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Title: An overview of basic molecular biology of SARS-CoV-2 and current COVID-19 prevention strategies

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Abstract: COVID-19 manifests regarding extreme acute respiratory conditions caused by a novel beta coronavirus (SARS-CoV-2) which is reported to be the seventh coronavirus to infect humans. Like other SARS-CoVs it has a large positive-stranded RNA genome. But specific furin site in the spike protein, mutation prone and phylogenetically mess Orf1ab separates SARS-CoV-2 from other RNA viruses. Since, the outbreak (February - March 2020) which originated in China, researchers, scientists, and medical professionals are inspecting all possible facts from every possible aspect including its replication, detection, and prevention strategies. This led to the prompt identification of its basic biology, genome characterization, structural based functional information of proteins, and strategies to prevent its spread. Due to the rapid mutation rate, the functional characterization of a few proteins is still lagging. This review summarizes the recent updates on the basic molecular biology of SARS-CoV-2 and prevention strategies undertaken worldwide to tackle COVID-19. This recent information can be implemented for the development and designing of therapeutics against SARS-CoV-2.

Keywords: SARS-CoV-2; Genome organization and expression; Polyproteins; Prevention strategies

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Introduction:

Severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) are the two known lethal coronaviruses that were in news worldwide. In December 2019, the local health center of Wuhan, Hubei Province, China reported that a group of people was suffering from severe pneumonia whose cause was unknown to the health center. The expert from Centres for Disease Control (CDC) identified the disease to be new and described it as a novel coronavirus pneumonia [1]. Initially, WHO named the virus as a novel coronavirus (2019-CoV). Based on phylogenetic analysis of related coronaviruses, on Feb 11, 2020, the Coronavirus Study Group (CSG) of the International Committee on Virus Taxonomy renamed the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to similarity with the one that caused the SARS outbreak (SARS-CoVs), and the disease has been renamed as Coronavirus Disease 2019 (COVID-19) (WHO). The rapid increase and spread of COVID-19, which is characterized by dry cough, high body temperature, breathe shortness, and pneumonia, led the researchers to look into its epidemiology and transmission [2]. Three forms of transmission have been recorded so far – a) Symptomatic transmission with symptoms varying from mild (fever and dry cough), severe (shortness of breath, dyspnea, respiratory frequency of ≥30/minute, blood oxygen saturation of ≤93%, lung infiltrates of >50% within 24-48 hours) [3] or critical (septic shock, respiratory failure or multiple organ dysfunction/failure) [4]. b) Presymptomatic transmission (transmission of the virus from infected COVID-19 patients before significant symptoms occur [5, 6] and c) asymptomatic transmission (transmission of the virus from individuals who do not develop symptoms) [7]. Later two forms of transmission may fail proper diagnosis and individuals may step out in the crowd assuming the absence of the virus in their body, thus having the potential of spreading COVID-19 silently. Based on the airborne nature of diseases like tuberculosis, chickenpox, and measles, a study was conducted to figure out the nature of airborne transmission of the disease after the SARS epidemic in Hong Kong [8]. This led a group of the research team to carry out the same for novel SARS-CoV-2 from two designated hospital and public areas of Wuhan and they found that SARS-CoV-2 RNA amount elevated at patient's toilet areas as compared to isolation wards and ventilated patient's room [9]. SARS-CoV-2 belongs to beta coronavirus and COVID -19 is the third zoonotic outbreak of beta-CoVs, the first outbreak was due to severe acute respiratory syndrome coronavirus (SARS-CoV) originated from a bat and civet cat occurred during 2002-2003 and second outbreak was due MERS-CoV, which began in Saudi Arabia with approximately 2,500 cases and 800 deaths and still counting [10, 11]. Structurally, SARS-CoV-2 has round or elliptic and often pleomorphic form, with a diameter of approximately 60–140 nm and is sensitive to ultraviolet rays and heat [12]. The complete SARS-CoV-2 consists of four components: spike (S), nucleocapsid (N), membrane (M), and envelop (E) (Fig. 1**)** [12]. The most possible animal reservoir hosts of SARS-CoV-2 are wild animal and bats [13], but it is not confirmed whether COVID-19 is directly transmitted by bat or wild animals or through some other intermediates [14]. Recent whole-genome analysis of SARS-CoV-2 shows that it has 96% similarly with bat coronavirus, which indicates that bat is the most possible host of SARS-CoV-2 [15]. But based on macro genomic, molecular biology detection and electron microscopy, SARS-CoV-2 isolated from pangolins show 99% similarity with pandemic causing human viral strain suggesting pangolins may also be the possible potential host of SARS-CoV-2 [16]. Recently mutations have been reported in the novel strain in orf1ab, ORF8, and in 116 different positions, suggesting the reason for its global spread and severity [17]. A recent article entitled "Covid-19 evolution: One strain overwhelms others" published in Deccan Herald mentioned the evolution of 11 types of virus including ancestral type-O of Wuhan origin during transmission to different countries of the world and eventually type (A2a) turned out to be most dominant over others and reported in most of the regions [18].

Coronaviruses are enveloped, positive-sense, single-stranded RNA virus with 3' and 5' cap structure. The approximate genome size is 30kb in length [19]. Based on the genome structure and expression of the family Coronaviridae, it is divided into four genera (alfa, beta, gamma, and delta) coronavirus [20]. Alphacoronavirus and betacoronavirus infect mammals and show similarity to other positive-sense, single-stranded RNA virus. In this review, we intend to summarize the current Molecular biology of SARS-CoV-2 which includes its basic biology inside the host, genome organization, and expression, enzymes involved in the replication. Recent advances in

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prevention and control strategies across the globe to date have also been summarized.

