REVIEW ARTICLE

Coronavirus as silent killer: recent advancement to pathogenesis, therapeutic strategy and future perspectives

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Abstract The present outbreak associated with corona virus [CoVs] in China which is believed to be one of the massive eruptions towards mankind in 2019–2020. In the present scenario CoVs has been transmitted to the European and American regions through the travellers from wide spread countries like China and Japan. The viral disease is spreading through the contact in any form by the infected persons or patients and creating huge risk of mortality. CoVs are a single positive-sense RNA virus; mutation rates are higher than DNA viruses and indicate a more effective survival adaption mechanism. Human CoVs can cause common cold and influenza-like illness and a variety of severe acute respiratory disease such as pneumonia. Early in infection, CoVs infects epithelial cells, macrophages, T-cells, dendritic cells and also can affect the development and implantation of pro-inflammatory cytokines and chemokines. It mainly produces the melanoma differentiation associated with protein-5, retinoic acid inducible gene-1 and endosomal toll-like receptor 3. How CoVs affects the function of the immune system is still unclear due to lack of this knowledge. No Food and Drug Administration approved treatment is available till date. In this review, we are tried to explore the epidemiology, pathogenesis and current treatment of CoVs infection. The promising therapeutics molecules against CoVs and future prospective have been also discussed which will be helpful for researchers to find out the new molecules for the treatment of CoVs disease.

Keywords CoVs · China · Epidemiology · Pathogenesis and current treatment · Vaccines

Introduction

Coronaviruses [CoVs] belong to the coronavirinae subfamily, in the nidovirales order corona viridae family. There are four genera: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus* and *Gammacoronavirus* [41]. CoVs are a single positive-sense RNA virus, mutation rate is higher than DNA viruses and indicate a more effective survival adaption mechanism [37].The genome codes for at least 4 major proteins: membrane [M], spike [S], nucleocapside [N], envelope [E] proteins and other accessory proteins that help replicative process and facilitate entry into cells [14]. CoVs can be divided into 3 categories they are category 1 [including human coronavirus 229E [HCoV 229E] and transmissible gastric enteritis virus [TGEV], category 2 [including human CoVs-OC43 [HCoV-OC43], murine hepatitis virus [MHV], and bovine CoVs [BCoV], and category 3 including avain infectious bronchitis virus [IBV] [13]. After the emergence of severe acute respiratory syndrome CoVs [SARS-CoV] in 2003, category 2 coronavirus were further categories into 2 subgroups, termed 2A and 2B. The classical category 2 viruses constitute subgroup 2A, while the newly emergent SARS-CoV and its animal counterparts from sub-category 2B. Category 1 and 2 of CoVs has a greater impact on human health then category 3, since category 3 of CoVs [such an avian IBV] can affect only avian organisms [18].

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Human CoVs can cause the common cold and influenza-like illnesses. A variety of more severe acute respiratory disease such as pneumonia is also associated with CoVs and other respiratory viruses [43]. During the end of 2019 and early 2020, there were only six human cases of novel CoVs infection that could infect human and cause respiratory disease: HCoV-229E, HCoV-OC43, HCoV-NL63, HKU1, SARS-CoV, and MES-CoV. SARS-CoV and MERS-CoV coronaviruses can cause serious respiratory syndrome in humans. Figure 1 summarises the structure of the coronavirus, and the structural protein feature.

Novel coronavirus [2019-nCoV]

On 31 December, 2019, Chinese authorities alerted the World Health Organization [WHO] to a number of pneumonia like cases in the city of Wuhan, a London-sized city with about 11 million people. This was identified that the first human infections were possibly from the Human Seafood Market in Wuhan. Two weeks later, scientists from China, and WHO declared a new type of coronavirus, detected through genomic sequencing, was the problem of pneumonia [31]. The symptoms of coronaviruses infection include, dry cough, shortness of breath and respiratory distress. The number of cases has risen over the weekends of 18 and January, and health workers are more vulnerable for infection. As of 23 January, 2020, 622 were globally infected with 17 death all located inside China [44]. Thailand, Korea and Japan have been confirmed the detection of a human infection with 2019-nCoV from China country. Novel corona viruses have emerged

periodically in different areas around the world over the last few years. Severe acute coronavirus respiratory syndrome [SARS-CoV] occurred in 2002, with 8422 officially infected [5].

