



Potential approaches to combat COVID-19: a mini-review

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Abstract

The outbreak of a novel coronavirus namely SARS-CoV-2, which first emerged from Wuhan, China, has wreaked havoc not only in China but the whole world that now has been engulfed in its wrath. In a short lapse of time, this virus was successful in spreading at a blistering pace throughout the globe, hence raising the flag of pandemic status. The mounting number of deaths with each elapsing day has summoned researchers from all around the world to play their part in driving this SARS-CoV-2 pandemic to an end. As of now, multiple research teams are immersed in either scrutinizing various antiviral drugs for their efficacy or developing different types of vaccines that will be capable of providing long-term immunity against this deadly virus. The mini-review sheds light on the possible approaches that can be undertaken to curb the COVID-19 spread. Possible strategies comprise viral vector-based, nucleic acid-based, protein-based, inactivated and weakened virus vaccines; COVID-19 vaccine being developed by deploying Hyleukin-7 technology; plant-based chimeric protein and subunit vaccines; humanized nano-bodies and human antibodies; intravenous immunoglobulin (IVIG) infusion therapy; inhibitors for ACE-2, Angiotensin 1 receptor (AT1R), complement system, viral proteins, host cell protease and endocytosis; shield immunity; IL-6R, NKG2A and hACE2-SARS-CoV-2-RBD interaction blocking monoclonal antibodies; SARS-CoV RdRp-based drugs, traditional Chinese medicine, repositioned and anti-viral drugs. These vaccines and drugs are currently being screened in the clinical trials as several of them have manifested positive results, hence increasing the probability of becoming one of the potential treatments for this disease.

Keywords SARS-CoV-2 · COVID-19 · Epidemiology · Vaccines · Combat strategies · Approaches

Introduction

From Wuhan, China, Coronavirus disease 2019 (COVID-19) first emerged out which is known to trigger acute respiratory diseases like lung failure and pneumonia [1, 2]. The causative factor of COVID-19 is identified as a novel coronavirus, which is now recognized as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. SARS-CoV-2 has been classified as a betacoronavirus, belonging to the Coronaviridae family. Its characteristics include genome size of nearly 30 kb, bearing single-stranded, positive-sense RNA and an envelope. The entry of SARS-CoV-2 into the cell relies on glycoprotein - spike protein (S) which is present in each CoV. It is apparent that SARS-CoV-2 gains its access

inside the cell through an enzyme that is amply expressed on the surface of the cell termed as ACE2 (Angiotensin-converting enzyme 2). Against certain critical organs such as lungs, gastrointestinal tract, kidneys and airways, SARS-CoV-2 exhibits potent tropism [3].

Through a couple of symptoms which varies from showing mild or no manifestations to acute sickness or even death, COVID-19 occurrence can be indicated. Its usual symptoms comprise dyspnea, fever and cough. Further documented symptoms include fatigue, pharyngitis, olfactory dysfunction, ageusia, pain in muscles and distress of respiratory system [4]. Additional significant pathologies were noticed, in particular clotting ailment, failure of liver and kidney and nervous system disorder. Their criticality and implications lately have been started to be apprehended [3].

There is increased probability of SARS-CoV-2 having been stemmed from zoonotic coronaviruses, such as SARS-CoV, which appeared in 2002. Just a couple of months of the very first study, SARS-CoV-2 had spread through China and across the globe, approaching a pandemic juncture. Because

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COVID-19 has incurred significant economic damages and prodigious human deaths [2] (that are 991, 224 in number as of 27 September, 2020) [5], presenting a global peril, the assessment of the current scenario and designing strategies to curb the dissemination of the fatal virus is strongly obligated [2] as no clinically-proven vaccines and antiviral drugs are available against SARS-CoV-2 [6]. This mini-review outlines the potential approaches to combat noxious COVID-19.

Probable strategies

COVID-19 vaccines

SARS-CoV-2 pandemic has called on research teams from all around the world to develop vaccines at a swift pace in order to wane off this alarming ordeal. As of now, research organizations which are sprawled worldwide are engrossed in developing more than 90 vaccines altogether. Currently, eight different kinds of vaccines are being assessed for COVID-19. These include: inactivated and weakened virus vaccines on which seven groups are working on; the approach of nucleic acid vaccine (either in the DNA or RNA form) is being employed by 20 groups at least; 25 research teams are concentrated on developing viral vector-based vaccines (which can include either replicating or non-replicating viral vector) and 33 teams are forming protein-based vaccines (which can either be protein subunit vaccine on which 28 groups are focused on or virus like particles approach which is being exploited by 5 teams in particular) [7].