Genome arrangement:

Two notable features of the SARS-CoV-2 genome based on structural and biochemical studies were described upon comparison with alphacoronavirus [13, 21, 22]. The genome of SARS-CoV-2 consists of 29903 nucleotides, ssRNA linear possessing 13 open reading frames (ORF) which were annotated using homology to SARS-CoV NC_004718.3 [23]. 5'UTR region is predicted from (1 to 265)nt, orf1ab (266 to 21555)nt, ORF3a (25393 to 26220)nt, E (26245 to 26572)nt, M (26523 to 27191), ORF6 (27202 to 27387), ORF7a (27394 to 27759)nt, ORF7b (27756 to 27887)nt, ORF8 (27894 to 28259), N (28274 to 29533)nt, ORF10 (29558 to 29674) and 3'UTR (29675 to 29903)nt (based on computation analysis and prediction of NCBI Gene bank Accession no NC_045512). The genome organization of the betacoronavirus clearly states that the genome contains a 5' cap, ORF 1ab, replicase gene, S (spike orf), E (envelop orf), M (membrane orf), N (nucleocapsid orf) along with 3' poly-A tail which is near to similar to that of the organization of SARS-CoV-2 [24, 17]. The predicted functions of the ORFs of SARS-CoV-2 are based on the known coronaviruses and are not yet experimentally proved [21]. The proteins encoded by the S, E, M, and N gene represent the structural proteins of the coronavirus [25]. The accessory proteins encoded by ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 [26] may provide a collective advantage in the infected host and pathogenesis.

Receptors:

The viral entry into the host cell is mediated by special spike protein, which is composed of three domains, outer N-terminal domain having unit S1 and S2, a cytoplasmic C-terminal domain, and a transmembrane domain [27, 28] (Fig. 2). S1 and S2 are highly conserved and are glycosylated. Priming of S protein by cellular proteases (Transmembrane protease, serine 2, TMPRSS2), results in cleavage at a specific site and S2 subsequently mediates the fusion of viral and host membrane [29]. A receptor-binding domain (RBD) presents in the spike protein precisely binds to the angiotensin-converting enzyme 2 (ACE2) present in the host membrane [30, 31]. Determination of the crystal structure of the RBD domain bound to ACE2 at 2.45 A˚ resolution showed that the binding mode of the novel virus is nearly identical to that of the SARS-CoV RBD [27]. But a functional polybasic (furin) is reported to be present in SARS-CoV-2 which is optimized to effectively bind to ACE2 [**22**]. This special cleavage site is reported to be missing in other SARS-like CoVs [32]. A recent computational analysis predicted that a single N501T mutation may lead to an increased binding affinity of SARS-CoV2 RBD and ACE2 present in the human cell [21]. The same research group also described the similarity (73.8-74.9 percent amino acid) of the predicted structure of the spike RDB with the crystal structure of the spike RBD of SARS-CoV (PDB 2GHV) complexed with human ACE2. Moreover, determination of the crystal structure of the RBD, revealed that the compact hACE2 binding ridge in SARS-CoV-2 RBD stabilizes the binding of two viruses which increases the hACE2 binding affinity and this may be the reason of animal to human transmission of the novel strain [33]. Interestingly, a group of scientists recovered a mutant strain (Del-mut-1, 10amino acid) in S1/S2 junction from Vero-E6 cells which did not show any major change in lung pathology and hence could be a lead for attenuated vaccine [34].

Genome expression:

The viral genome injecting into the host cytoplasm is facilitated by S protein, which is the translation product encoded by S gene, a protein of 1273 amino acids. It mediates the fusion of envelope with the host's cell membrane [35]. Protein information of SARS-CoV-2 is summarized based on homology to SARS-CoV NC_004718.3 [23]. The product encoded by orf1a is polyprotein 1a (pp1a, 4405 amino acid residue). Addition of 1b encoded sequence to 1a, yield 7096 amino acid residue protein (pp1ab). It occurs as a result of ribosomal shifting of -1 reading frame [23]. Thus, orf1ab encodes a huge replicase polyprotein. The four structural genes S, E, M, and N are predicted to code Spike protein (1273 amino acid), Envelope protein (75 amino acid), Membrane protein (222 amino acid), and Nucleocapsid phosphoprotein (419 amino acid) respectively. An identical leader sequence (protein of 180 amino acids) is carried by each mRNA [23, 36, 37]. ORFs has not yet been experimentally verified, six accessory proteins (ORF3a, 275 aa; ORF6, 61 aa; ORF7a, 121 aa; ORF7b, 43 aa; ORF8, 121 aa; and ORF10, 38 aa) through computation analysis has been predicted [17, 23] (Fig. 3). Positive-sense genomic RNA (gRNA) and subgenomic RNAs (sgRNAs) are utilized to generate negative-sense RNA intermediates. A recent study [37] suggested internal modification sites concerning the poly (A) tail of the transcript of ORF1ab and S which is predicted to be playing a

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crucial role in RNA stability inside the host. Due to the complex regulation of viral RNA synthesis and quick recombination, SARS-CoV-2 may show flexibility in specificity and sensitivity.

Proteins involved in genome replication and transcription:

Unlike other RNA viruses, CoVs have the largest RNA genome [38], which gets translated into structural and non-structural proteins (nsps). Complex polyproteins of SARS-Cov-2 and other coronaviruses are due to the construction of diverse RNA sequences with varied enzymatic activities. The nsps undergo post-translational changes and regulate the activities of the replicative proteins [28]. ORF1a produces pp1a (440-500 kDa) which gets processed into 11nsps and ribosomal frameshift results in the continuation of translation of ORF1b producing a huge polypeptide pp1ab (740-810 kDa) which gets processed into 15 nsps [37] (Fig. 4). Usually, coronaviruses including SARS-CoV possess a papain-like protease for the processing of polyproteins [39]. But a recent genome-wide structure and functional modeling by Zhang lab, University of Michigan revealed an additional 3C-like protease (3CL-PRO) in SARS-CoV-2, which may be responsible for cleaving the C-terminus of replicase polyprotein and also predicted that 3Cl-PRO recognizes substrate containing sequence ILMVF-Q-I-SGACNN and also can bind an ADP-ribose-1-phosphate [40*]. Huang and his team also demonstrated de novo designing of peptide that blocks the interaction between SARS-CoV-2 spike and human ACE2 [41]. Replication and transcription of the viral genomic RNA is carried out by nsp12 possessing RNA-dependent RNA polymerase (RdRp) activity [42]. The cryo-EM structure of SARS-CoV-2 with the complex nsp12-nsp7-nsp8 at 2.9-A˚ and N terminal beta-hairpin domain has been discussed [43]. The complete structure, almost all residues is revealed, where residues 4 to 28 and 51 to 249 include 8 helices and 5-stranded beta-sheet respectively towards the N terminus. The three conserved structure of the polymerase viz. Finger domain (L366-A581), palm domain (T582-P620 and T680-Q815), and a thumb domain (H816-E920) are also shown where palm domain forms the active site of the enzyme [43]. The structural features especially nsp12 can be utilized as a potential drug target and possibly move towards the control of the COVID-19 pandemic. No experimental analysis for the other proteins have been reported till date but many computational prediction and information from earlier SARS-CoV is underway for revealing the biology, molecular structure, and function of the SARS-CoV-2, which hopefully would contribute to therapeutics and drug research in near times. SARS-CoV helicase is considered to be a multi-functional protein harboring a zing-binding domain in N-terminus showing RNA and DNA duplex-unwinding activities (5′-3′) [44]. Similarly, Exoribonuclease in SARS-CoV-2 is predicted to have ssRNA and dsRNA 3′-5′ proofreading exoribonuclease and N7- guanine methyltransferase activity. Uridylate-specific Endoribonuclease (NendoU) harbor uridylate specific enzyme in the presence of Mn2+, 2′-O-methylase is predicted to mediate mRNA cap 2′-O-ribose methylation to the 5′-cap of viral mRNA [40*]. Coronaviruses ExoN has been predicted to be involved in repair mechanisms [42].