Countries affected with 2019 novel coronavirus [2019-NCoV]

In the 1960s, CoV was identified, initially exposed in chicken as infectious bronchitis virus and secondly from the nasal cavities of human patients with the common cold that were subsequently named as human CoV [229E and OC43] [33]. other family members of coronavirus were indentified, including Severe Acute Respiratory Syndrome-coronavirus [SARS-CoV] in 2003, Human coronavirus-NL [HCoV NL63] in 2004, Human coronavirus-HKU1 [HCoV-HKU1] in 2005, Middle East Respiratory Syndrome Coronavirus [MERS-CoV] in 2012 and Severe Acute Respiratory Syndrome Coronavirus [SARS-CoV-2[commonly known as 2019-nCoV novel Coronavirus] [38]. The detailed report of epidemic case across the world [except China] listed in Fig. 2. The re-emergence of CoVs infections is currently ongoing in China which is shown in Fig. 3.

Mode of transmission of COVs

CoVs gets transmitted from animals to humans (Fig. 4). CoVs are generally found in pigs, camels and bats, establishing a common host system [28]. These CoVs by various unwanted or by various means they move from animals to

Fig. 1 Structure of corona virus

SPIKE PROTEIN

MEMBRANE PROTEIN

RNA

NUCLEOCAPSID PROTEIN

ENVELOPE PROTEIN
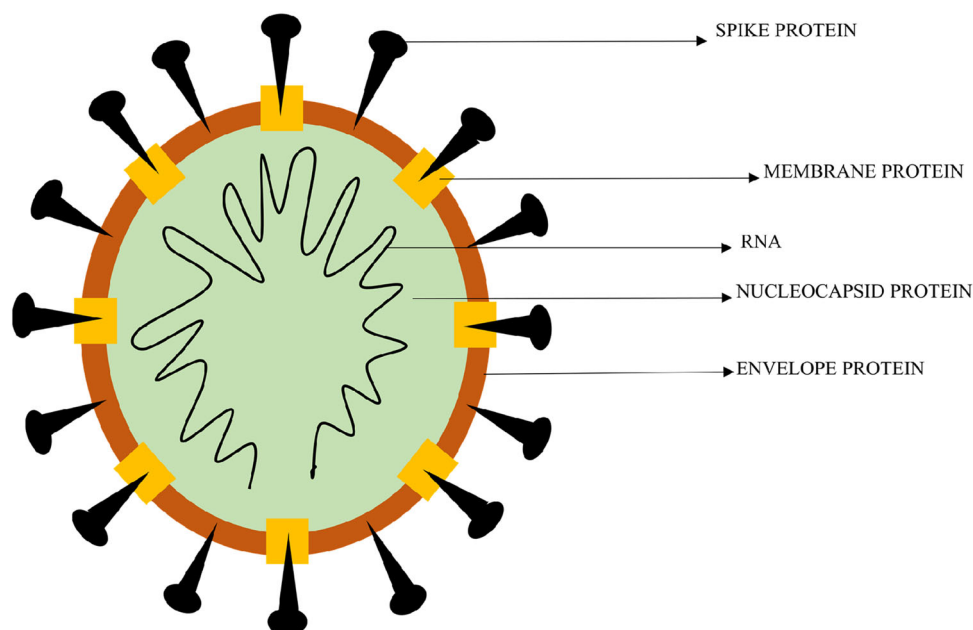
humans. Humans, who are closer to those animals contains more susceptible to CoV infection, thus forming the disease reservoir [24]. At first the expressions for CoV are not well expressed and as the mutation, replication goes on they produce to show syndromes. These viruses are transferred from one individual to another by cough, snezing, shaking hands; and thus, settle themselves at the respiratory tract [16].