Weakened virus, non-replicating viral vector and protein-based vaccines

A couple of methodologies are being utilized for SARS-CoV-2 vaccine development. In an inactivated vaccine, by means of heat or chemicals, a virus is made non-contagious. Beijing's Sinovac Biotech has commenced testing this vaccine in humans [7]. The vaccine contains an entire particle of virus that is inactivated chemically. A booster of immune system termed as alum is also being added in this vaccine of SARS-CoV-2 [8]. Furthermore, a weakened virus vaccine uses a virus that has been weakened conventionally via passing it through the cells of humans or animals until mutations develop or arise in it, thus lessening its potential to induce a disease. In addition, non-replicating viral vector vaccine is being developed by Johnson & Johnson, though no authorized vaccine available in the market has ever used such approach to manufacture one. Another method, virus-like particles (VLP) deploy vacant shells of virus which are intended to simulate the structure of the virus. VLPs are also able to elicit robust immune reactions [7].

DNA and viral vector-based vaccines

Inovio Pharmaceuticals' DNA vaccine (INO-4800) stimulates T cell activation by introducing plasmid DNA which displays the spike of SARS-CoV-2 [2]. This DNA vaccine is currently in the phase I of the clinical trial. Moreover, CanSino Biologics has developed a viral vector-based vaccine known as 'Adenovirus type 5 vector vaccine' (Ad5-nCoV) that can express protein S of SARS-CoV-2 [9]. Contained in this vaccine is an adenovirus 5 present in non-replicating form which plays the role of a vector in order to transfer the SARS-CoV-2 spike protein's gene [8]. Presently, its efficacy is under assessment in the first clinical trial. Such viral vector-based vaccines provide an abiding stability, great degree of expression of proteins and elicit robust immune reactions [9].

COVID-19 vaccine incorporating Hyleukin-7 technology

A COVID-19 vaccine being designed by Genexine Inc. employs the technology of the Hyleukin-7 system. Hyleukin-7 System improves immune reactions by combining interleukin-7 (IL-7) to hy-Fc, engineered for hybridization of IgG4 and IgD in order to achieve prolonged Fc fusion protein's effect. A flexible hinge area is possessed by IgD that optimizes the Fc-fusion protein's biological function. IgG4 contains an unexposed junction area that helps reduce detrimental immunogenicity by impeding complement-dependent and antibody-dependent cellular cytotoxicity [2]. Genexine Inc. has documented enhanced vaccine effectiveness in the influenza A virus model, exhibiting aggregation of lung-resident T cells and a rise in the number of plasmacytoid dendritic cells through Fc-coalesced IL-7 therapy [10].

Recombinant subunit vaccine

Clover Biopharmaceuticals has been pre-clinically experimenting on a recombinant subunit vaccine which is focused on the SARS-CoV-2 trimeric S protein [2]. Protein S comprises a trimeric S2 base and three S1 heads [11]. In the mammalian cell culture-based expression model, Clover Pharmaceuticals verified the formation of an indigenous trimeric viral spike and the presence of neutralizing antibodies which are specific to the antigen in the completely recuperated patients' sera. Also, the University of Queensland is creating subunit vaccines, utilizing the groundbreaking techniques of the molecular clamp. In this method, the surface protein is secured by a polypeptide namely molecular clamp which enhances the identification of the appropriate antigen, hence resulting in better immune responses [2].

Protein S-centered vaccines

Numerous approaches are enacted for designing vaccines for CoV as majority of those vaccines focus glycoprotein spike (S) (which is exposed on the surface) as the neutralizing antibodies' primary stimulator. A few protein S-centered approaches are being undertaken in order to manufacture vaccines for CoV, for example, deploying S1-receptor-binding domain (RBD) or complete-length protein S [12, 13]. The two subunits namely S2 and S1 are found within protein S molecule. RBD is held by subunit S1 as RBD comes into contact with angiotensin-converting enzyme 2 (ACE2), its host cell receptor, whereas subunit S2 facilitates host-virus cell membrane fusion for transferring viral RNA to the cytoplasm in order to aid the replication process. Therefore, protein S-centered vaccines would produce antibodies that not only inhibit viral receptor's attachment but also virus genome's uncoating event [13, 14].

Avian IBV vaccine

The coronavirus that affects chicken is Avian Infectious Bronchitis Virus (IBV). It was hypothesized that IBV (strain H) live avian virus vaccine could be beneficial for SARS [15], provided that immunity generated by strain H is hinged on neutralizing antibody formation and various responses of immune cells. Therefore, after determining its efficacy, the IBV avian vaccine can be regarded as one of the alternatives for COVID-19 vaccine [13, 16].