Viral entry and host immune responses:

The entry process of 2019 coronavirus (SARS-CoV-2) follows the usual pattern of a common virus life cycle, which starts with the attachment of the spike protein with the host ACE2 receptor. The cellular Human Airway-Trypsin like protease (HAT) [45] cathepsins and Transmembrane protease serine 2 (TMPRSS2) breaks the S-protein [46] and facilitate the fusion of cellular and viral membrane through the endosomal pathway and release of SARS-CoV-2 RNA into the host cell takes place [24]. The receptor-binding region (RBD) of SARS-CoV-2 uses the hACE2 receptor as an entry key [21]. The replicase gene of the genomic RNA of the virion, once inside the host cell cytoplasm, is translated using host cell machinery. After the formation of nsp by proteolytic cleavage, some of the nsp combine with the sense strand (RNA+) to form the replicase transcriptase complex which facilitates RNA replication. When RNA+ strand is replicated, it produces genomic RNA but that happens to be antisense RNA (RNA-). The antisense RNA strand can be replicated back into the genomic (RNA+) strand or can be transcribed into sub-genomic RNAs by discontinuous transcription [47]. The sub-genomic RNAs are mRNAs that can be translated into viral structural proteins. Many different structures are proposed which regulate alternate RNA synthesis stages which including seven stem-loop structures at the 5’-UTR [48-51]; a bulged stem-loop, a pseudoknot, and a hypervariable region at the 3’-UTR [52-55]. CoVs genomic RNA replication is mediated by RNA-directed RNA polymerase (Pol/RdRp) which is also responsible for transcription of the viral RNA genome. There is experimental evidence for SARS-CoV that nsp7 and nsp8 activate and confer processivity to the RNA-synthesizing

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activity of the Polymerase [46]. The synthesis of sub-genomic RNA through the discontinuous extension of the antisense RNA strand is mediated by the fusion of leader TRS and body TRS. It has been found that Pol/RdRp when reaches at any one of the body TRS, it pauses and then either continues elongation to the next TRS or jumps to the leader TRS, thus terminating transcription [21]. Following the formation of sub-genomic and genomic RNAs, the viral structural proteins encoded by the sub-genomic RNAs are translated. These proteins are then trafficked to the Endoplasmic Reticulum followed by the entry into the Golgi Intermediate Compartment via the secretory pathway. The viral genomes are encapsulated by the N-protein into the membranes of the ER-Golgi intermediate compartment (ERGIC) where both the structural proteins and viral genome form mature virus particles [56, 57]. Both the M and E proteins function together to form the coronavirus envelops [57]. Finally, the mature virions transported via vesicles and released out of the cell through exocytosis.

While the virus completes its life cycle in the host cell, the signaling molecules of the host immune system already starts its action either by regulating the expression of the genes associated with immune response or by initiating cascade of reactions necessary for immune response. In humans, after cell infection, the up-regulation of Interferon stimulated genes (ISGs) is necessary for the induction of Interferons for antiviral defense [58]. The binding of the transcription factor STAT1 homodimers to ISGs are moderated by the various IFNs (IFNa, IFNb, IFNg, and IFNl) and thus, play a crucial role in host defense (signaling the nearby cells) [59, 60]. Recently, [61, 62] suggested that uses of approved IFN in clinical therapy against SARS-CoV2 may either vanish or worsen the symptoms of COVID-19. Experimentally it has been shown that IFNa drives the up-regulation of ISGs in ACE2 expressing cells and high expression of the same in influenza-infected upper epithelial cells [63]. Thus, this information can be considered for designing IFN-system targeted therapeutics. It is clear that upon an attack of coronaviruses, due to the huge activation of immune molecules in the host cell, a cytokine storm is generated which results in adverse damage of both targeted and nearby tissues (**Fig. 5)** [12, 64]. A study suggested that interleukin 6 (IL-6) which is generated by leukocytes plays a leading role in inducing the storm [12]. Moreover, IL6 is known to be associated with inflammatory response, disease, disorders, differentiation of B lymphocytes, cell growth regulation, and more importantly with cytokine release syndrome (CRS) [12, 64]. People with diabetes, heart disease, pulmonary disease, and kidney problem when infected with SARS-CoV-2 have shown worse outcomes because of the plasmin and proteases which tend to break the S protein (furin site) which eventually increases its virulence [65]. However, this system could be a target for future therapeutics.