Pathogenesis

The CoVs enters into the host body with the help of Spike [S] protein, a type I transmembrane glycoprotein [45]. The V484-L567 of receptor binding domain [RBD] is present on the outer sub-domain, and the central sub-domain involves anti-parallel β-sheet [five-stranded] with six helices in the middle. The external sub-domain of the CoV RBD comprises a β-sheet together with one small and three bulky strands which are arranged in an antiparallel way. Unique intervening loops connect the RBD core to this region, and a clamp at the lower and upper areas attaches it to the core subdomain. Three central helices [HR1] and three near-core chains [HR2] encourage the secretion of the viral particle into the cytoplasm and support the development of the infection [11]. Coronavirus can also reach the cell through transmembrane proteases, via an auxiliary pathway on the cell surface. The host protease divides the S protein of CoV in two distinctive functional domains in the N-terminal region [for the S1 subunit] consisting of RBD, and in C-terminal portion [S2 subunit] consisting of a fusion peptide, two heptad repeats [HR2 and HR1] and a trans membranous [TM] domain [21].
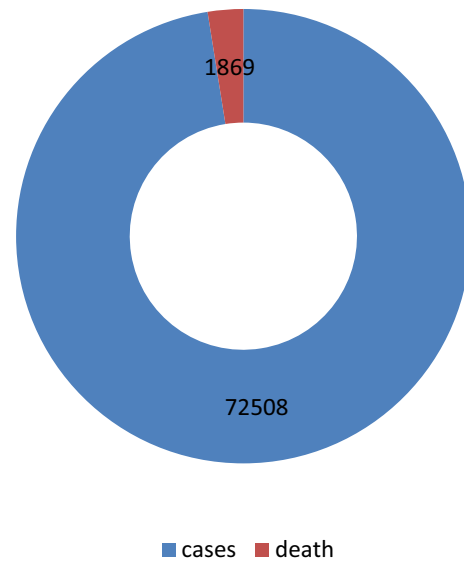

Fig. 3 The total number of reported cases with Corona virus in China

The post-mortem histopathological tests of 33-year-old male T cell lymphoma patient showed the presence of CoV particles in his pulmonary and extra pulmonary tissues [3]. The histopathological examination of the biopsies from lung, brain, liver, heart, kidney, and skeletal muscle revealed diffuse pulmonary alveolar trauma, necrotizing pneumonia, acute kidney injury, portal and lobular hepatitis, and myositis with atrophic muscle changes. Viral particles were observed to be present in pulmonary macrophages, pneumocytes, proximal renal tubular epithelial cells and macrophages thorough skeletal muscle [35].

The CoV pathogenesis in humans and animals is primarily due to (1) DPP4 [dipeptidyl peptidase-4] mediated mechanism, (2) papain such as protease PLpro mediated