Epitope-based vaccine

For being used in the vaccine for COVID-19, the immunoinformatics method may be deployed to recognize epitopes. Immuno-informatics has lately been employed to detect major B-cell epitopes and cytotoxic T lymphocytes (CTLs) in S proteins of SARS-CoV-2. Encounters among these epitopes and their respective class I MHC molecules had been additionally explored through molecular dynamic simulations and discovered that epitopes of CTL attach to peptide-binding grooves of MHC class I by numerous contacts, implying their ability to generate immune responses. These epitopes have the ideal properties of being included in the COVID-19 vaccine [13]. Moreover, E protein's possible B cell epitopes and Nucleocapsid (N) protein of MERS-CoV are proposed as potential immuno-protective sites which elicit neutralizing antibody reactions, also, responses of T-cell [17, 18].

Live-attenuated virus vaccine

Via removing or lessening alive virus's virulence, live-attenuated vaccines can be developed employing site-directed

or chemical-led mutagenesis; hence, virus has the ability to induce a potent infection, however, ensuing illness can either get eliminated or diminished. This vaccine holds the potential to generate adaptive and innate immune reactions, thus providing long-lasting protection. In this, reverse genetic techniques have been auspiciously deployed to disable the non-structural protein 14 exonuclease consequences or remove the SARS envelope protein [19]. In addition, a live-attenuated vaccine provides host with numerous antigenic constituents and could hence presumably bring about various immunological effectors in opposition to pathogens. Codagenix Inc. has stated that it is collaborating with India's Serum Institute, Ltd. for producing live-attenuated SARS-CoV-2 vaccine [20]. Codon deoptimization process on virus is carried out or employed to produce a live-attenuated vaccine that is "rationally engineered" [20, 21].

Plant-based chimeric protein and subunit vaccines

Since plant-based biopharmaceuticals offer efficient and price-effective approaches to guard against novel contagious illnesses, plant expression systems may be used to develop COVID-19 vaccines. Transient expression in plants may be tailored for biopharmaceutical protein generation as it is required to manufacture 'fast response vaccines' being capable of producing more protein in a brief period. Against COVID-19, biopharmaceutical manufacturing based on plants should entail the detection of possible epitopes and the generation of complete-length viral surface proteins found in the envelope area or the development of chimeric proteins or subunit vaccines [22]. Because coronavirus employs ACE2 that aids its entry into the cell, monoclonal antibodies could be generated in plants which are discovered and validated to be efficacious against proteins of SARS or ACE-2-specific-proteins and later nCoV efficacy would be assessed [22].

mRNA vaccine

The mRNA vaccines are a propitious substitute to existing vaccines owing to their increased efficacy, rapid generation span, inexpensive processing and harmless administration. The protocol for the production of mRNA vaccine comprises identification of antigens, gene optimization, scanning the altered nucleotides, tuning the delivery methods, assessing immune responses and lastly, testing for its safety [23]. In particular, no mRNA vaccine has so far reached the market, hence more time may be required for the establishment of quality standards and safety assessment. Moderna has developed the SARS-CoV-2 mRNA vaccine which contains mRNA-1273 that encodes protein S [20]. It makes use of protein S mRNA which is recombined in vitro in accordance with the sequence of the gene.

For successful conveyance, this mRNA is encapsulated by LNPs (lipid nanoparticle). A myocyte picks up LNP until infused into a muscle. Afterwards, for protein S translation, mRNAs get delivered to cytoplasm. Such internally produced protein S would be released which would trigger cellular and humoral immune reactions [24]. This vaccine has been introduced in animal models and clinical batch generation [20].

Antigen-based vaccine

The S2 subunit's FP domain is responsible for the virus's membrane fusion, this being a crucial stage in the pathogenicity of the virus [25]. It can also act as a candidate antigen for the vaccine. Currently, RBD-FP fusion protein has been developed by Tianjin University. These proteins when inoculated in mice have shown to produce a large titer of antibodies and now its efficacy is under review [20].

SARS-CoV M protein, the amplest protein present on its surface, is involved in the assembly of the virus [26]. It has been stated that in SARS patients, complete-length M protein immunization is capable of producing neutralizing antibodies [27]. The structural and immunogenic analysis has disclosed that the M-protein transmembrane domain comprises a T-cell epitope cluster capable of inducing a potent cellular immune response [28]. Between various species, M protein is often extremely conserved in evolution [26]. Hence, it can become a candidate antigen for the production of the SARS-CoV-2 vaccine [20].