Techniques in COVID-19 detection:

The shortage of first-hand testing kits and the unavailability of reliable and efficient diagnostic tools also elevated the risk of spreading COVID-19 infection. So far, several diagnostic tools were developed to manage the COVID-19 outbreak. Information at the molecular level and the use of molecular biology tools have always been remaining promising in detecting viral diseases [66]. Techniques adopted till date around the world for diagnosis and controls of COVID-19 are summarized:

Molecular biology-based diagnostic tools:

Molecular biology-based diagnostic tools require information regarding the genomic and transcriptomics of the target organism and understanding of biology at the molecular level not only provides the detection of such pathogens but also opens the way to target and combat the same. One such technique is the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) [67] which has been used since long in the detection of pathogenic viruses causing respiratory infection [68, 69, 70]. Various scientists from different regions adopted various assays of RT-PCR approved by WHO, CDC, and FDA EUA [71-73, 29, 74]. However, the availability of RT-PCR tools and reagent kits are not up to the demand. Moreover, PCR technology fails to identify asymptomatic patients with SARS-CoV-2 infection recovery [75], and hence, optimization of protocols and its validation is in the foremost need for the proper diagnosis of COVID-19 infection. Isothermal amplification techniques including recombinase polymerase amplification [76], loop-mediated isothermal amplification (LAMP) [77], Reverse Transcriptase LAMP (RT-LAMP) [78, 79], helicase dependent amplification [80] among a few are in the developmental stage, some of which has been clinically tested for SARS-CoV-2 detection. Medical imaging through X-ray and chest CT scan provides better screening and diagnosis of COVID-19 and at the same time elevates the risk of spreading the disease especially to the medical staff [81, 82].

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Artificial Intelligence techniques can be coupled with a Chest CT scan for safe, efficient, and accurate image acquisition, segmentation, and diagnosis of COVID-19 [83]. Besides, CRISPR-Cas technology has been adopted for RNA sensing that uses Cas12 in a combination of isothermal amplification and DETECTOR technology [84] and Cas13a/C2c2 ribonuclease proteins [85] following Cas13 based detection methodology and SHERLOCK detection protocol [86].

Host-antibody and viral protein-based diagnosis:

One of the common types of rapid diagnosis of COVID-19 is that of the detection of the presence of antigens and antibodies (in the blood samples of the individuals that have likely been infected by SARS-CoV-2) [87]. However, antibodies produced against SARS-CoV-2 may counteract with other pathogens resulting in false-positive detection. Also, several factors such as the severity of the disease, age of the infected individual, and medication may result in hindrance in the actual diagnosis of COVID-19 infection [88, 89]. Similarly, rapid diagnostics using antigens (viral protein) may not provide full-proof diagnostics which may be due to limitations in quality and time of sample collection, concentration of target antigen in the sample, and so forth [90]. In a note of this, uses of antibodies and viral proteins for rapid diagnosis of COVID-19 have not been yet approved by WHO although encouraged scientists and researchers to utilize it in disease surveillance and epidemiologic research. Very recently, first indigenous antibody detection kit (human IgG ELISA test kit) was developed by National Institute of Virology (NIV), Pune which will aid in the surveillance of SARS-CoV-2 infected population [91]

Non-laboratory based diagnosis:

Diagnosis at the preliminary level is at most important o estimate as well as resists the spread of the virus from an infected person to a healthy individual. Though RT-PCR based diagnosis is more accurate its time-consuming property leads to the need for non-laboratory rapid testing kits. Several pharmaceutical industries and companies have come up with rapid antibody (IgM/IgG) testing kit and Real-time PCR mediated SARS-CoC-2 diagnostic kits. US-FDA and CE have approved several real-time PCR kits and CE marked a rapid antibody test for SARS-CoV-2 diagnosis and can be used directly after due marketing approval from DCGI [92]. CSIR India has come up with low-cost paper-strip test kits that use a cutting-edge-gene-editing tool (Crispr-Cas9) to target and identify the genome sequences of the novel coronavirus in the samples of suspected individuals within an hour [93]. As of 1st May 2020, 45 real-time PCR kits (10 are Indian based company) and 23 antibody-based rapid test (9 from Indian based company) have been validated by ICMR and NIV Pune respectively [94, 95].

Prevention and control strategies of COVID-19 to date:

No such vaccine is available till date against COVID-19, but research is in its peak to develop an efficient and effective solution considering molecular biology and genetic makeup of both the virus and host system. Based on approaches undertaken by different countries around the globe, the strategies have been summarized into Western, Homeopathy, and traditional. Certain innovative ways undertaken to prevent the disease from spreading are also summarized.

Western:

The first human trial for COVID-19 vaccine mRNA-1273 was done by the USA which was developed by the NIAID scientists and their collaborators at the biotechnology company Moderna, Inc., based in Cambridge [96]. Following that, a series of trials have been carried out considering both host and viral proteins to prevent the virus to replicate inside the host [97]. The first vaccine mRNA-1273 generally targets the spike protein of the COVID-19 virus which is necessary to bind with the host receptor AEC2 (Angiotensin-converting enzyme 2) [98, 99]. On the other hand, researchers have also focused on the use of Hydroxychloroquine and Azithromycin since it raises the pH of the cell because of which the enzymatic activity halts. The increasing pH in cytoplasm resists the binding of Spike-AEC2 protein and also because of the rise in pH in cell organelles, their activity halted because of which virus cannot produce its copies [100-103]. Chloroquine and Zinc also function the same as that of Hydroxychloroquine and Azithromycin but instead, the Chloroquine acts as a channel to diffuse zinc to the cell since [100, 101, 104, 105]. Several Pharmaceutical industries also consider Arbidol Hydrochloride, Ivermectin, Fingolimod, Methylprednisolone, and Ritonavir, etc. which may be a possible cure for Covid-19 pandemic [106]. Israel Institute of Biological Science recently reported that they are working on a monoclonal antibody which can attack and neutralize the virus in the patient's body [107]. Recently a UK-based biotech company Synairgen has been granted to go-ahead to take its lead candidate SNG001 into a trial for the treatment of COVID-19 patients [108] (List of

vaccines under clinical trial developed by different Pharmaceutical Company is depicted in Table 1) [103,104,107,108,109,114-130]. Recently some of the leading pharmaceutical company and research institute stated about their ongoing clinical trial, among them BioNet Asia, Zydus Cadila, Greffex, Cipla, Council of Scientific and Industrial Research (India), Centro Nacional Biotecnologia (Spain), IMV Inc, University of Pittsburgh, AJ Vaccines, Flow Pharma Inc, Baylor College of Medicine, OncoGen, University Of Alberta, etc. are most prominent [124]. Council of Scientific and Industrial Research (India) has received approval for the clinical trial of two drugs – Favipiravir and Phytopharmaceutical by Drug Control General of India (DGCI) to combat coronavirus [126]