Fig. 2 The list of countries (except China) reported with 2019 novel coronavirus
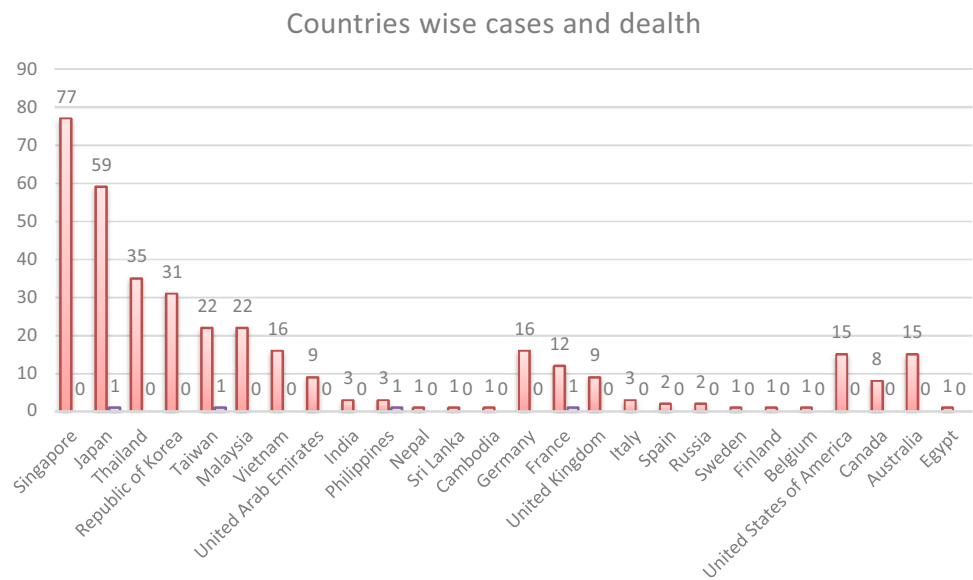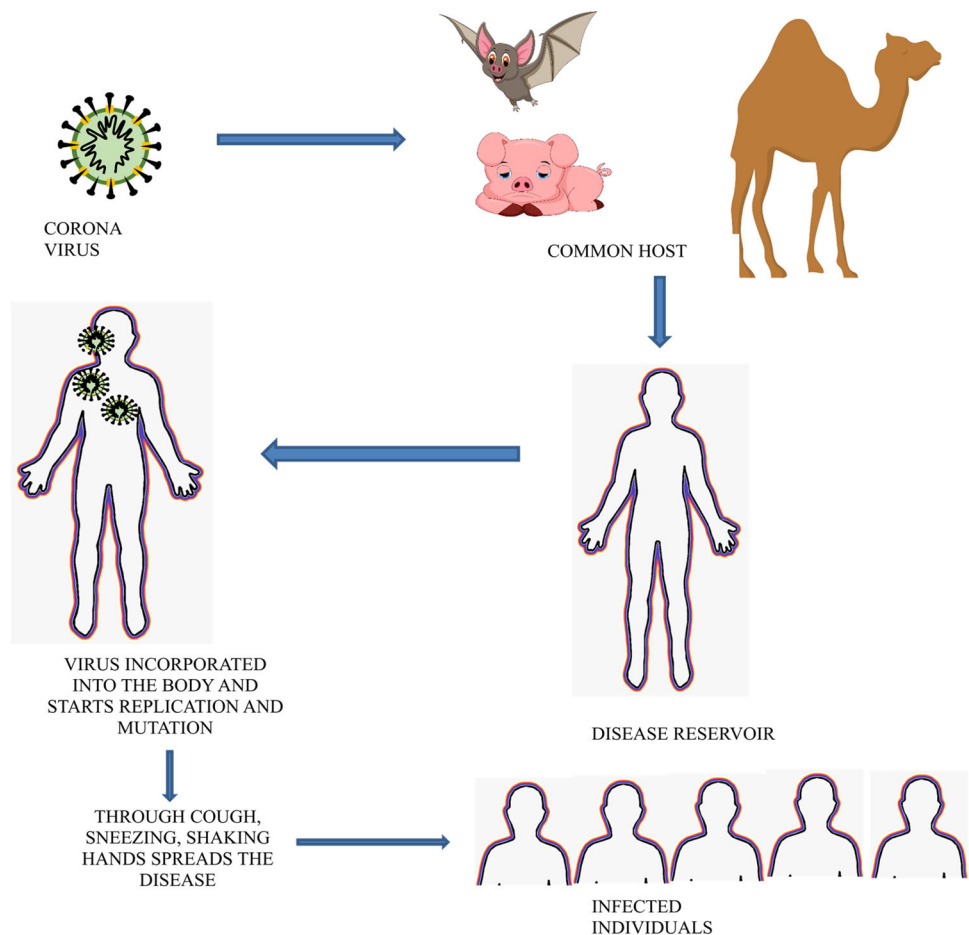
Fig. 4 Transmission, cause and effects of corona virus