Intravenous immunoglobulin (IVIG) infusion therapy

IVIG (Intravenous Immunoglobulin) is derived from a significant volume of sera which is obtained from convalescent individuals with the viral infection. It has been suggested that specific IVIG (sIVIG) can be used from which IgG could be obtained and used in minimum quantities. It is plausible to purify this IVIG by affinity either on a column composed with peptides that form the virus spikes or with peptides considered to be virus components. It tends to be a possible solution by transmitting the normal innate immune system to compromised individuals from healthy ones [29].

ACE-2 inhibitor

ACE-2 receptor aids the entry of SARS-CoV-2 into the cell, thus ACE-2 can be targeted to hamper SARS-CoV-2 replication, as this strategy brings into play another therapeutic alternative against SARS-CoV-2. A new hypothesis has been

proposed which speaks of ACE inhibitors like enalapril and captopril that may prove advantageous for patients suffering from SARS-CoV-2-induced-pneumonia. These inhibitors yet need to be clinically verified [11].

The reduction of the ACE-2 rates is of immense importance in the battle against COVID-19. Against COV-stimulated lung damage, ACE-2 may elicit a protective effect by promoting the synthesis of vasodilator angiotensin. When CoV spike protein binds to the receptor ACE-2, it prompts a decline in ACE-2 amounts, which is more likely to cause lung damage [30]. As stated by Xu et al., vitamin D might be considered as a possible therapeutic option to alleviate lipopolysaccharide-provoked severe lung injury through regulating the angiotensin-renin mechanism [31]. Hence, it could be proposed that vitamin D can restrict the CoV spread by inhibiting the receptor ACE-2. Also, the infusion of ACE-2 has been recommended to inhibit the contact of CoV with non-infected cells and regenerate ACE-2 in the infected cells [30].

Angiotensin 1 receptor (AT1R) inhibitor

A neoteric supposition claimed that inhibitors for angiotensin 1 receptor (AT1R) may be effective for patients diagnosed with COVID-19, suffering pneumonia. It has been shown that coronavirus's spike protein attachment to ACE-2 receptor, the cellular site for its binding, contributes to ACE-2 being downregulated. As an upshot of this effect, increased formation of angiotensin by the associated enzyme ACE results, although small amount of ACE-2 can change it to the vasodilator angiotensin 1–7 heptapeptide. Its influence leads to lung damage because angiotensin-stimulated AT1R provokes decreased pulmonary vascular permeability, facilitating enhanced lung pathology. Thus, elevated ACE-2 expression which is followed by chronically mediated SARS-CoV-2 in affected patients with AT1R blockers prevents severe lung damage in them instead of placing them at a greater risk of infection [32].

The two complementing processes can help explain the aforementioned phenomenon: disabling immoderate angiotensin-conciliated AT1R activity that has been fueled via virus infection, which is coupled with ACE-2 upregulation, subsequently decreasing ACE-produced angiotensin and enhancing the vasodilator angiotensin 1–7 development. The interim proposition to use antagonists of AT1R like telmisartan and losartan as SARS-CoV-2 drugs to treat patients subsequent to the severe respiratory syndrome onset stands as a possibility till it is tested [32].

Complement system inhibitors

In a new preprint research, it has been documented that biopsy samples of lung tissue taken from severely affected COVID-19 patients manifested that there is an extensive activation of complement system which is distinguished by C3-fragment removal and C3a formation. In addition to it, levels of C5a were also elevated in these patients' sera. This activation of complement system is coupled with acute respiratory distress syndrome (ARDS) pathophysiology as its activation might lead to maladjusted inflammatory reactions, hence exacerbating the disease. In order to repulse complement activation, inhibitors of C3 can be deployed, in particular, AMY-101. Its effectiveness is under inspection in COVID-19 patients. The inhibition of C3 can concomitantly impede C5a and C3a synthesis, thus improving injury in lungs. Also, C5 inhibitor can be exploited as a therapeutic agent against extensive complement activation [33].

Viral protein and host cell protease inhibitors, ribozyme, anti-sense RNA and siRNA approach

Therapeutic approaches that may be tested and employed for COVID-19 comprise inhibitors targeting particular enzymes engaged in viral transcription and replication, helicase or vital protease inhibitors, inhibitors for host cell protease and endocytosis, ribozyme, anti-sense RNA and siRNA [34].