Homeopathy:

Few countries including several states of India are considering Homeopathic medicines against COVID-19. Arsenic album is regarded as an immune booster by Central Council for Research in Homoeopathy [127]. The world-first clinical trial of Homeopathic medicine for the treatment of COVID-19 has begun at Neminath Homeopathic Medical College, Agra (India) [128]. Cuba, a North American country also declares about the distribution of Prevengovir, a new homeopathic immunological booster to fight against COVID-19 [129]. Some Scientists mentioned the utilization of several homeopathic medicines (Arsenicum Album, Phosphorus Flavus, Atropa Belladonna, Antimonium Tartaricum, Eupatorium Perfoliatum, Hepar Sulphur, Lycopodium Clavatum, Kalium Phosphoricum) in Italy which is the major hotspot to Covid-19 and suggested positive results. Even though few countries consider this branch of medical science, huge research is required in this field which may lead our battle against COVID-19 into new horizons and may prevent and control this deadly disease more efficiently [130].

Traditional:

China, where the pandemic originated used traditional medicines to treat approximately 58.3% of their confirmed cases, reported promising results, and succeed in reducing recovery time up to 1.7 days [131,132]. A joint study done by the Shanghai Institute of Materia Medica and Wuhan Institute of Virology found that Shuanghuanglian oral liquid could inhibit SARS-CoV-2 [102, 103, 133]. Lianhuaqingwen capsule reducing the level of inflammatory factors and can regulate the immune response of the virus [102,103]. Yupingfeng San, ancient medicine that possesses three herbs namely; Astragalus, Fangfeng, and Atractylodes helps to maintain healthy Qi (substance require for proper body functions) of lungs and spleen. It can also remove pathogenic Qi (substance pathogenic to body) and improve immune response [133]. Sangju yin and Yinqiao san are two Chinese traditional medicines that are used to clear lung heat, expel phlegm, relieve cough, regulate the patient's lungs and restore normal lung function and also enhance the immune system in the body [133]. Maxingshigan tang and Baihegujin tang are reported to expel the pathogenic Qi and enhance lung Qi of the body [133]. Besides these, several other Chinese traditional used medicines namely; Qingfei paidu decoction, Gancaoganjiang decoction, Sheganmahuang decoction, Qingfei touxie fuzheng recipe, etc. where Qingfei paidu decoction shows an efficiency of 90% against COVID-19 symptoms. It can enhance immune response and found to inhibit the replication of SARS-CoV-2 by acting on ribosomal proteins [131]. A group of Scientist further mentioned few Chinese traditional remedies which include Qingfei Paidu Tang, Xiang Sha Liu Junzi Tang, Shen Ling Baizhu San, Qingfei Paidu Tang, Qingwen Baidu Yin, Shen Fu Tang, Ma Xing Shi Gan Tang, Da Yuan Yin, Xia Ling Tang and described their medicinal value against COVID-19 [134].

India also had a great treasure of traditional knowledge in medical science which is known as Ayurveda. Several herbs namely; *Allium sativum*, *Curcuma longa*, *Trachyspermum ammi*, resin of *Styrax benzoin*, and *Boswellia* species are used for disinfection purposes [135]. Rasayana, a classic Ayurvedic therapy function as an antioxidant, anti-stress, anti-inflammatory, anti-microbial, vaccine adjuvant, and can boost immunity against diseases. Sanjivani vati Ayurvedic medicine had a great role during the treatment against fever, cold, cough, and indigestion. Except this, *Lakshmi Vilas Rasa*, *Pippali rasayana*, *Chitrakadi vati*, *Go jihvaadi Kashaya*, *Vyaghri haritaki*, *Kantakaari Avaleha*, *Dashamul kwath*, *Sitopaladi*, *Talishadi*, *Yashtimadhu*, *Pippali rasayana*, *Laghu Vasant Malati*, *Sanjeevani vati*, *Tribhuvan keerti rasa*, *Brihata Vata Chintamni rasa*, *Mrityunjaya rasa*, and *Siddha makardhvaja rasa* can also be used to treat mild to severe COVID-19 patient [135]. The Indian Health Ministry (Ministry of Health and Family Welfare) recently stated that Council of Scientific and Industrial Research (CSIR), India under the guidance of Indian Council of Medical Research (ICMR), New Delhi have started conducting clinical trials for traditional Ayurvedic medicines like

Ashwagandha, Guduchi Pippali, Ayush-64, and Yashtimadhu [136,137].

Innovative prevention strategies against COVID-19:

Dealing with the COVID-19 outbreak is of high concern and from the initial stage of the onset of this pandemic, researchers, high-tech manufacturers, designers came forward with innovative equipment for the prevention of SARS-CoV-2 virus. PPEs like masks, gloves, and other essentials like rapid testing facilities, ventilators are needed in extraordinary quantities especially for the frontline coronavirus warriors. Chinese company based Anti-virus snood, smart helmet, 3D printed isolation wards, drones; Italian company based 3D printed ventilator valves; South Korean based coronavirus booth; Czech based 3D printed face shield [138]; Indian company based cost-effective novel mask [139], bag valve mask ventilator (Ambu bag) by Mahindra and Mahindra Company, DRDO based UV disinfection tower [140], mobile virology research lab and diagnostic laboratory (MMVR) [141], portable or fixed microwave sterilizer – ATULYA [142], IIT based incubation boxes [143] to name a few are being designed for prevention and surveillance of COVID-19 outbreak.