mechanism and (3) accessory proteins such as p4a and membrane M protein mediated mechanism. Studies have suggested that CoVs pathogenesis in humans and animals is primarily due to three mechanisms, such as DPP4 [dipeptidyl peptidase-4] mediated mechanism, papain such as protease PLpro mediated mechanism and accessory proteins such as p4a and membrane M protein mediated mechanism.

Dpp4 mediated mechanism

The structural analysis disclosed that this receptor has a α/β-hydrolase realm and a β-propeller site with eight blades, which managed to bind the RBD in CoVs [9]. A DPP4 binding enzyme, adenosine deaminase, is known as one of the key blockers of CoV's S protein [30]. Two ecto-domains of the S protein can be classified into fusion-catalyzing [FD] and RBD. The S RBDs adhere to DPP4, after which they reveal the FDs by unfolding. The unfolding FD has allowed host proteases to cleavage S protein. Cleavage at S1/S2 [specific proteolytic cleavage sites between the FD and RBD] location is necessary for CoVs infection in Calu3 cells. It was discovered that after infection,

monocytes chemo attractants protein-1 and IFN-γ-inducible protein-10 were stimulated and the multiplication of human myeloid progenitor cells was inhibited. CoV can contaminate T-cells from mammalian lymphoid organs and trigger peripheral blood to induce apoptosis via intrinsic and extrinsic tracts [2]. A study showed that the inactivation of DPP4 minimized the initiation of PPARπ [transcriptional repressor] and IRAKM [negative TLR signaling regulator] which suggested that DPP4 controlled the immunosuppressive intervention of S glycoprotein [6].

Papain like protease PLpro mediated mechanism

Two viral proteases, PLpro and 3C-like proteinase [3CLpro] process ORFs and construct 16 non-structural proteins that are essential for the membrane-associated duplication complex. Besides protease activity in CoV, the PLpro was observed to be multipurpose enzymes with deISGylating [deletion of host cell ISG15 conjugates] and deubiquitinating [cleavage of host cell ubiquitin] properties [34]. This contributed to antagonization of the host antiviral immune response and to the development of viral replication. PLpro can hinder the behavior of the IFN-β

reporter triggered by the mitochondrial anti-viral signaling protein and decrease the event of TNF-α-induced NF-κB reporter.

Accessory proteins mediated mechanism

The viral genome is acknowledged as pathogen-associated molecular patterns through the melanoma differentiation associated with protein-5 [MDA5], retinoic acid inducible gene-1 [RIG-1] and endosomal toll-like receptor 3 [TLR3]. In the exclusion of MDA5 and RIG-1, CoVs utilized protein such as 4a [p4a], containing RNA-binding domain to attachment [4]. The motif covers the CoVs dsRNA by a simple attachment. The p4a has αββββα folding with the β1-β2 loop and α1helix binding to the small dsRNA groove. In a study, it was proposed that NF-κB persist in the cytoplasm of infected cells while 4b was figured to be linked to the nucleus. In the lack of 4b, it was found that the distribution of pro-inflammatory cytokines was translocated to the nucleus as NF-κB. This was also found in the case of 4b mutants which do not have a nuclear localization signal, suggesting that the 4b controlled mechanism of nuclear localization signal is necessary for the expression of NF-κB [29]. In the ER-Golgi complex the proteins M, E, and S interact with N protein. This interaction hampers the fusion of cellular and viral membranes [23]. CoV M protein associated with TRAF3 and involvement of disturbed TRAF3–TBK1 contribute to decreased stimulation of IRF3 [50].

