural proteins are translocated into the endoplasmic reticulum (ER) and travel through the ER-to-Golgi intermediate compartment (ERGIC), assemble with the genomic RNA, and the progeny virions are released from the infected cells. Several steps in the viral life cycle represent important therapeutic targets: (a) viral entry interfered with anti-spike monoclonal antibodies; (b) the RNA genome replication, prematurely stopped by nucleoside analogs binding to the viral RNA polymerase; (c) synthesis of the nonstructural proteins by inhibitors of the main viral protease (Mpro).

2. Broadly Neutralizing Antibodies

Broadly neutralizing antibodies, active against different variants of SARS-CoV-2, including Omicron, were isolated from convalescent plasma donors or vaccinated individuals [8]. Cryo-EM studies showed antibodies that were cross-reactive between sarbeco-, merbeco- and embecoviruses, and have flexible binding modes, targeting both the “up” and “down” conformations of the RBD [9]. The development of such ultrapotent antibodies directed towards conserved viral epitopes, with broad-spectrum activity against both wild-type and mutant virus strains, is an important strategy for COVID treatment [10][11] and a step forward towards a pan-coronavirus vaccine. In addition, innovative antibody delivery techniques, such as inhaled antibodies, might offer a convenient, highly accessible method for COVID-19 prevention.

Nanobodies (Nbs) are single-domain antibodies, similar to the heavy-chain-only antibodies initially isolated from camelids and cartilaginous fish [12]. Nbs have a truncated structure, without any light chains and with a single variable domain in the two heavy chains (VHH), representing the antigen-binding region. Nbs exhibit ideal attributes for large-scale manufacture and have numerous advantages over classical human antibodies: ultra-high antigen-binding affinity, due to a very long CDR3, that can access otherwise inaccessible epitopes; recognition of a higher diversity of paratopes; good physicochemical qualities with increased solubility; good tissue penetration; and high stability, allowing for oral or inhalation administration. Bi- or multi-specific heavy chain antibodies and nanobody-drug conjugates are tested as antitumoral therapeutic strategies and can be used to prevent or treat inflammatory and infectious diseases [13].

Caplacizumab, a bivalent single-domain antibody, is the first nanobody-based medicine approved by the EMA and FDA in adults with thrombotic thrombocytopenic purpura and thrombosis in November 2018, and February 2019, respectively [14]. Due to their high antigen affinity and stability, nanobodies can be administered in oral or inhaled versions and might be beneficial for COVID-19 non-hospitalized patients, during the early stages of the disease, acquiring high pulmonary concentrations with minimal systemic adverse effects [15][16].

Nanobodies able to recognize the RBD of different variants of SARS-CoV-2 were identified using phage display libraries derived from camels and llamas immunized with SARS-CoV-2 spike protein or receptor-binding domain [17]. Engineered multivalent nanobodies constructs with superior neutralizing activity can block SARS-CoV-2 entry, either by inhibition of receptor binding or by inducing conformational modifications that prevent viral–cell fusion [18]. In experimental mice models, prophylactically administered combinations of bivalent nanobody-Fc fusions, recognizing different epitopes in SARS-CoV-2 RBD, were able to decrease viral replication [19].

Nanobodies that target chemokines or cytokines, can be customized to modify inflammatory responses in COVID-19 disease [20]. Previously, several studies using the phage display method to elicit nanobodies directed towards cytokines were published, proving higher efficacy compared to the traditional cytokine blocking antibodies [21][22].

3. Novel Viral Entry Inhibitors

Bemcentinib is a selective inhibitor of the AXL receptor tyrosine kinase, that mediates uptake of the apoptotic bodies, used by SARS-CoV-2 in a process of apoptotic mimicry, to adhere to and internalize into the host cells. Bemcentinib is currently tested in two phase 2b clinical trials in hospitalized COVID-19 patients. The first study recently reported the short-term efficacy results, with minor benefits in the primary trial endpoints (time to improvement by two points on the WHO ordinal scale or time to discharge), but with potentially significant clinical benefits in a key secondary endpoint (avoidance of clinical deterioration) [23].

4. Inhibitors of Host Transmembrane Surface Protease TMPRSS2

Camostat mesylate, an oral serine protease inhibitor, primarily used for symptomatic treatment in gastrointestinal tract disorders, is a potent inhibitor of the TMPRSS2 protease used by SARS-CoV-2 to prime and activate the spike protein. Randomized, double-blinded studies, with clinical endpoints including viral load, number of hospitalization days, and mortality, show that camostat mesylate might be a promising repurposed drug, with a very good safety profile in humans [24].

N-0385 is a small peptidomimetic molecule, an inhibitor of TMPRSS2, that shows high efficacy in vitro on several SARS-CoV-2 variants (Alpha, Beta, Gamma, Delta) at low, nanomolar concentrations. The drug demonstrated a potential prophylactic and therapeutic effect during experimental intranasal infection in a transgenic mouse model, that expresses the human ACE2 receptor driven by a keratin promoter [25]. Further studies are necessary to evaluate the efficacy of this compound on the Omicron variant, which was shown to have a decreased use of TMPRSS2 and a preference for endocytosis dependent cell entry, with altered spike processing and reduced fusogenicity [26].

5. Interferons

A limited and delayed interferon (IFN) response might stimulate an uncontrolled viral replication and an aberrant immune response, leading to severe forms of SARS-CoV-2 infection. Patients with errors in the type I IFN activating pathways and those with autoantibodies neutralizing type I IFN are prone to a severe course of COVID-19 [27][28].

Systemic and inhaled IFN alpha and beta were administered in hospitalized patients, either alone or in combinations with antivirals, such as remdesivir or ribavirin, without major clinical benefits [29][30].

Interferon lambda has a limited inflammatory activity, due to a more restricted distribution of its IFNLR1/IL10R2 receptors, on epithelial and immune cells [31]. Small randomized clinical trials with peginterferon lambda did not show significant clinical benefits for non-hospitalized patients [32], although an accelerated suppression of viral replication was demonstrated [33].

Interferons can inhibit cell division, as such, treatment is associated with flu-like symptoms, nausea, fatigue, weight loss, hematological toxicities, alopecia, elevated transaminases, and psychiatric problems (e.g., depression, suicidal ideation) can most often occur. Concomitant treatment with immunomodulatory drugs or chemotherapeutic agents is not recommended, due to an increased risk of toxicity. Administration in pregnancy is not safe, as congenital anomalies in the fetus or spontaneous abortion may occur. There are insufficient data for interferons' administration in children [34].

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