Humanized nano-bodies and human antibodies

For producing humanized-nanobodies such as sdAb (single-domain antibodies) and VH/VHH or purely human antibodies like Hu-scFvs (human single-chain antibodies), technology is present that can help these antibodies or nanobodies cross through the virus-infected cell's membrane. In addition to it, they can attach or intervene with the biological activity of viral proteins that are being replicated. As a result, they can contribute to impeding the viral replication [35]. It is, therefore, possible to design human antibodies or humanized nano-bodies against intracellular CoV proteins namely cysteine-like protease (3CLpro), papain-like protease (PLpro), or other non-structural proteins (nsps) which are instrumental for transcription and replication of the coronavirus. When aimed at CoV-infected subjects, these antibodies or nano-bodies can provide non-immunogenic, safe yet highly efficient passive immunization [13].

Drugs

There are a number of anti-CoV candidates, mainly pre-clinical compounds that still need to be analyzed as potential COVID-19 drugs. A few of them have reached phase III clinical trials such as ritonavir, cobicistat, lopinavir, darunavir, remdesivir, HIV protease inhibitor (ASC09F) and oseltamivir [36]. Already available SARS-CoV or MERS inhibitors may be checked for effectiveness [13].

SARS-CoV RdRp-based drugs

The SARS-CoV-2 sequence of RNA-dependent RNA polymerase (RdRp) has displayed 96 percent similarity to the SARS-CoV RdRp sequence. It is a crucial discovery because drugs formulated for SARS-CoV RdRp may exhibit efficacy close to RdRp of SARS-CoV-2 [13, 37].

Repositioned drugs, traditional Chinese medicine and other therapeutics

When coupled with ribavirin, the two inhibitors of protease namely ritonavir and lopinavir had shown positive clinical results in SARS patients, implying their therapeutic potential [38]. A controlled trial of interferon- α 2b treatment and ritonavir-boosted lopinavir for hospitalized patients has been reported in China as a preliminary endeavor to test such repositioned drugs for COVID-19 [39]. Moreover, inhibitors of RNA synthesis like Tenofovir (TDF) and Lamivudine (3TC), abidol, neuraminidase inhibitors, anti-inflammatory medicines, peptide (EK1), traditional Chinese drugs such as Shufeng Jiedu and Lianhua qingwen capsules maybe become potential options for COVID-19 treatment [40].

Antiviral drug

It has been reported that carmofur, an antineoplastic medicine which generally treats colorectal cancer, can act as an inhibitor of main protease of SARS-CoV-2, therefore, having the potential to be added to the list of novel antiviral drugs. The virus main protease's crystal structure bound with carmofur, obtained by X-ray crystallography, has unveiled that this coupled molecule has the ability to directly alter catalytic Cys145 of main protease of SARS-CoV-2. This modification in protease can be achieved by carmofur's carbonyl group binding covalently to Cys145. Hence, carmofur can play a major role in hindering replication of SARS-CoV-2 [41].

Endovenous mesenchymal stem cell (MSC) infusion

Dr. Zhao and his fellow workers' research revealed that endovenous mesenchymal stem cell (MSC) infusion can

minimize excessive immune system activation and promote recovery by regulating the lungs' microenvironment, surprisingly in older patients, following the infection by SARS-CoV-2. Endovenous MSCs injection usually tends to concentrate these stem cells in the lungs where several paracrine factors are secreted [42, 43]. These factors are likely to have played a crucial part in revitalizing or protecting epithelial alveolar cells, enhancing lung function and mitigating fibrosis. Infusion of MSC will probably be advantageous for elderly people, with both and without co-morbidity, who have been afflicted with SARS-CoV-2, since this demographic becomes more vulnerable to SARS-CoV-2-caused pneumonia, thus leading to immunosenescence-induced death and serious respiratory failure [43–47].

Improved activity following MSC infusions in various conditions of the disease has primarily been imputed to immunomodulatory impacts, since these cells secrete a number of paracrine factors that interfere with immune cells, causing immunomodulation. In COVID-19 patients, the processes fundamental to the ameliorations following MSC infusion also turned out to be the strong anti-inflammatory action of MSCs [43]. These mechanisms were apparent from various favorable outcomes, including a decrease in C-reactive protein, a rise in the number of peripheral lymphocytes, and a reduction in over activated cytokine secreting immune cells such as CXCR3 + NK cells, CXCR3 + CD8 + T cells and CXCR3 + CD4 + T cells. When observed in circulating blood, this outcome manifested within 3–6 days [43, 48].