CoVs and immune response

Coronavirus can easily contaminate human epithelial cells, macrophages, T-cells, dendritic cells and can affect the development and implantation of pro-inflammatory cytokines and chemokines [25]. A study defined that CoV-infected cells showed unique alterations in chromatin frameworks such as repressive histone markers, which could restrict transcription factors from attaching to interferon-stimulated gene promoter regions [26]. The alteration in DNA methylation have been shown to be one of the factors for IFN-γ-associated antigen-presenting gene declining after infection [46]. CoV proteins such as 4a, 4b, M and PLpro were observed to inhibit interferon initiation [20]. Viral infection was identified with delayed activation of pro-inflammatory cytokines/chemokines such as IL6, IL-8 and IL-1β [7, 49]. Immature dendritic cells could not activate T-cells after antigen absorption, preventing the triggering of T-cells and allowing for more viral replication [7]. Human macrophage infection has led to the interpretation of pro-inflammatory cytokines/chemokines, such as MCP-1/CCL-2, IFN-α2, IFN-α2, MIP-1α/CCL-3, IP10/CXCL-10, RANTES/CCL-5, IL-8, TNF-α, IL-12p40, and IL-6 [19, 47]. The contaminated dendritic cells stimulated IL-12, RANTES/CCL-5, IP-10/CXCL10 and IFN-γ expression, and displayed a weak IFN-α expression and no IFN-β expression [47]. The type-I IFN stimulated secretion of IL-10 and CXCL10 can direct T-cells to the infection site. Their unregulated and powerful interpretation the IFNγ and IL-12 expression was hindered and the uptake of T-helper cells was prohibited. The functions of T-cells get hindered with down-regulated antigen exposure pathways. The sequestered T cells had therefore failed to target the virus [40].

Treatment and promising therapeutics interventions

Recently, HIV drugs are found to be repurposed for the treatment of CoV, Remdesivir [combination of lopinavir and ritonavir] these two HIV drugs block enzymes that viruses need to replicate [32]. The antimalarial drug chloroquine is found effective for CoV by diminishing inflammation in people with severe COVID-19, or cause harm. Chloroquine inhibited replication of SARS in cell culture [1]. Remdesivir, a prodrug, gets metabolizes to its dynamic form GS-441524. GS-441524 is an adenosine nucleotide analog that confuses the viral RNA polymerase and avoids reading by viral exoribonuclease [ExoN], producing a diminution in viral RNA production [22]. It inhibits viral polymerase activity, shutting down transcription and synthesis of viral RNA [36]. The intravenous remdesivir [200 mg on day 1 and 100 mg once daily for 9 days] in patients with 2019-nCoV [NCT04252664 and NCT04257656], with estimated to be completed in April 2020 [27]. Recently, on this February, an antiviral drug named Favilavir [Fapilavir] was found effective against CoV. It is the first drug to get approved for marketing as a novel anti-CoV drug. Therapeutic approach/clinical trial approach of drugs have been shown in Table 1.

Vaccine development and possible drug targets

The production of virus like particles against the Hepatitis B virus human papilloma virus, parvovirus, Norwalk virus, Rotavirus provides the essential information about the success ratio [15]. Virus like particle administration can stimulate the formation of lymphocyte specific CD8 + T antibodies and virus counteractors. In addition, viruses like particles have the capacity to stimulate the adaptive immune response that made them risk-free and functional in clinical trials [12]. In the case of CoVs, the lack of information on the interactions between the host immune system and the virus has made it difficult to create vaccine.