Emerging strategies for COVID-19

In a sea of different platforms for the possible treatment of COVID-19, CRISPR-Cas mediated gene-editing technology remains promising which can be used to manipulate target gene using guide RNA and Cas protein (cleavage protein) [144-146]. CRISPR technology using Cas9 [147], Cas12a [84], and Cas13 [148] protein have shown to be used to knock out genes in coronaviruses. Use of CRISPR-Cas13 is advantageous over CRISPR-Cas9 as thousands of Cas13 target sites have been identified in some human infectious coronaviruses [148] and can be programmed and easily updates CRISPR RNA sequence in response to the change in viral genome sequence which may be due to response to therapy, thus inhibiting viral escape by evolving resistance [149]. Delivery of CRISPER-Cas to the target RNA in a living individual could be using lipid nanoparticles [150], HEDGES platform [151], or ribonucleoprotein complex [152] but is still challenging. The Cas13d can be harnessed to target a wide range of ssRNA viruses including SARS-CoV-2 along with CARVER (Cas13-assisted restriction of viral expression and readout) for rapid diagnostics and anti-viral drug development [149].

Besides this molecular platform, nanotechnology-based (silver and gold-based) therapy [153-157], ultrashort pulsed laser irradiation technology using short-lived reactive oxygen species (singlet oxygen) [158, 159] and ultrasound-based therapy [160] could be employed in the medicament of COVID-19. Although these emerging techniques are under development, extensive research, and laboratory diagnosis with proper validation are prerequisite to bringing into play in combating COVID-19. Moreover, the administration of convalescent plasma therapy into SARS-CoV-2 infected patients may be of clinical benefit of viral etiology [161]. Since other treatments might affect the relationship between convalescent plasma and antibody level including intravenous immunoglobulins [162], it might be worthwhile to test the safety and efficacy of convalescent plasma transfusion in SARS-CoV-2 infected individual. Very recently, certain guidelines have been issued by the FDA to provide recommendations to healthcare providers and investigators on the administration and study of investigational COVID-19 convalescent plasma [163]. Another emerging approach is the use of llama derived single domain antibodies called VHH and bacterial super glue to form specific multimeric VHH which has the potential to be used as antiviral tools [164, 165]. This novel approach opens up new opportunities to optimize, reformate, and validate novel tools. Another promising treatment that may regenerate lung disease and reduce inflammation due to COVID-19 infection could be the use of stem cells. This very recent research approach is under trial while waiting for its validation [166].

Challenges in pandemic diseases

A major challenge in answering to emerging pandemic diseases is that vaccine may not extant or effectual against them. Each new strain requires a novel vaccine. It requires several months to years to design and mass-produce a safe and effective vaccine. Moreover, in emergency and severe illness difficulties may arise during transport and storage, vaccine security, maintenance of low temperature may become compromised. Besides, meeting supply-demand, distribution, and uptake of vaccines where vaccination is commonly not practiced are the significant challenges that the government may face during emerging pandemic diseases.

Of note, many of the highlighted specifics in the review are in the developmental or experimental stage which requires clinical validation and some specifics may change with increasing data and more studies on COVID-19.

Update and summary

Synthetic DNA of SARS-CoV-2 have been recently cloned into a yeast-based platform and reverse genetics maybe use to through light into the functional molecular biology and pathogenesis of emerging viruses including SARS-CoV-2 [167] Genomic sequence was used for identifying novel drug targets against COVID-19 through viral-human protein interaction analysis and then cheminformatic analysis of existing compounds was carried resulting in the identification of 66 human proteins that can be druggable [168]. The new SARS-CoV-2 outbreak has brought out many outstanding research work and studies. It includes understanding its infections, transmission, history, genome, and employment of previously reported drugs and many clinical trials with a single goal of designing a drug/vaccine to help our immune system fight better. Information regarding molecular function and structure of the viral proteins has helped greatly in the detection and evolution of new potent and efficient medicines. The rapid increase of information about SARS-CoV-2 along with highly sensitive and reproducible diagnostic and proper obeying of health authority's awareness and guidelines should bring about control of COVID-19 soon.

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 doi:10.20944/preprints202006.0086.v2

Figure Legends:

Fig. 1 Structure of SARS-CoV-2

Fig. 2 Structure of SARS-CoV-2 spike protein, spike protein of 1300 amino acid with NTD, N terminal domain; RBD, receptor binding domain, RBM, receptor binding motif; SD1, subdomain 1; SD2, subdomain 2 are the components of S1 subunit, Left side and FP, fusion peptide; HR1, heptad repeat 1; HR2, hepted repeat 2; TM, transmembrane region; IC, intracellular domain are the components of S2 subunit, right side

Fig. 3 Genome organization and RNA synthesis of SARS-CoV-2. The replicase gene comprises of ORF 1a and 1b which are required for the replication of the genome and the synthesis of sgRNAs. The structural genes, Accessory genes in the genome and leader sequence derived from 5 prime end of the genome are depicted

Fig. 4 Overview of proteolytic processing of SARS-CoV-2 replicase polyprotein pp1ab (740-810 kDa) which gets processed into non structural proteins (nsps).The 3C-like protease (3CL-PRO, marked in red) in SARS-CoV-2, may be responsible for cleaving the C-terminus of replicase polyprotein. The polyproteins are processed by papain like protease (presented in red). RNA-dependent RNA polymerase (RdRp), responsible for viral genome replication and transcription is indicated in red color. The information provided is based on genome wide structure and functional modeling of SARS-CoV-2) [23, 37, 40]

Fig. 5 Overview of viral entry and host immune response leading to cytokine storm and multiple tissue damage; **A.** SARS-CoV-2 entry, replication, transcription, translation and release outside (indicated within red circle) a. SARS-CoV-2 virus, b. binding of spike protein with ACE2 receptor, c. Endocytosis and release of viral genome into the host cell, d. synthesis of nsp from polyproteins by genome RNA translation using host machinery, e. replication and transcription of antisense RNA (formation of viral structural proteins), f. assembly of the RNA(+) strand and structural proteins into Golgi bodies, g. transport of the mature virus particles to the outside of the cell via vesicles, h. exocytosis and release of SARS-CoV-2 virus; **B.** Host immune response leads to Cytokine storm in COVID-19 (indicated within blue circle) [12, 64].