Shield immunity

For newly arising SARS-CoV-2 infections, public health sector has employed two strategies for controlling its spread: suppression and mitigation [49]. Both approaches concentrate on curtailing recent infections via restricting interactions between humans. A strategy which has been proposed to reduce COVID-19 spread speaks of a model of epidemiological intervention that requires serological testing to pinpoint individuals who have recovered from SARS-CoV-2 infection, possessing antibodies against this virus. Through interaction substitution, the recuperated subjects can play a pivotal role in facilitating and maintaining un harmful interactions, hence aiding introduction of 'shield immunity' at the level of community. Its aim is to increase the number of interactions of recuperated subjects with infected and vulnerable subjects, essentially, exchanging interactions with vulnerable and infected subjects for interaction with convalesce subjects, supposing that convalesce subjects are able to interact safely with both the subjects. This approach can potentially curb the transmission of COVID-19 [50].

Monoclonal antibodies

hACE2-SARS-CoV-2-RBD interaction blocking monoclonal antibodies

By isolating memory B cells from COVID-19 recovered patients, with B cells being specific to RBD of SARS-CoV-2, two blocking, human mAbs have been cloned successfully. Both the mAbs could particularly attach to RBD of SARS-CoV-2, hence obstructing the contact between receptor hACE2 and RBD of SARS-CoV-2 and effectuating 'neutralizing effect' on protein S of SARS-CoV-2 pseudo-typed viral infection. These human mAbs, with capacity to block hACE2-SARS-CoV-2-RBD interaction, are reported for the first time, holding massive potential to be included in the list of therapeutic options against SARS-CoV-2 ongoing pandemic [51].

IL-6R blocking monoclonal antibody

When epithelial alveolar cells get infected with SARS-CoV-2, this virus provokes either the innate and adaptive immune system, inducing massive cytokine release or promoting cytokine release syndrome (CRS) with IL-6 being the key cytokine. IL-6 can bind to IL-6 receptor which exists in both transmembrane and soluble form, thus initiating intracellular signaling cascade such as JAK/STAT pathway [52]. As observed in SARS patients, inflammatory cytokine, IL-6, is produced in excessive quantities by macrophages and plasmacytoid dendritic cells. Because IL-6 triggers the severity of the disease, its extensive signaling can pave way towards multiple effects that leads to the damage of the organ. The effects include reduction in contractility of myocardium, vascular endothelial growth factor (VEGF) expression observed in epithelial cells, maturation of effector T cells from naïve T cells and an increase in permeability of vessel [53] which causes the alveoli to be filled with blood cells and fluid, hence kindling dyspnea. The humanized recombinant monoclonal antibody namely Tocilizumab, antagonist of IL-6R, can attach to both the forms of IL-6R, inhibiting the intracellular signaling pathway that leads to CRS. So, Tocilizumab can help dampen CRS caused by SARS-CoV-2 in severely affected patients [52]. Its clinical trials are ongoing to analyze the merits of IL-6R antagonist, Tocilizumab [1].

CD94/NK group 2 member A (NKG2A) blocking monoclonal antibody

In severe COVID-19 patients, populations of CD8 + T and natural killer (NK) cells are reported to be lower when compared with those having mild infection. These immune cells are mostly associated with antiviral response to

SARS-CoV-2. But the SARS-CoV-2 infection elicits functionally exhausted NK and CD8 + T cells in which receptor CD94/NK group 2 member A (NKG2A) expression has been surged [54]. Hence, NKG2A expression can prompt weakened antiviral superintendence as hypothesized by the researchers. Because of inhibitory activity of NKG2A, it quells the immune cells' cytotoxic functionality, thus fostering the spread of viral infection. Due to scant NKG2A expression in neutrophils whereas increased in lymphocytes (NK and CD8 + T cells), NKG2A restricts the proliferation of lymphocytes but does not affect that of neutrophil's, thus disrupting the normal ratio of neutrophil to lymphocyte. Additionally, inflated IL-10 and IL-6 release as observed in COVID-19 patients can aggravate the disproportionality of immune cells with NKG2A expression being up-regulated, ensuing NKG2A's inhibitory activity on the lymphocyte population. To counter this, monoclonal antibody against NKG2A namely monalizumab is presently being experimented in clinical trials [55]. Table 1 summarizes the key probable strategies against COVID-19.

Future prospects

There is a mutual consent that the abiding resolution for infections caused by viruses is an efficacious vaccine. Probability is present that approaches exceeding one in number might be auspicious, few approaches may suit to a particular class of patients and the evolution of virus nature may impede efforts poured in for development of vaccine. Moreover, possibility stands that lasting immunization may not entirely develop in few people. RNAi therapy can act as a substitute when an unintended vaccination consequence arises [3].