Table 1 Therapeutic approach/clinical trial approach

S. no.	Companies	Name/type/treatment for medication	Approach/to overcome condition	Working procedure
1.	Zydus Cadila	Vaccine for Covid-19	1. Development of a DNA vaccine against the viral membrane protein of the virus 2. A live attenuated recombinant measles virus (rMV) vectored vaccine will be developed	The rMV-based vaccine works by inducing specific neutraling antibodies, which will provide protection from the coronavirus infection
2.	NanoViricides	A treatment for nCoV-2019 using its nanoviricide® technology	The company's technology is used to develop ligands	These ligands can bind to the virus in the same way as a cognate receptor and attack various points of the virus
3.	Vir Biotechnology	Developing two monoclonal antibodies	These antibodies can bind to the virus that causes COVID-19	The antibodies target the spike (S) protein of the virus by entering through the cellular receptor ACE2
4.	Inovio Pharmaceuticals has collaborated with Beijing Advaccine Biotechnology Company	Development of the former's vaccine, INO-4800	Not disclosed till now	Not disclosed till now
4	Clover Biopharmaceuticals	Developing a recombinant subunit vaccine using patented Trimer-Tag© technology	Development of the vaccine is based on the trimeric S protein (S-Trimer) of the 2019-nCoV virus, which is responsible for binding with the host cell and causing a viral infection	Antigen-antibody reaction
5	Vaxart	Developing an oral recombinant vaccine in tablet formulation using its proprietary oral vaccine platform, VAAST	Development of vaccines will be based on the published genome of 2019-nCOV which needs to be tested in pre-clinical models for mucosal and systemic immune responses	Not developed
6	Cyto Dyn	Leronlimab (PRO 140), a CCR5 antagonist	The drug is in phase 2 clinical trial	PRO 140 is an entry inhibitor. PRO 140 binds to the CCR5 receptor on the CD4 cells, and interferes with virus ability to enter the cell and stop viral replication
7	Linea Rx and Takis Biotech	Develop a linear DNA vaccine	They will use Polymerase Chain Reaction (PCR)-based DNA manufacturing technology to develop the vaccine	Not yet disclosed
8	BIOXYTRAN	BX-25, as a treatment for Acute Respiratory Distress Syndrome (ARDS) in late-stage patients infected with the coronavirus	The diffusion of oxygen to the blood gets decreased in patients suffering from ARDS leading to fluid build-up in the lungs	BX-25 is designed to be 5000 times smaller than blood cells and efficiently transport oxygen through the body for a period of 9 h before being processed by the liver. The drug can help in supplying oxygen to the vital organs and enable the patient to recover and survive
9	Novavax	MERS coronavirus vaccine	It inhibited infection by inducing immune responses in the laboratory studies	The candidate is designed to primarily bind to the major surface S-protein and developed using the company's recombinant nanoparticle vaccine technology

Table 1 continued

S. no.	Companies	Name/type/treatment for medication	Approach/to overcome condition	Working procedure
10	Inovio and Gene One Life Science	DNA immunotherapy, INO-4700 (GLS-5300)	The vaccine was well-tolerated and demonstrated high immune responses against the MERS-CoV in 94% of patients in the early-stage clinical trial in July 2019	Generated broad-based T cell responses
11	Gilead Sciences	Remdesivir (GS-5734)	Suppressing viral RNA production	Remdesivir is metabolizes into its active form GS-441524. GS-441524 is an adenosine nucleotide analog that confuses viral RNA polymerase and evades proofreading by viral exoribonuclease (ExoN), causing a decrease in viral RNA production
12.	Biocryst Pharma	Antiviral drug Galidesivir (BCX4430)	Broad-spectrum antiviral activity	It is a nucleoside RNA polymerase inhibitor that disrupts the process of viral replication
13.	Regeneron	Combination of monoclonal antibodies REGN3048 and REGN3051 through intravenous administration	Inhibit binding of virus with the host cells	Both the antibodies bind to S-protein of MERS coronavirus

The development of vaccines is a key component in preventing widespread viral infection and reducing morbidity and mortality associated with numerous viral infections [48]. Coronavirus vaccines may be live attenuated coronavirus, inactivated coronavirus or S protein-based. Vaccines have been developed which target several animal coronavirus and some have been shown to be effective in preventing viral infection. The mechanism is no fully understood, but is believed to result from increase absorption and spread of virus by biding virus-antibody immune complexes to Fc receptors on the macrophage surface; low-title [subneutralizing] antibodies directed against the S protein are primarily responsible for this [5].Coronavirus infection, and studies of vaccines and passive immunoprophylaxis conducted with mice and hamsters indicate that previous exposure and the existences of Nabs provide protection [48].