Table:

Table 1 List of vaccines under clinical trial developed by different Pharmaceutical Company*

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Figures:

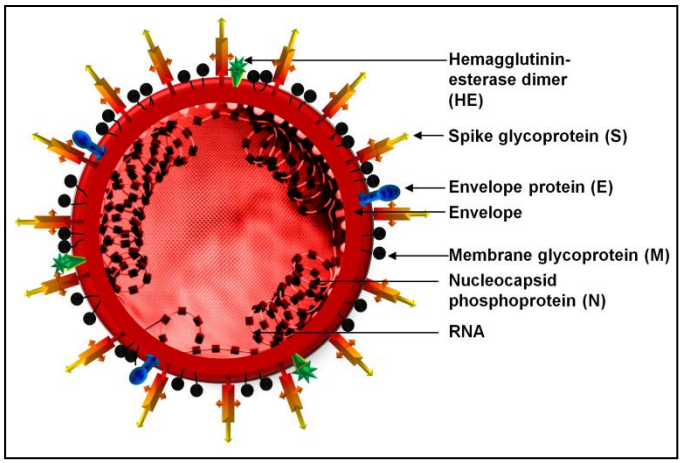

Fig. 1

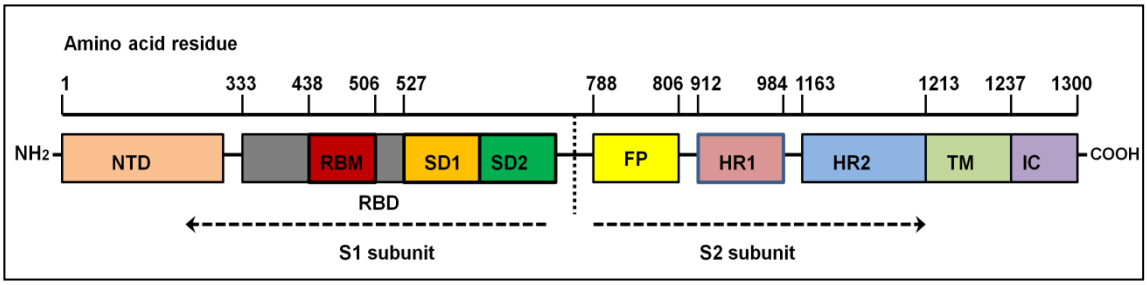

Fig. 2

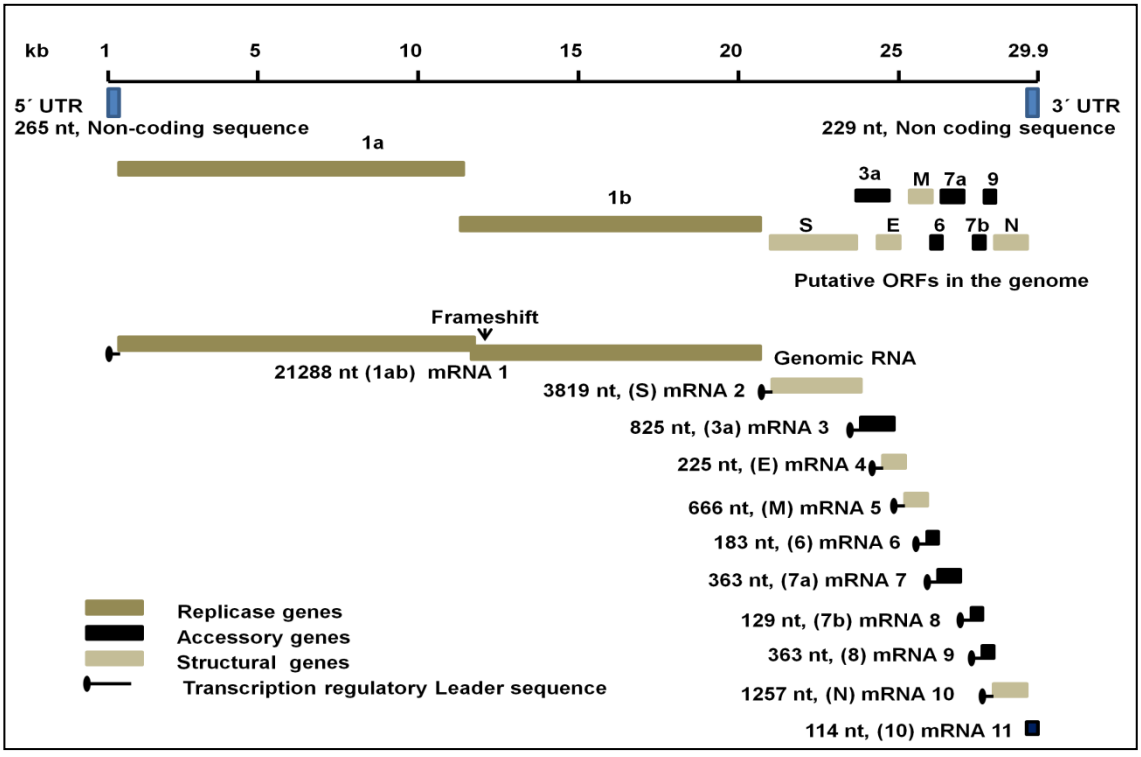

Fig. 3

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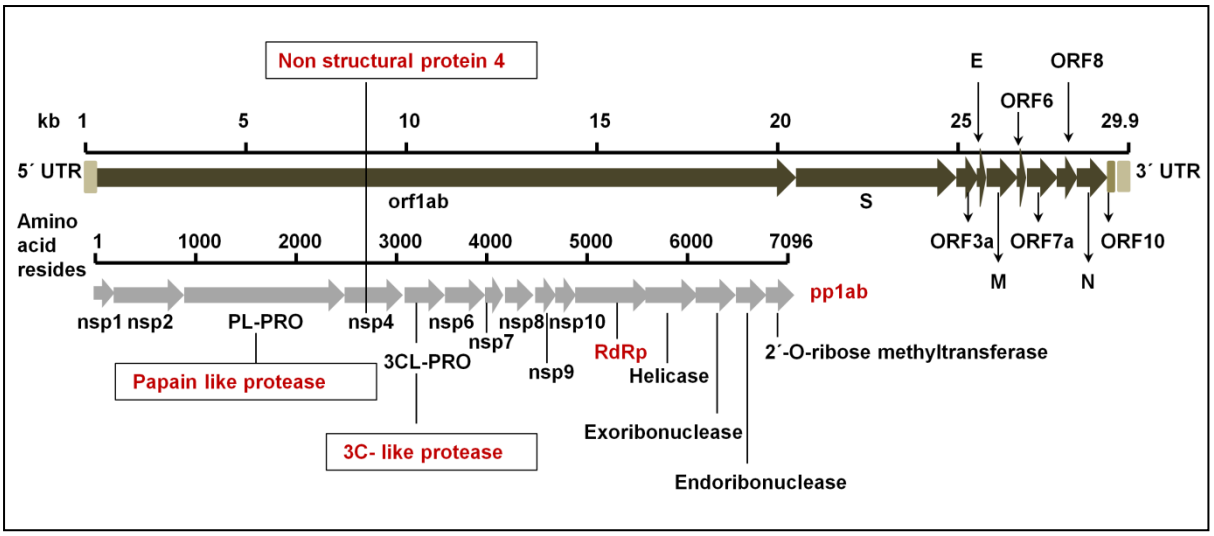