For RNAi-provoked silencing, few of the accessory proteins (such as non-structural protein 15 (nsp15) that displays anti-apoptotic role through inhibition of apoptosis induced by MAVS [3, 56]; ORF-3a, ORF-4a that might play a role of ion channel which might trigger release of virus [3, 57] and ORF-9b that is involved in manipulation of mitochondria which resists responses of IFN [3, 58]) encoded by SARS-CoV can function as targets. The significance of the aforementioned potential targets still needs to be probed. In the epithelium of air passage, miR-1246 (non-coding microRNA) has been lately related to ACE-2 expression modulation. A few miRs are projected to attach to the genome of SARS-CoV-2. These miRs might behave as targets for RNAi. They could be directly employed (whether as anti-miRs or mimics of miR), or indirectly in consequence to pharmacological entity regulation [3].

Recently, a strategy based on CRISPR-Cas13d system namely PAC-MAN (prophylactic antiviral CRISPR in human cells) has been developed that has the potential to

degrade highly conserved RNA sequences of SARS-CoV-2 in an effective manner. In this approach, crRNAs (CRISPR-associated RNAs) are employed by Cas13d system. This system can be directed to specified molecules of RNA by crRNA spacer sequence (a configurable 22 nucleotide long sequence) for degradation of selected RNA [59].

In order to target viral conserved parts and identify functioning crRNAs that target SARS-CoV-2, crRNAs were developed and examined. Its bioinformatic scan revealed that greater than ninety percent of entire family of coronaviruses can be targeted by a set comprising six crRNAs only. PAC-MAN bears the ability to act as a significant inhibition tool for all coronaviruses as soon as effective and safe delivery method for respiratory system develops. Before PAC-MAN could be clinically tested in trials for potentially treating COVID-19, numerous mandatory measures need to be taken, for instance authenticating crRNAs selectivity and efficacy for hampering alive SARS-CoV-2 from infecting cells of respiratory system [59].

Conclusions

In this mini-review, we outlined the approaches that can potentially curb the spread of deadly COVID-19. In a battle against time where SARS-CoV-2 outbreak has played havoc with human lives, different research teams from all around the globe are immersed in finding the ultimate solution for COVID-19 pandemic by developing various vaccines with a major focus on spike protein of SARS-CoV-2, repurposing already approved drugs, employing inhibitors such as enalapril and captopril for human receptor ACE-2 which aids SARS-CoV-2 entry into the cell and utilizing humanized nano-bodies and human antibodies that intervene with the biological activity of viral proteins e.g. 3CLpro, PLpro and Nsps which ultimately impede viral replication.

The efficacy of probable vaccine or drug candidates against COVID-19 is presently being analyzed in the clinical trials. Even with all these endeavors being put into practice, there is currently no authorized vaccine or drug available for combatting against this fatal disease, though a few of the vaccines and antiviral agents have manifested positive results in the clinical trials, indicating that an approved COVID-19 therapy might be available soon. The already performed COVID-19 researches might unveil potential therapeutics against forthcoming pandemics as its merits and demerits will already be assessed in the clinical trials set against COVID-19. This will help bring forth a therapeutic candidate capable of eliciting positive response in a timely manner, well before the pandemic encapsulates the humans in a disastrous matrix of death.