Inactivated coronavirus vaccine

In experimental animals the immunogenicity and effectiveness of inactivated SARS-CoV vaccines has been established, and one such vaccine is being evaluated in the clinical trial [17].

Live attenuated coronavirus vaccine

Live attenuated SARs-CoV vaccines have not been evaluated until now. However, system was developed to generate cDNAs encoding the CoV genomes, including SARS-CoV. Through in vitro ligation, the array of cDNAs covering the entire CoV genome can be system length cDNAs from which recombinant viruses can be saved [10]. This system was used for genetic analysis of SARS-CoV protein functions and will be enabled researchers to engineer specific attenuating mutations or modifications into the genome of the virus to develop live attenuated vaccines [10]. Although live attenuated vaccines targeting respiratory viruses, including adenoviruses and influenza, were approved for use in humans. The infectious virus accumulated in the feces of individuals diagnosed with SARS-CoV raises concerns that a live attenuated SARS-CoV vaccine stain may also be deposited in feces, with the potential to spread to unvaccinated people [39]. Another concern is the risk of recombining a live attenuated vaccine virus with wild-type CoV; moreover, ways of engineering the vaccine virus genome to minimize this risk [12].

S protein-based coronavirus vaccine

S protein's roles in receptor binding and membrane fusion indicates-protein based vaccines that induce antibodies to block the binding and fusion of viruses, or to neutralise virus infection. Among all SARS-CoV structural protein is the primary antigenic component responsible for inducing host immune responses, neutralizing antibodies and/or protecting immunity from viral infection. Hence, S protein

has been chosen as an important target for vaccine development and anti-viral development [10].

DNA vaccines against coronavirus

In animal model, specifically in mice, DNA vaccines have shown strong induction of immune responses to viral pathogens; however, clinical data on DNA vaccines in human subjects are limited [8]. DNA vaccines encoding the E, M, N, M and SSARS-CoV proteins had been assessed in mice. The vaccination with vaccines with M-, N-, and S-encoding DNA induced combination vaccines against coronavirus. Among all SARS-CoVs structural proteins, S protein is the primary antigenic component responsible for neutralizing antibodies, inducing host immune responses and/or protecting immunity against infection by viruses [42]. It also evaluated combination vaccines for their ability to increase immune responses. Two dose of a DNA vaccine encoding the S protein, followed by immunization with inactivated whole virus, were shown more immunogenic in mice than either type of vaccine alone [48]. The combination vaccine triggered high immune responses, both humorally and cell-mediated. Combination vaccines can increase the effectiveness of the candidates for the DNA vaccine. In addition, the lessons learned from these vaccinations will aid in the development of future vaccines against established and newly identified coronavirus [8, 10, 42].

Conclusion and future perspectives

The severe acute respiratory syndrome associated with CoVs is the major health risk factor of current destructive outbreak in China which resulted in huge rate of mortality. The present outbreak started in Thailand, Korea and Japan have been confirmed the detection of a human infection with 2019-nCoV from China country. Pathogenesis of coronavirus infection and mechanism of transmission are still not cleared so, no approved therapy is available for treatment of coronavirus [42]. However, DNA vaccines, monoclonal antibodies, and different combinations of HIV drugs, and herbal medications have been used for its treatment under expanded access program. Different nations are providing huge funds for the development of anti-CoV drug which encourage researchers for extensive studies. Presently many promising drug molecules are under preclinical and clinical phase. Hopefully these molecules will successfully pass all phases of clinical trial and will reach public as early as possible to overcome the suffering from CoVs disease.

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