Fig. 4

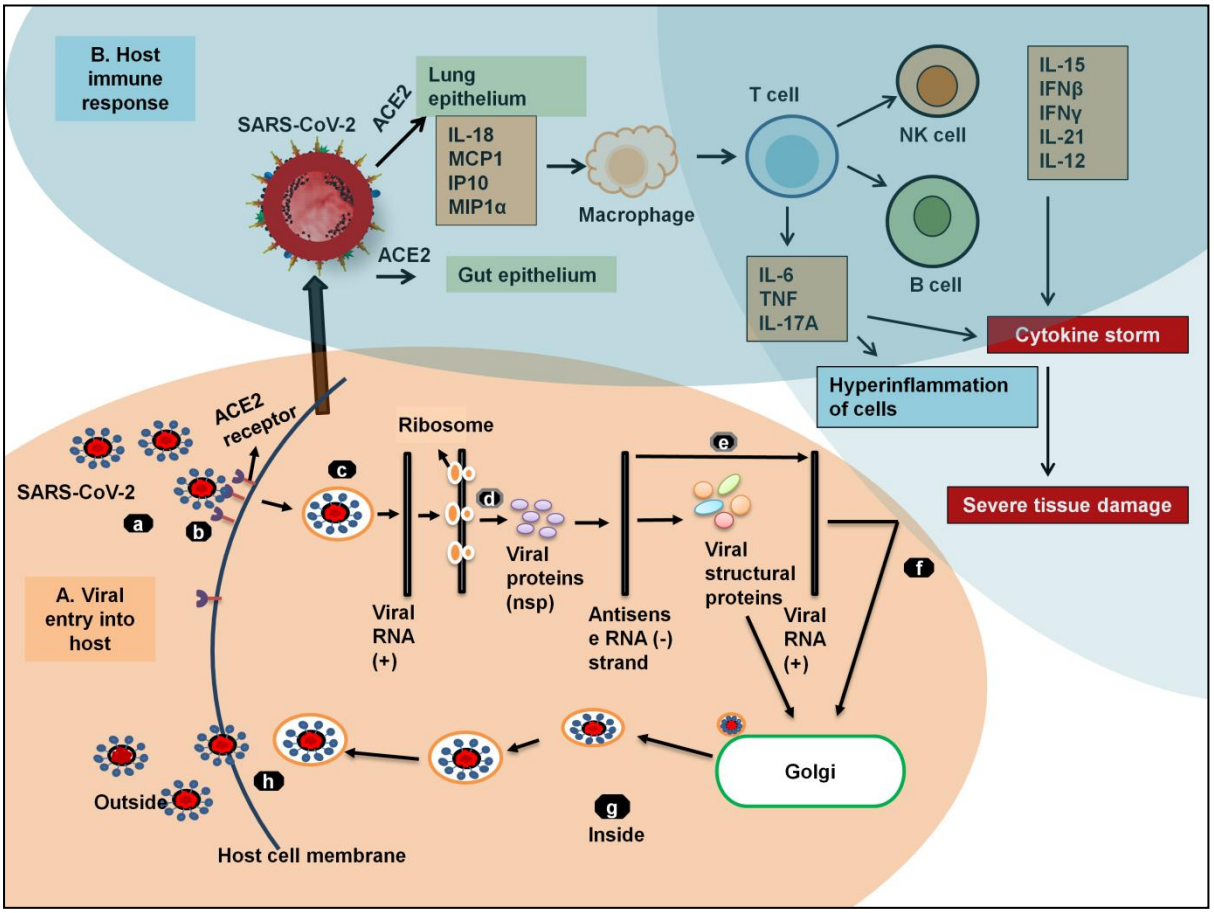

Fig. 5

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Tables:

Table 1

Vaccine Candidate	Developer	Technology	Phase of trial	Location
Ad5-nCoV	CanSino Biologics, Institute of Biotechnology of the Academy of Military Medical Science	Recombinant adenovirus type 5 vector	Phase I-II	China
ChAdOx1 nCoV-19	University of Oxford	Adenovirus vector	Phase I-II	United Kingdom
BNT162	BioNtech, FoSun Pharma, Pfizer	RNA	Phase I-II	Germany
Unnamed	Sinovac Biotech	Inactivated	Phase I-II	China
INO-4800	Inovio Pharmaceuticals	By electroporation DNA plasmid vaccine delivered	Phase I-II	USA, South Korea
Unnamed	Beijing Institute of Biological Products, Wuhan Institute of Biological Products	Inactivated	Phase I	China
bac TRL-Spike	Symvivo Corporation, University of British Columbia, Dalhousie University	DNA, bacterium medium	Phase I	Canada
Covid-19/aAPC	Shenzhen Geno-Immune Medical Institute	Lentiviral Vector, pathogen-specific artificial antigen presenting dendritic cells	Phase I	China
mRNA-1273	Moderna, US National Institute of Allergy and Infectious Diseases	**Targets the spike protein of the COVID-19 virus**	Phase I	United States
LV-SMENP-DC	Shenzhen Geno-Immune Medical Institute	Lentiviral minigene vaccine	Phase I	China
Remdesivir (GS-5734)	Gilead Science	Inhibit RNA dependent RNA Polymerase	Phase III	United States

*[103,104,107,108,109,114-130]