Table 1 Probable strategies against COVID-19

No.	Probable strategy against COVID-19	Mechanism of action of potential strategy	References
1	DNA vaccine (INO-4800)	Stimulates T cell activation by introducing plasmid DNA which displays the spike of SARS-CoV-2	[2]
2	Hyleukin-7 Technology-based COVID-19	Immune reactions are improved by combining interleukin-7 (IL-7) to hy-Fc, hybridizing IgG4 and IgD where unexposed junction area of IgG4 helps reduce detrimental immunogenicity	[2]
3	Recombinant subunit vaccine	An indigenous trimeric viral spike to which the presence of antigen-specific neutralizing antibodies was validated	[2]
4	Protein subunit vaccine	A molecular clamp secures a surface protein, enhancing the identification of an appropriate antigen which results in better immune responses	[2]
5	Inactivated SARS-CoV-2 vaccine	Containing an entire particle of virus that is inactivated chemically with the addition of immune system booster termed as alum into this vaccine	[8]
6	Viral vector-based vaccine -Adenovirus type 5 vector vaccine (Ad5- nCoV)	Contained in this vaccine is an adenovirus 5 present in non-replicating form which plays the role of a vector in order to transfer the SARS-CoV-2 spike protein's gene	[8, 9]
7	ACE-2 inhibitor	Enalapril and captopril may inhibit SARS-CoV-2 entry in patients suffering from SARS-CoV-2-induced-pneumonia	[11]
8	Protein S-centered vaccines	Protein S-centered vaccines based on S1-RBD or complete-length protein S would produce antibodies that inhibit viral receptor's attachment and its genome's uncoating event	[12–14]
9	Epitope-based vaccine	Epitopes of CTL (cytotoxic T lymphocytes) can attach to peptide-binding grooves of MHC class I, implying their ability to generate immune responses	[13]
10	Humanized nano-bodies and human antibodies	Attach to or intervene with the biological activity of viral proteins e.g. 3CLpro (cysteine-like protease), PLpro (papain-like protease) and Nsps (non-structural proteins) that are being replicated, hence impeding viral replication and transcription	[13, 35]
11	Epitope-based vaccine	E protein's possible B cell epitopes and Nucleocapsid (N) protein of MERS-CoV can elicit neutralizing antibody reactions and responses of T-cell	[17, 18]
12	Live-attenuated vaccine	Deploying reverse genetic techniques to disable the non-structural protein 14 exonuclease consequences or remove the SARS envelope protein	[19]
13	Antigen-based vaccine	RBD-FP fusion protein has been developed that has shown to produce a large titer of antibodies in mice	[20]
14	Live-attenuated vaccine	Codon deoptimization process on virus is carried out or employed to produce a live-attenuated SARS-CoV-2 vaccine	[20, 21]
15	mRNA vaccine (mRNA-1273)	LNP-enclosed protein S mRNA gets delivered into cytoplasm where protein S translation occurs, thus triggering cellular and humoral immune reactions	[20, 24]
16	Plant-based chimeric protein and subunit vaccines	Using plants to generate complete-length viral surface proteins found in the envelope area, chimeric proteins or subunit vaccines and monoclonal antibodies against ACE2 at a faster pace	[22]
17	Antigen-based vaccine	M-protein transmembrane domain comprises a T-cell epitope cluster capable of inducing a potent cellular immune response	[28]
18	Intravenous immunoglobulin (IVIG) infusion therapy	From sera of convalescent individuals with the viral infection, IVIG could be derived from which IgG could be obtained, transmitting normal innate immune system to compromised individuals	[29]

Table 1 (continued)

No.	Probable strategy against COVID-19	Mechanism of action of potential strategy	References
19	Vitamin D	Vitamin D might restrict the CoV spread by inhibiting the receptor ACE-2 through regulation of angiotensin-renin mechanism which might alleviate lipopolysaccharide-provoked severe lung injury	[30, 31]
20	Angiotensin 1 receptor (AT1R) inhibitor	Using antagonists of AT1R like telmisartan and losartan to disable immoderate angiotensin-conciliated AT1R activity that has been fueled via virus infection	[32]
21	Complement system inhibitors	For repulsing complement activation, inhibitors of C3 can be deployed which can concomitantly impede C5a and C3a synthesis, thus improving injury in lungs	[33]
22	Carmofur Antiviral drug	SARS-CoV-2 main protease when bound with carmofur has the ability to directly alter catalytic Cys145 of main protease of SARS-CoV-2, thus inhibiting its main protease and hindering its replication	[41]
23	Endovenous mesenchymal stem cell (MSC) infusion	MSC infusion can minimize excessive immune system activation and promote recovery by regulating the lungs microenvironment	[42, 43]
24	Shield immunity	Through interaction substitution, the recuperated subjects who possess antibodies should interact with infected and vulnerable subjects, aiding to introduce 'shield immunity' at the level of community	[50]
25	hACE2-SARS-CoV-2-RBD interaction blocking monoclonal antibody	Two blocking, human mAbs have been cloned which could attach to RBD of SARS-CoV-2, hence obstructing the contact between receptor hACE2 and RBD of SARS-CoV-2	[51]
26	IL-6R blocking monoclonal antibody	Tocilizumab, antagonist of IL-6R, can attach to transmembrane and soluble forms of IL-6R, inhibiting the intracellular signaling pathway that leads to CRS (Cytokine Release Syndrome) which is caused by SARS-CoV-2 in severely affected patients	[52]
27	CD94/NK group 2 member A (NKG2A) blocking monoclonal antibody	To counter inhibitory activity of NKG2A which quells the immune cell's cytotoxic functionality, monoclonal antibody against NKG2A namely monalizumab is being experimented	[55]

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Compliance with ethical standards

Conflict of interest It is declared that there is no conflict of interest as all authors contributed equally.

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